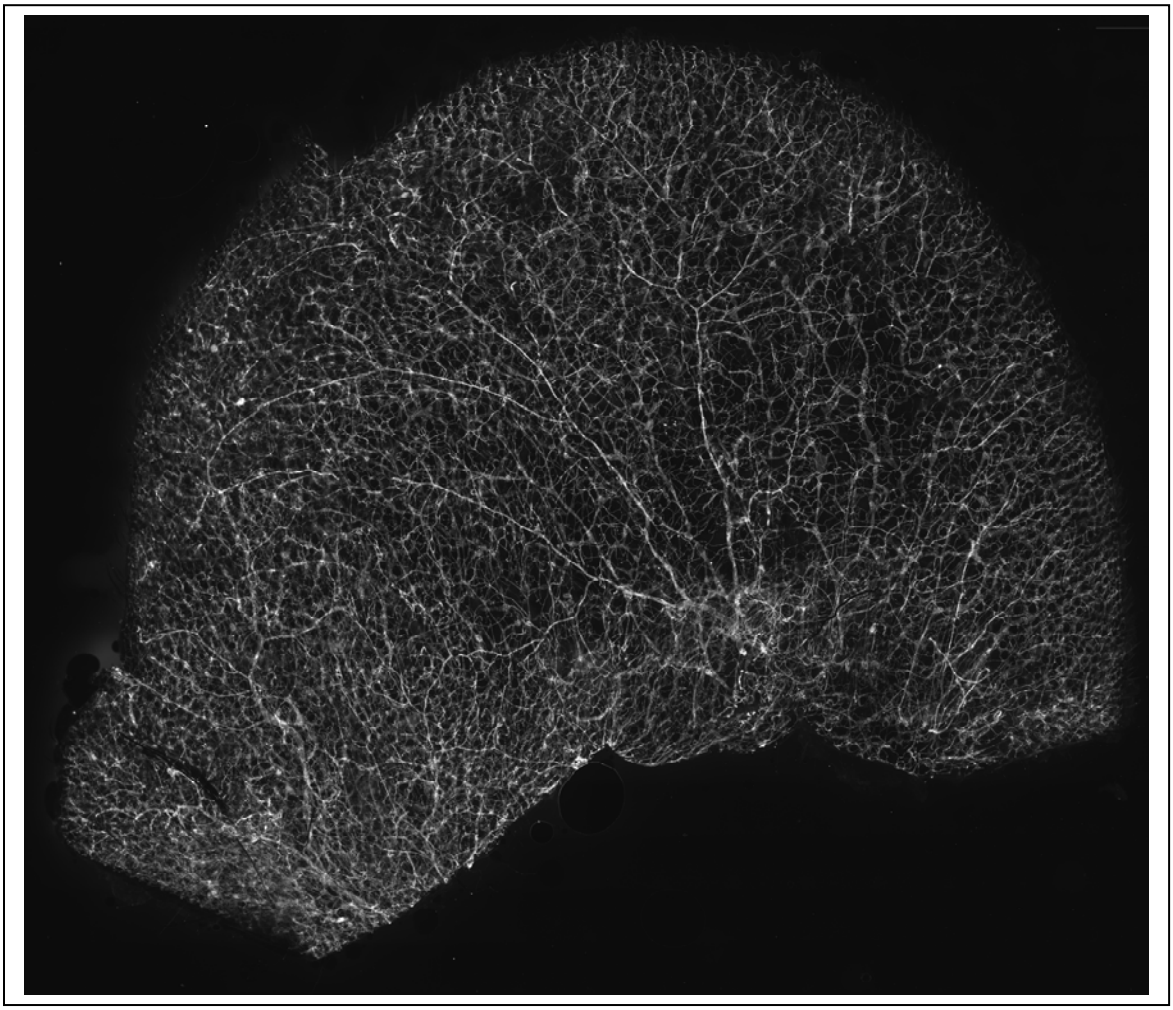
YALE UNIVERSITY SCHOOL OF MEDICINE

Vascular Biology and Therapeutics Program



Annual Report 2007- 2008

Vascular biology
& therapeutics

TABLE OF CONTENTS

On the Cover: Photograph courtesy of Jun Yu, M.D.
PECAM-1 staining showing normal mouse vascular in the ear.

Vascular Biology and Therapeutics Program

Annual Report 2007 – 2008

Message from the Director

The past academic year has been filled with exciting developments in VBT. After many months of significant advance planning, thirteen PIs were relocated to the third and fourth floors of the new Amistad Research Building as a new home for many VBT faculty. This bright, spacious building permitted the acquisition of integrated resources and potential interactions with the Yale Stem Cell Center (YSC) and the Human Translational Immunology (HTI) Program. The move to Amistad was highlighted by an impressive building dedication ceremony capped off by plenary lectures presented by thought leaders representing each of the three programs in Amistad. This was followed by a combined retreat with faculty and staff from VBT, YSC and HTI. In addition, VBT hosted a conference on Williams Syndrome sponsored by the Foundation Chloe's Quest, that was attended by many outstanding scientists. We welcomed new VBT members Drs. Arnar Geirsson and Richard Kim, to the Program and look forward to our interactions in 2008.

PROGRAM OPERATIONS

VBT Steering Committee

The Steering Committee serves as the principal advisory and leadership group for the program for the program. The current membership of the Steering Committee is listed in Table 1.

Administrative Operations

Dr. Sessa assumed his new role as Director of VBT on July 1, 2007. Ms. Carol Muzzey continues as the Program Manager and the program is served by the Central Administration Business Office.

Table I. VBT Steering Committee

Jeffrey R. Bender, M.D., Professor of Internal Medicine (Cadiovascular Medicine) and Immunobiology
Alfred L.M. Bothwell, Ph.D., Professor of Immunobiology
Jack A. Elias, M.D., Zedwitz Professor of Medicine and Section Chief, Pulmonary and Critical Care Medicine
Frank Giordano, M.D., Associate Professor of Internal Medicine (Cardiology)
Joseph A. Madri, M.D., Ph.D., Professor of Pathology
Laura Niklason, M.D., Ph.D., Associate Professor of Anesthesiology and Biomedical Engineering
Jordan S. Pober, M.D., Ph.D., Vice Chair, Immunobiology for Section of Human and Translational Immunology, Professor of Pathology, Immunobiology and Dermatology
Nancy H. Ruddle, Ph.D., John Rodman Paul Professor, Epidemiology and Public Health, Professor of Immunobiology
W. Mark Saltzman, Ph.D., Professor of Chemical and Biomedical Engineering, Chair of Biomedical Engineering
William C. Sessa, Ph.D., Director VBT, Professor and Vice Chair of Pharmacology
George Tellides, M.D., Ph.D., Professor of Surgery (Cardiothoracic) and Chief, Cardiothoracic Surgery

Program Faculty Membership

All faculties at Yale with a significant interest in vascular biology and/or therapeutics are eligible to join VBT. New VBT members in academic year 2007-2008 are:

Arnar Geirsson, M.D., Assistant Professor, Department of Surgery

Richard W. Kim, M.D., Assistant Professor, Department of Surgery

Table 2 VBT Membership

Jeffrey R. Bender, M.D., Professor of Internal Medicine (Cadiovascular Medicine) and Immunobiology	Patty J. Lee, M.D., Associate Professor of Internal Medicine (Pulmonary)
Anton Bennett, Ph.D., Associate Professor of Pharmacology	Joseph A. Madri, M.D., Ph.D., Professor and Director Medical Studies Pathology
Alfred L.M. Bothwell, Ph.D., Professor of Immunobiology	Laura R. Ment, M.D., Professor of Pediatrics (Neurology)
Christopher Breuer, M.D., Assistant Professor of Surgery (Pediatrics)	Wang Min, Ph.D., Associate Professor of Pathology
David Calderwood, Ph.D., Assistant Professor of Pharmacology	Laura Niklason, M.D., Ph.D., Vice Chair for Research, Anesthesiology, Associate Professor of Anesthesiology and Biomedical Engineering
Alan Dardik, Ph.D., M.D., Associate Professor of Vascular Surgery	Jordan S. Pober, M.D., Ph.D., Vice Chair, Immunobiology for Section of Human and Translational Immunology, Professor of Immunobiology, Pathology and Dermatology
Jack A. Elias, M.D., Zedwitz Professor of Medicine and Section Chief, Pulmonary and Critical Care Medicine	David M. Rothstein, M.D., Associate Professor of Internal Medicine (Nephrology)
Tarek Fahmy, Ph.D., Assistant Professor of Biomedical Engineering and Chemical Engineering	Nancy H. Ruddle, Ph.D., John Rodman Paul Professor, Epidemiology and Public Health and Immunobiology
Richard Flavell, Ph.D., FRS, Sterling Professor and Chairman of Immunobiology, Investigator of Howard Hughes Medical Institute	Kerry S. Russell, M.D., Ph.D., Associate Professor of Medicine (Cardiology)
Arnar Geirsson, M.D., Assistant Professor, Department of Surgery	Mehran M. Sadeghi, M.D., Associate Research Scientist of Internal Medicine (Cardiovascular Medicine)
Frank J. Giordano, M.D., Associate Professor Internal Medicine (Cardiovascular Medicine)	W. Mark Saltzman, Ph.D., Professor of Chemical and Biomedical Engineering, Chair of Biomedical Engineering
Daniel R. Goldstein, M.D., Assistant Professor Internal Medicine (Cardiovascular Medicine)	William C. Sessa, Ph.D., Director VBT and Professor and Vice Chair of Pharmacology
Murat Gunel, M.D., Associate Professor of Neurosurgery	Albert J. Sinusas, M.D., F.A.C.C., Professor of Internal Medicine (Cardiovascular Medicine) and Diagnostic Radiology
Richard W. Kim, M.D., Assistant Professor, Department of Surgery	Jeffrey Sklar, M.D., Ph.D., Professor of Pathology and Lab Medicine
Martin Kluger, Ph.D., Research Scientist (Dermatology)	Edward Snyder, M.D., Professor Laboratory Medicine, Director, Apheresis/Cell Processing VBT Core Facility
Diane Krause, M.D., Ph.D., Professor, Departments of Laboratory Medicine, Pathology and Cell Biology	Bing Su, Ph.D., Associate Professor of Immunobiology
Sanjay Kulkarni, M.D., Associate Professor of Surgery (Transplantation & Immunology)	George Tellides, M.D., Ph.D., Professor of Surgery (Cardiothoracic)
Themis Kyriakides, Ph.D., Assistant Professor of Pathology	Agnes Vignery, DDS, Ph.D., Associate Professor of Orthopaedics and Rehabilitation
Erin Lavik, Sc.D., Associate Professor of Biomedical Engineering	Dianqing (Dan) Wu, Ph.D., Professor of Pharmacology

PROGRAM ACTIVITIES

Seminar Series

The VBT Monday afternoon seminars continue to serve as an intellectual focus of the vascular biology community at Yale. The series also serves as venue for assistance in the recruitment of faculty with research in vascular biology to various departments at Yale. The seminars are run by Dr. Themis Kyriakides and have maintained CME accreditation. A list of seminar speakers and their titles is show in Table 3.

Table 3 – VBT 2007-2008 Seminar Series

October
Michael Simons, M.D., Chief, Section of Cardiology, Director, Angiogenesis Research Center, Dartmouth Medical School, "FGF-dependent regulation of vascular integrity"
Timothy Hla, Ph.D., Professor of Cell Biology, Genetics & Developmental Biology, Director, Center for Vascular Biology, UCONN School of Medicine, "Sphingosine 1-phosphate regulation of vascular phenotype"
November
Paul Lizardi, Ph.D., Professor of Pathology, Yale University, "Mapping DNA methylation changes in genes, noncoding NA, and DNA repeats: Metrics for the onset of genomic instability in tissues"
Andrew Lichtman, M.D., Ph.D., Department of Pathology, Brigham and Women's Hospital, "Regulation of T cell responses in the cardiovascular system"
January
Stanley Hazen, M.D., Ph.D., Section Head, Preventive Cardiology & Rehabilitation, Cleveland Clinic, "Myeloperoxidase and impairment in HDL function during atherosclerosis"
February
Edward A. Fisher, M.D., Ph.D., M.P.H., Leon H. Charney Professor of Cardiovascular Medicine, Director, Marc and Ruti Bell Vascular Biology and Disease Program, NY University School of Medicine, "The regression of atherosclerosis: insights from muse models and clinical studies"
March
Jeffrey R. Bender, M.D., Robert I. Levy Professor of Cardiology, Professor of Medicine and Immunobiology, Associate Chief, Division of Cardiovascular Medicine, Yale University School of Medicine, "Integrin-dependent cytokine and angiogenic factor gene expression: Relevance in vascular and pulmonary pathology"
Leslie M. Loew, Ph.D., Professor of Cell Biology, Director, Center for Cell Analysis and Modeling, UCONN Health Center, "The virtual cell project"
Dario Fauza, M.D., Associate in Surgery, Assistant Professor of Surgery, Children's Hospital, Harvard Medical School, "Fetal tissue engineering for the treatment of congenital anomalies"
Bart Edward Muhs, M.D., Ph.D., Associate Professor, Department of Vascular Surgery, Yale University School of Medicine, "Endovascular dynamics of the aorta and its side branches; implications for aortic aneurysm repair"
Francis William Luscinskas, Ph.D., Associate Professor, Department of Pathology, Harvard Medical School, Associate Director, Vascular Research Division, Brigham and Women's Hospital, "Adhesion dependent signaling and mechanical forces induced during leukocyte diapedesis"
April
Edward Damiano, Ph.D., Associate Professor of Biomedical Engineering, Boston University College of Engineering, "The role of the endothelial glycocalyx in cardiovascular health and disease"
Heidi Stuhlmann, Ph.D., Professor of Cell & Developmental Biology, Weill Cornell Medical College, "Role of VEZF1 and EGFL7 in vascular development"
May
Jeffrey Holmes, Ph.D., Associate Professor of Biomedical Engineering and Medicine, University of Virginia, "The mechanics of healing myocardial infarcts and tissue-engineered analogs"
Joel Pachter, Ph.D., Professor of Cell Biology, UCONN Health Center, "CCL2 interactions at the blood-brain barrier: A subtle means to an inflammatory end"
June
Louis M. Messina, M.D., Professor of Surgery, Chief, Division of Vascular Surgery, Vice Chair, Department of Surgery, University of Massachusetts Medical School, "eNOS and collateral artery enlargement"
July
Calum A. MacRae, M.D., Ph.D., Assistant Professor of Medicine Harvard Medical School and Massachusetts General Hospital, "common cardiovascular disease: complementary approaches in human and zebrafish"

Retreat

The annual retreat continues to be an extremely popular activity, bringing together over one hundred scientists from the laboratories of VBT faculty members. This past year, the retreat was held on October 6. 2007 at the Anlyan Center for Medical Research and Education (TAC) in conjunction with Human and Translational Immunology Program and Yale Stem Cell Center. In addition to three sessions by VBT, HTI and Stem Cell, the retreat continued the poster session competition with prizes for the best posters by a graduate student and by a post-doctoral fellow.

Guests at this years retreat were Marc Feldmann, FMedSci, FRS, Director, Kennedy Institute of Rheumatology, Imperial College, London, Douglas Melton, Ph.D., Thomas Dudley Cabot Professor of the Natural Sciences, Investigator, Howard Hughes Medical Institute, Harvard University and Dr. Salvador Moncada, MD, PhD, DSc, FRCP, FRS, Director, Wolfson Institute for Biomedical Research, University College, London.

The retreat was sponsored by an unrestricted gift from Boehringer-Ingelheim Pharmaceuticals, Inc. the retreat Program is listed in Appendix 1.

Williams Syndrome Conference

The Vascular Biology and Therapeutics (VBT) Program of the Yale University School of Medicine and The Chloe's Quest Foundation organized a scientific meeting on the subject of "Cardiovascular disease in Williams-Beuren syndrome that was held at Yale University in May 2008.

The Chloe's Quest Foundation has been established to support basic research with the principal aim of identifying treatments that can be offered in clinical trials. Since the VBT Program at Yale is committed to applying the discoveries of vascular biology to advances in medicine, it joined with The Chloe's Quest Foundation to sponsor this meeting. The program for this meeting is listed in Appendix 2.

Yale-Cambridge Program in Cardiovascular Disease

The research alliance with Cambridge has continued as an important activity, with 17 faculty from Cambridge visiting Yale in September 2007 for a two day scientific meeting. The program for this retreat is listed in Appendix 3. A visit by Yale members to Cambridge is scheduled for September 2008.

Tissue Engineering Group

This biweekly forum, sponsored by VBT and organized by Dr. Themis Kyriakides, brings together investigators from Yale Medical School and Yale's central campus to exchange updates in research in progress and to foster new research collaborations.

Jeffrey R. Bender, M.D.
Professor of Internal Medicine (Cardiovascular Medicine) and Immunobiology

Leukocyte-endothelial cell (EC) interactions are thought to play a role in a variety of pathological processes including inflammation, allograft rejection, and atherosclerosis. As the contiguous barrier to circulating immunocompetent cells in vascularized allografts, donor endothelium is a major stimulator and target of alloimmune responses by recipient lymphocytes, resulting in graft rejection, one form of which is transplant coronary arteriosclerosis. Furthermore, non-transplant atherosclerosis is now recognized as a multifactorial complex process that bears many similarities to chronic inflammatory conditions as demonstrated by the focal accumulation of leukocytes. The gender/hormonal influences on the development of atherosclerosis may be manifested in alterations in these inflammatory components. The efforts of my laboratory are directed at defining cellular and molecular mechanisms that govern leukocyte-EC interactions, and to test these molecular discoveries in animal vascular pathology models. Furthermore, we are studying physiologic and pathologic modulators of endothelial function. More specifically, there are three major areas of investigation: (1) leukocyte integrins and T cell/macrophage gene expression; (2) effects of metabolic syndrome-associated lipids on endothelial function; and (3) influence of ovarian steroid hormones of endothelial activation and endothelial progenitor cell function.

Specific Research Accomplishments in the last 12 months: (1) through proteomics analysis, we determined that the leukocyte integrin-modulated mRNA-binding protein, HuR, interacts with another nuclear protein, RNP-C, which affects the proinflammatory cytokine and angiogenic factor mRNA stabilization response; (2) macrophage-specific deletion of HuR results in (a) impaired neovascular responses to an inflammatory stimulus and, (b) abrogation of pulmonary inflammation in response to an aerosolized challenge; (3) through TIRF microscopy approaches, we documented the rapid recruitment of the estrogen receptor splice isoform, ER46, to the plasma membrane in response to hormone; (4) through the induction of ceramide-activated protein phosphatases, free fatty acid exposure results in a state of endothelial "VEGF resistance"; (5) a leukocute integrin-associated component of the COP9 signalsome is critical in early stages of T cell development; and (6) endothelial progenitor cells highly express ER46 and incorporate into vascular structures (*in vitro* and *in vivo*) in response to estrogen. These findings have mechanistic implications in allograft rejection, atherosclerosis and angiogenesis, all major clinical targets for the VBT Program.

Publications:

Wang, J., Collinge, M., Ramgolam, V. Ayalon, A., Xinhao, C.F., Pardi, R., **Bender, J.R**.: LFA-1-Dependent HuR Nuclear Export and Cytokine mRNA Stabilization in T Cell Activation.. J. Immunol. 176(4):2105-2113, 2006.

Sadgehi, M.M., **Bender, J.R.**: Targeting □v□3 in vascular remodeling. Trends in Cardiovascular Medicine. 17:5-10, 2007.

Smith, D., Sadeghi, M.M., **Bender, J.R.:** Imaging Targets in Atherosclerosis. In: Textbook of Cardiovascular Molecular Imaging. Informa Healthcare Publishing. Ed. Sinusas A, Gropler R, Glover D, Taegtmeyer H. Ch. 18, p. 189-202, 2007.

Sadeghi, M., Esmailzadeh, L., Zhang, J., Guo, Z., Asadi, A., Krassilnikova, S., Fassaei, H.R., Luo, G., Danesh, F.R., Al-Lamki R.S.M., Takahashi, T., Tellides, G., **Bender, J.R.,** Rodriguez E.R.: Endothelial and smooth muscle cell-derived neuropilin-like protein is a marker and regulator of cell proliferation in vascular remodeling. Am. J. Transplant., 7(9):2098-2015, 2007

Li, L., Hisamoto, K., Haynes, P., Bauer, P., Sanjay, A., Baron,R., Sessa, W.C., **Bender, J.R.:** Variant Estrogen Receptor -c-Src Molecular Interdependence and c-Src Structural Requirements for eNOS activation. Proc. Natl. Acad. Sci, USA, 104(42):16468-16473, 2007.

Savio, M., Rotondo, G., Rossetti, G., **Bender, J.R.**, Pardi, R. A novel alternatively spliced constitutive photomorphogenic-1 (COP-1) product stabilizes UV stress-induced C-jun by inhibiting COP1. Oncogene, 27:2401-2411, 2008.

Panattoni, M., Sanvito, F., Basso, V., **Bender, J.R.**, Doglioni, C., Casorati, G., Mondino, A., Pardi, R.: Targeted Inactivation Of The COP9 Signalosome Impairs Multiple Stages Of T Cell Development. J Exp Med., 205:465-477, 2008.

Kim, K.H., Moriarty, K., **Bender, J.R**.: Vascular Cell Signaling by Membrane Estrogen Receptors. Steroids 73:864-869, 2008.

Rao, G.K., **Bender, J.R.:** Rac, PAK and eNOS ACTion. Circ. Res., 103:328-330, 2008.

Ramgolam, V., DeGregorio, S., Subaran, S., Pardi, R., Collinge, M., **Bender, J.R.:** LFA-1 Engagement Induces HuR-dependent Cytokine mRNA Stabilization Through a Vav-1, Rac, p38 Cascade. Submitted (in revision).

Anton M. Bennett, Ph.D.
Associate Professor, Department of Pharmacology

The broad research plan of this laboratory is to define the molecular mechanisms, physiological and pathophysiological roles of the protein tyrosine phosphatase (PTP) family of enzymes. Given that the maintenance of the vasculature is dependent upon pathways controlled by tyrosine phosphorylation, PTPs are likely to play essential roles in cardiovascular function. We are interested in how the tyrosine-specific PTP termed, SHP-2, controls growth factor receptor signaling. In addition, we are focusing on the pathophysiological mechanisms of SHP-2 in Noonan syndrome (NS), an autosomal dominant disorder in which activating SHP-2 mutations are found in ~50% of cases. NS patients exhibit a diverse array of clinical manifestations, most notably, congenital heart disease. We are also pursuing the physiological function and pathophysiological roles of the dual-specificity phosphatases, known as MAP kinase phosphatases, which directly inactivate the MAP kinases, thereby terminating this pathway. We are investigating the role of the MKPs in tissue injury and repair.

Specific Research Accomplishments in the last 12 months:
In our ongoing efforts to define the pathophysiological mechanisms of how NS-associated SHP-2 mutations give rise to cardiovascular disease, we have discovered critical target proteins of the NS-associated SHP-2 mutations. These target proteins were found to be aberrantly regulated in the myocardium of a mouse model of NS, suggesting that these proteins might play critical roles in the development of NS-associated SHP-2 mutant signaling in the heart. In addition, we have identified that SHP-2 plays a critical role in endothelial cell signaling downstream of the VEGF receptor to control Src-mediated signaling. Collectively, these two studies strongly suggest an important role for SHP-2 in disease-related signaling in the myocardium and VEGF-mediated signaling in endothelial cells.

Significance of Key Findings Relevant for the Mission of VBT:
Congenital heart disease occurs in up to 80% of NS patients, making *PTPN11*/SHP-2 mutations the most common non-chromosomal cause of congenital heart disease. Identification of target proteins of the disease-associated mutations of SHP-2 as well as a role for SHP-2 in VEGF signaling is anticipated to provide significant insight in to the pathways that become dysregulated in the heart of NS patients. We anticipate that once fully characterized these signaling pathways may provide insight in to potential therapeutic strategies for the treatment of some forms of cardiovascular disease.

Publications:

Rodrigues, M. A., Dawidson, G.A., Leite, F., Grant, W., Zhang, L., Lam, W., Cheng, Y-C Cheng, Bennett, A.M. and Nathanson, M.H., (2007) Nucleoplasmic Calcium is Required for Cell Proliferation, *J. Biol. Chem.*, 282: 17061-17068.

Dawidson, G.A., Rodrigues, M.A., Leite, M.F., Gomez, M.V., Balla, T., Bennett, A.M. and Nathanson, M.H. (2008) c-met must translocate to the nucleus to initiate calcium signals, *J. Biol. Chem.*, 283: 4344-4351.

Ha, C.H., Bennett, A.M. and Jin, Z.G. (2008) A novel role of vascular endothelial cadherin in modulating c-Src activation and downstream signaling of vascular endothelial growth factor., *J. Biol. Chem.*, 283: 7261-7270.

Eminaga, S. **and Bennett, A.M.** (2008) Noonan Syndrome-associated SHP-2/*Ptpn11* Mutants Enhance SIRPα and PZR Tyrosyl Phosphorylation and Promote Adhesion-mediated ERK Activation, *J. Biol. Chem.*, 283: 15328-15338.

Tyner, K. J., Boadu, E., Mercan, F., Zhang, L., Hall, J.K., Antwine, T., Olwin, B.B. and Bennett, A.M. (2008) MAP Kinase Phosphatase-1 Coordinates Myogenic Progression and is Essential for Skeletal Muscle Regeneration, *J. Cell Biol.*, in revision.

Alfred L.M. Bothwell, Ph.D.
Professor, Dept. of Immunobiology

The research goals of the lab are to characterize the development and function of regulatory T cells and characterize mechanisms that affect autoimmunity, inflammation, transplantation and recruitment into vascular sites. Both mouse and human responses are studied in vitro and in vivo which includes the development and use of humanized mice. In addition, our experience with synthetic microvessels has lead to a translational project to revascularize islets to treat type I diabetes.

Specific accomplishments in the last year:

PPARs: Peroxysome proliferated activated receptors (PPARs) represent a group of transcription factors that are critical in regulating glucose and lipid metabolism. Ligands of PPARγ inhibit metabolically induced arteriosclerosis and also prevent the development of inflammatory disorders in several experimental mouse models including EAE, asthma, rheumatic arthritis and sepsis. The role of PPARγ in graft arteriosclerosis (GA) has not been characterized. We therefore tested the *in vivo* effects of administration of the endogenously occurring ligand, 15 deoxy-prostaglandin-J_2 (15-d-PGJ_2), on vascular remodeling of human artery induced by alloreactive PBMC and the IFN-γ model. The data indicate that 15-d-PGJ_2 inhibits human GA in our *in vivo* human arterial graft model in immunodeficient mice. Since the interactions between endothelial cells (EC) and lymphocytes initiate vascular rejection, we investigated the role of PPARγ in these primary human cells. During the last year we have optimized the isolation and expansion of human Treg and trasnduced them with lentivurus vectors that alter expression of PPARγ. We hope to identify the important signaling pathways that affect PPARγ function in T cells, EC and SMC.

Microorgan Islet Grafts: The goal of this project is to bioengineer pancreatic beta cell-containing implants for treatment of diabetes. Casting the islets together with EC in collagen gels effectively revascularizes the islets in SCID/bg mice. Indeed, our pilot data indicate that these human islet-EC microorgans secrete human insulin into the peripheral blood of mice for periods of at least 4 months and demonstrate responsiveness to glucose in glucose tolerance tests. We are characterizing the structure/function properties of these microorgans in detail, including the microvessel structure and the stability of the microvessels with time. During the last year we have shown (with Serge Kobsa and M. Saltzman) that Hepatocyte Growth Factor (HGF) has significant anti-apoptotic activity on islets in vitro. We will utilize synthetic molecular scaffolds to increase the size and function and microspheres will be optimized for the delivery of factors like HGF that will promote beta cell survival and function.

Publications:

Muthukumarana, P., Chae, W.-J., Maher, S.E., Rosengard, B.R., Bothwell, A.L.M. and Metcalfe, S.M. (2007). Regulatory transplantation tolerance and "stemness": evidence that Foxp3 may play a regulatory role in SOCS-3 gene transcription. Transplantation 84:S6-S11, PMID: 17632414.

Bai Y, Ahmad U, Wang Y, Li JH, Choy JC, Kim RW, Kirkiles-Smith N, Maher SE, Karras JG, Bennett CF, Bothwell AL, Pober JS, Tellides G. (2008). Interferon-{gamma} Induces X-linked Inhibitor of Apoptosis-associated Factor-1 and Noxa Expression and Potentiates Human Vascular Smooth Muscle Cell Apoptosis by STAT3 Activation. J. Biol. Chem. 283:6832-6842.

Yi, T., Rao, D.A., Tang' P.C.Y., Wang, Y., Cuchara, L., Bothwell, A.L.M., Colangelo, C.M., Tellides, G., Pober, J.S. and Lorber, M.I. Amelioration of human allograft arterial injury by atorvastatin or simvastatin correlates with reduction of interferon-γ production by infiltrating T cells. Transplantation, in press.

Alfred L.M. Bothwell, Ph.D.

Das, G., Das, J., Ren, G., Zhang, L., Bothwell, A.L.M., Van Kaer, L., Roberts, A.I. and Shi, Y. Impaired Transforming Growth Factor-□ signaling in $CD4^+$ T cells leads to the spontaneous development of Th17 cells. Submitted.

Tobiasova, Z., Van der Lingen, K.H.B., Scahill, L.D., Anderson, G., Leckman, J.F. Zhang, Y., McCracken, J.T., Vitiello, B., Tierney, E., Aman, M., McDougle, C., Katsovich, L., Mulder, E.J., Hoekstra, P., de Bildt, A., Minderaa, R.B., Bothwell, A.L.M., and Kawikova, I. Altered levels of EGF and IL-13 in children with autism are not associated with severity of the disorder. Submitted.

Choi, J.-M., Kim, S.-H., Shin, J.-H., Gibson, T., Yoon, B.-S., Lee, D.-H., Lee, S.-K., Bothwell, A.L.M., Lim, J.-S. and Lee, S.-K. Cell permeable ccytoplasmic domain of CTLA-4 recombinant protein inhibits TcR-specific membrane proximal activation signals and prevents collagen-induced arthritis. Submitted.

Giambra, V., Volpi, S., Emelyanov, A., Pflugh, D.L., Bothwell, A.L.M., Norio, P., Fan, Y., Skoultchi, A.I., Hardy, R.R., Frezza, D. and Birshtein, B.K. Pax5 and linker histone H1 coordinate DNA methylation and histone modifications in the 3' regulatory region of the immunoglobulin heavy chain locus. Moll. Cell. Boil., in press.

Chen, W., Begum, S., Garyu, J., Gibson, T.F., Bothwell, A.L.M., Virginia E. Papaioannou, V.E. and Kevan C. Herold, K.C. Promotion of β-Cell Differentiation in Pancreatic Precursor Cells by Adult Islet Cells. Submitted.

Nakayama, Y., Schultz, V., Karmakar, S., Mahajan, M.C., Euskirchen, G., Tuck, D., Synder, M., Weissman, S.M. and Bothwell, A.L.M. The role of IRF4 in the transcriptional network in B cell development. Submitted.

Choi, J., Shin, J.-H., Lee, C.G., Lee, S.-K. and Bothwell, A.L.M. Foxp3 protein transduction of mice inhibits IBD and asthma. Submitted.

Christopher Breuer, M.D.
Assistant Professor, Department of Surgery

The overall goal of my research program is to use tissue-engineering techniques to create autologous, living blood cardiovascular neotissues that can be used for reconstructive surgical applications such as bypass grafting.

Specific Research Accomplishments in the last 12 months:
In the last year I received the Doris Duke Charitable Foundation clinical scientist development award, the American College of Surgeon's Jacobson Promising Investigator Award and a Connecticut Stem Cell Research grant.

Significance of Key Findings Relevant for the Mission of VBT:
The key findings related to our research and relevant to the VBT mission include: (1) confirmation that tissue engineered vascular grafts demonstrate evidence of normal, non – pathologic growth and development, (2) development of a cellular probe that enables us to monitor tissue engineered vascular grafts using magnetic resonance imaging *in vivo*, (3) discovery and demonstration that vascular neotissue formation arises from a paracrine effect and is MCP-1 dependent.

Publications:

Kudo FA, Muto A, Maloney SP, Pimiento JM, Bergaya S, Fitzgerald TN, Westvik TS, Frattini JC, **Breuer CK**, Cha CH, Nishibe T, Tellides G, Sess WC, DardikA.Venous identity is lost but arterial identity is not gained during vein graft adaptation. *Arterioscler Thromb Vasc Biol.* 2007*,* 27(7):1562-71.

Roh JD, Nelson GN, Udelsman BV, Brennan MP, Lockhart B, Fong PM, **Breuer CK**. Centrigugal seeding increases seeding efficiency and cellular distribution of bone marrow stromal cells in porous biodegradable scaffolds. *Tissue Eng.* 2007, 13(11):2743-9.

Roh JD, Nelson GN, Brennan MP, Mirensky T, Yi T, Hazlet T, Tellides G, Sinusas AJ, Pober JS, Saltzman WM, Kyriakides TR, **Breuer CK**. Small diameter biodegradable scaffolds for vascular tissue engineering in the mouse model. *Biomaterials.* 2007, 29(10):1454-63.

Nelson GN, Mirensky T, Brennan MP, Roh JD, Yi T, Wang Y, **Breuer CK**. Functional small-diameter human tissue engineered arterial grafts in an immunodeficient mouse model: Preliminary findings. *Arch Surg*. 2008, 143(5):488-94.

Mirensky T, **Breuer CK**. The development of tissue engineered grafts for reconstructive cardiothoracic surgical applications. *Pediatr Res.* 2008, 63(5):559-68.

Qian X, Brennan M, Dione D, Dobrucki L, Jackowski M, Breuer C, Sinusas AJ, Papademitris X. Detection of complex vascular structures using polar neighborhood intensity profile. *Med Image Anal*, 2008 (in press).

Brennan MP, Dardik A, Hibino N, Roh JD, Nelson GN, Papademitris X, Shinoka T, **Breuer CK**. Tissue engineered vascular grafts demonstrate evidence of growth and development when implanted in a juvenile animal model. *Ann Surg* 2008 (in press)

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David A. Calderwood, Ph.D.
Assistant Professor, Department of Pharmacology

The overall goal of my lab is to understand integrin adhesion receptors and their links to the actin cytoskeleton. The binding of integrin extracellular domains to diverse protein ligands mediates cell-cell and cell-substratum adhesion however, cellular control of these adhesive interactions and their translation into dynamic responses, such as cell spreading or migration, requires the integrin cytoplasmic tails. These short tails bind intracellular ligands that connect integrins to signaling pathways and cytoskeletal networks. Thus, by binding both extracellular and intracellular ligands, integrins provide a link for the bidirectional transmission of mechanical force and biochemical signals across the plasma membrane. Tight regulation of integrin function is essential because it controls cell adhesion, migration, and assembly of an extracellular matrix and so is a critical step in angiogenesis, embryonic development, cardiac function, the immune response and tumor metastasis.

Specific Research Accomplishments in the last 12 months: Progress has been on 2 main fronts: characterization of integrin filamin (FLN) interactions and regulation of integrin activation by talin. **i**) Human FLNs are large proteins composed of an N-terminal actin-binding domain followed by 24 Ig-like domains (IgFLN). We showed that FLN binding to integrin β subunit tails regulates cell migration, and have since localized the major integrin-binding site to IgFLN21, and characterized this interaction structurally. This revealed several mechanisms likely to control integrin-filamin interactions. Recent structural studies of larger 3-domain FLN fragments revealed an unexpected domain arrangement resulting in masking of the integrin-binding site in IgFLN21 and auto-inhibition of binding to integrins. This is released in FLN splice variants and provides a mechanism by which ligand-binding may impact FLN structure. We have also continued our investigation of FLN in cell migration and recently found that loss of both FLNa and b is required to inhibit cell migration – the basis for this is under investigation. **ii**) The activation state of integrins determines their ligand binding affinity. We have shown that talin binding to integrin β tails governs activation and recently identified additional domains of talin that cooperate with the integrin-binding domain during β1 and β3 integrin activation. The basis for this effect is under investigation.

Significance of Key Findings Relevant for the Mission of VBT: We seek to understand the molecular basis of integrin activation which is critical for platelet aggregation, angiogenesis and leukocyte trafficking. We also seek to better understand IgFLN interactions with a particular interest in integrin-FLN interactions. In mice FLNa is essential for cardiac and vascular development, FLNb is required for skeletal and microvascular development, and FLNc is necessary for normal myogenesis. In humans, certain FLNa missense mutations cause familial cardiac valvular dystrophy and putative gain-of-function mutations result in a spectrum of congenital skeletal dysplasias. Mutations in FLNb cause abnormal vertebral segmentation, skeletogenesis and joint formation and a FLNc mutation causes an autosomal dominant myofibrillar myopathy. The diversity in phenotypes associated with different FLN mutations reveals that they perform a variety of essential functions and the current evidence suggests that specific disease phenotypes result from disruption of specific interactions between IgFLN domains and their partners.

Publications:

Lad, Y., Kiema T., Jiang, P., Pentikäinen, O. T., Coles, C. H., Campbell, I. D., **Calderwood, D. A.** and Ylänne, J. (2007) Structure of three tandem filamin domains reveals auto-inhibition of ligand binding. **EMBO J.** *26*, 3993-4004.

Bouaouina, M., Lad, Y., and **Calderwood D. A.** (2008) The N-terminal domains of talin co-operate with the PTB-like domain to activate β1 and β3 integrins. **J. Biol. Chem.**, *283* (10):6118-25.

Alan Dardik, M.D., Ph.D.
Associate Professor of Surgery (Vascular Surgery)

The Dardik laboratory continues to study the healing and function of blood vessels and synthetic blood vessel substitutes, such as used in patients having vascular bypass surgery, and with particular attention given to the role of aging in host responses.

Specific Research Accomplishments in the last 12 months:

We are trying to understand the fundamental molecular mechanisms by which vein graft adaptation results in positive remodeling and successful adaptation to the arterial environment, yet often proceeds, in the long-term, to neointimal hyperplasia and graft failure.

We previously published that Eph-B4, a determinant of venous identity during embryonic development that persists as a venous marker, decreases expression and immunodetectable protein during vein graft adaptation in both humans and aged rats. We have extended our findings to a mouse model and have found similar results. We are currently manipulating Eph-B4 in vivo to test our hypothesis that Eph-B4 remains functional during adult life and is a regulator of vein graft identity and adaptation to the arterial environment.

Significance of Key Findings Relevant for the Mission of VBT

Understanding vein graft adaptation to the arterial circulation is critical to improving vascular conduits for surgical use and minimizing conduit failure, consistent with the VBT mission to apply the insights of vascular biology to improve organ replacement therapy.

Publications:

Yoo PS, Mulkeen AL, **Dardik A**, Cha CH. A novel *in vitro* model of lymphatic metastasis from colorectal cancer. *Journal of Surgical Research* 143(1):94-98 (2007).

Nishibe T, Kondo Y, Muto A, **Dardik A**. Optimal prosthetic graft design for small diameter vascular grafts. *Vascular* 15(6):356-360 (2007).

Magri D, Fancher TT, Fitzgerald TN, Muto A, **Dardik A**. Endothelial progenitor cells: A primer for vascular surgeons. *Vascular* 15(6):384-394 (2007).

Fancher TT, Muto A, Fitzgerald TN, Magri D, Gortler D, Nishibe T, **Dardik A**. Control of blood vessel identity: From embryo to adult. *Annals of Vascular Diseases* 1(1):28-24 (2008).

Pimiento JM, Maloney SP, Tang PCY, Muto A, Westvik TS, Fitzgerald TN, Fancher TT, Tellides G, **Dardik A**. Endothelial nitric oxide synthase stimulates aneurysm growth in aged mice. *Journal of Vascular Research* 45(3):251-258 (2008).

Fitzgerald TN, Shepherd BR, Asada H, Teso D, Muto A, Fancher T, Pimiento JM, Maloney SP, **Dardik A**. Laminar shear stress stimulates vascular smooth muscle cell apoptosis via the Akt pathway. *Journal of Cellular Physiology* 216(2):389-395 (2008).

Jack A. Elias, M.D.
Waldemar von Zedtwitz Professor of Medicine, Professor of Immunobiology, Chairman of Internal Medicine

The Elias Laboratory is intensely interested in chronic inflammatory, injury and remodeling responses in the lung. To optimally address these issues, the laboratory has established the techniques that allow one to express transgenes in a lung-specific fashion. In addition, the laboratory established systems that allow transgenes to be eternally regulated giving the investigator the ability to selectively express a gene at a specific point in time during development and the ability to turn a gene on and off at will. Studies in the laboratory are presently focusing on the inflammation, vascular alterations and remodeling in asthma, COPD, the pathogenesis of pulmonary fibrosis and mechanisms of cytoprotection in acute lung injury. These studies are funded by multiple NIH RO1 grants, an NIH Program Project Grant (Dr. Elias is the Principal Investigator) and multiple industrial research awards.

Publications:

Kang MJ, Lee CG, Lee JY, Dela Cruz CS, Chen ZJ, Enelow R, Elias JA. Cigarette smoke selectively enhances viral PAMP- and virus-induced pulmonary innate immune and remodeling responses in mice. J Clin Invest. 2008 Aug;118(8):2771-84.

Lee CG, Hartl D, Matsuura H, Dunlop FM, Scotney PD, Fabri LJ, Nash AD, Chen NY, Tang CY, Chen Q, Homer RJ, Baca M, Elias JA. Endogenous IL-11 Signaling is Essential in Th2- and IL-13-induced Inflammation and Mucus Production. Am J Respir Cell Mol Biol. 2008 Jul 10. [Epub ahead of print]

Bhandari V, Choo-Wing R, Lee CG, Yusuf K, Nedrelow JH, Ambalavanan N, Malkus H, Homer RJ, Elias JA. Developmental Regulation of NO-mediated VEGF-induced Effects in the Lung. Am J Respir Cell Mol Biol. 2008 Apr 25.

Zheng T, Liu W, Oh SY, Zhu Z, Hu B, Homer RJ, Cohn L, Grusby MJ, Elias JA. IL-13 receptor alpha2 selectively inhibits IL-13-induced responses in the murine lung. J Immunol. 2008 Jan 1;180(1):522-9.

Chupp GL, Lee CG, Jarjour N, Shim YM, Holm CT, He S, Dziura JD, Reed J, Coyle AJ, Kiener P, Cullen M, Grandsaigne M, Dombret MC, Aubier M, Pretolani M, Elias JA. A chitinase-like protein in the lung and circulation of patients with severe asthma. N Engl J Med. 2007 Nov 15;357(20):2016-27.

Chapoval SP, Al-Garawi A, Lora JM, Strickland I, Ma B, Lee PJ, Homer RJ, Ghosh S, Coyle AJ, Elias JA. Inhibition of NF-kappaB activation reduces the tissue effects of transgenic IL-13. J Immunol. 2007 Nov 15;179(10):7030-41.

Kang HR, Lee CG, Homer RJ, Elias JA. Semaphorin 7A plays a critical role in TGF-beta1-induced pulmonary fibrosis. J Exp Med. 2007 May 14;204(5):1083-93. Epub 2007 May 7.

Tarek M. Fahmy, Ph.D.
Assistant Professor of Biomedical Engineering, Dept. of Biomedical Engineering

Our research program is focused on the engineering and application of novel biomaterials for modulation and detection of immune system cells. These materials range from biodegradable nanoparticles to semiconductor nanosensors and carbon nanotubes. Our program is currently active in three different areas: First, construction of artificial antigen-presenting systems that may by used for, a) detection of antigen-specific T cells, b) ex-vivo stimulation and expansion of those cells, c) delivery of drug to inhibit proliferation of those cells. A second area of research involves the design of modular nanoparticles that target dendritic cells for creation of adaptable vaccine delivery vehicles. Finally, we are integrating these approaches in the design of targeted particulate systems that can be imaged by a variety of modalities such as ultrasound, CT and magnetic resonance and that may be ultimately used for simultaneous tracking and drug/protein delivery to cells in vivo.

Specific accomplishments in the last year: (In relation to the VBT Program):
In relation to the VBT program we have focused on engineering non-invasive imaging modalities such as ultrasound and magnetic resonance imaging in targeted biodegradable particles. Our approach uses particulate contrast agents that are engineered with targeting ligands and made to be echogenic as well as paramagnetic, facilitating imaging of blood flow in target vessels by ultrasound and offering the potential for high resolution anatomical imaging of vessel architecture by magnetic resonance. This is critical to assessment of the success of approaches that induce vessel formation and blood perfusion in tissue after ischemic injury in vivo without the need for repetitive histology. In addition, we have designed fluorinated self-assembled nanoparticles that can accumulate in atherosclerotic plaques and that can detected by 19F MR imaging.

Publications:

Stern E, Routenberg D, Wyrembak P, Hamilton A, LaVan D, **Fahmy TM**, Reed MA. Label-Free Immunodetection with CMOS-Compatible Semiconducting Nanowires, *Nature*, 2007 Feb 1; 445, 519-522.

Fahmy TM, Schneck JP, Saltzman WM. A nanoscopic multivalent antigen-presenting carrier for sensitive detection and drug delivery to T cells. *Nanomedicine: Nanotech., Biology and Med*. 2007 Mar;3(1):75-85.

Fahmy TM, Fong P, Park J, Constable T, Saltzman WM, Nanosystems for simultaneous imaging and drug delivery to T cells. AAPS J. 2007 Jun 8;9(2):E171-80.

Shapiro E, Davis L, **Fahmy TM**, Dunbar C, Koretsky A. Antibody mediated cell labeling of peripheral T cells with micron sized iron-oxide particles (MPIOs) allows single cell detection by MRI. *Contrast Media & Molecular Imaging* 2007 May;2(3):147-53.

Stern E, Wagner R, Breaker R, Sigworth F, **Fahmy TM,** Reed MA. Importance of the Debye Screening Length on Nanowire Field Effect Transistor Sensors. *Nanoletters* 2007 Nov 14;7(11):3405-3409.

Samstein R, Perica K, Balderrama F, Look M, **Fahmy TM**, The use of deoxycholic acids for enhancing oral bioavailability of biodegradable particles. *Biomaterials*. 2007 Vol 29 (6) pp 703-708.

Mounzer R, Shkarin P, Papademetrious X, Constable T, Ruddle N, **Fahmy TM**, Dynamic Imaging of Lymphatic Vessels and Lymph Nodes Using a Bimodal Nanoparticulate Contrast Agent. *Lymphatic Research and Biology.* 2007 5(3) 151-155

Steenblock E, **Fahmy TM,** A comprehensive platform for T cell stimulation based on biodegradable artificial antigen-presenting cell microparticles. *Molecular Therapy*. 2008, Mar, 4 [Epub ahead of print]

Fadel TR, Haller G, Pfefferle L, **Fahmy TM**, Enhanced cellular activation with single-wall carbon nanotubes presenting antibody stimuli. *Nanoletters*. 2008 Jun 12. [Epub ahead of print]

Tarek M. Fahmy, Ph.D.

Fahmy TM, Mellman I, Caplan M, Saltzman WM, Design opportunities and challenges for Nanoparticle Vaccines. *Nanomedicine.* 2008 Jun;3(3):343-55

Nelson G, Papademeterious X, Shapiro E, Pober J, Saltzman WM, Fahmy TM, Breuer C. Initial evaluation of the use of USPIO cell labeling and noninvasive MR monitoring of human tissue-engineered vascular grafts in vivo. *Faseb J*. In Press.

Stern E, Steenblock E, Reed MA, **Fahmy TM**, Label-free electronic detection of the antigen-specific immune response. *Nanoletters*. In Press.

Stern E, Vacic A, Li C, Ishikawa F, Steenblock E, Zhou C, Reed MA, **Fahmy TM,** Cytokine detection with a nanoelectronic-enzyme linked immunosorbent assay (ne-ELISA). *Nanoletters.* (In Final Revision)

Criscione J, Stern E, Shkarin P, Papademetrious X, **Fahmy TM**. Enhancing 19F MRI high fluorine density self-assembled dendrimer particles. *Magnetic Resonance In Med.* Submitted.

Kress H, Park JG, Mejean, CO, Forster, JD, Park J, Walse SS, Weiner OD, **Fahmy TM**, Dufresne ER. Cell stimulation with optically manipulated microsources. *Nature Methods*. In Final Review

Arnar Geirsson, M.D.
Assistant Professor of Surgery

The laboratory has four research projects at various stages. First, we are assessing the role of microRNAs in cardiac remodeling in an ischemic murine model and by creating an inducible cardiac specific Dicer knockout mouse. Second project involves analyzing TGF-β signaling in mitral regurgitation and degenerative mitral valve disease. In a third project we are examining cardiac regeneration following ischemic injury in a murine model with specific emphasis on orthologous zebrafish genes. Fourth project involves assessing the role of negative gene regulation and MHC suppression in trophoblasts.

Specific Research Accomplishments in the Last Year:
We have established a reproducible ischemic model in mouse by coronary ligation. Operated mice are followed longitudinally by high-resolution ECHO. Micro RNA microarrays have demonstrated significant change in expression of key micro RNAs in a temporal fashion during early and late cardiac remodeling. We have successfully developed a tamoxifen-inducible cardiac-specific Dicer knockout mice (myh6-cre/Esr1;Dicer flox/flox). Preliminary results indicate that micro RNAs are necessary for maintenance of normal cardiac function, where tamoxifen-induction results in acute cardiomyopathy in the adult mouse. Preliminary analysis of TGF-β pathways in degenerative mitral valve disease indicates that specific genes of extracellular matrix origin and inflammatory pathways are involved.

Significance of Key Findings Relevant for the Mission of VBT:
There has been a significant interdepartmental collaboration in initiating and evolving all aspects of the research projects, fostering the development of new cardiac surgeon-scientist faculty.

Publications:

Szeto WY, Bavaria JE, Bowen FW, Geirsson A, Cornelius K, Hargrove WC, Pochettino A. Reoperative aortic root replacement in patients with previous aortic surgery. Ann Thorac Surg. 2007 Nov;84(5):1592-8; discussion 1598-9.

Geirsson A, Bavaria JE, Swarr D, Keane MG, Woo YJ, Szeto WY, Pochettino A. Fate of the residual distal and proximal aorta after acute type a dissection repair using a contemporary surgical reconstruction algorithm. Ann Thorac Surg. 2007 Dec;84(6):1955-64; discussion 1955-64.

Frank J. Giordano, M.D.
Associate Professor of Medicine, Section of Cardiovascular Medicine

Our program is focused on the application of basic and pre-clinical science in the development of translational strategies for the treatment and diagnosis of cardiovascular disease. Our basic science efforts are directed to better understand the mechanistic basis of angiogenesis, arteriogenesis, and vascular remodeling, and to understand the specific role of oxygen sensing in defining these processes. We have a specific focus on transcription, epigenetics, and the translational application of transcription factor engineering. Our efforts to date have led to the largest gene therapy clinical trial performed to date, and to a current Phase III international clinical trial. Our efforts in the development of engineered transcription factors has led to three clinical trials, and to the development of a specific engineered transcription repressor that augments contractility and normalizes calcium handling in the heart.

Publications:

Hao Z, Huang Y, Cleman J, Jovin IS, Vale WW, Bale TL, Giordano FJ. Urocortin2 inhibits tumor growth via effects on vascularization and cell proliferation. Proc Natl Acad Sci U S A. 2008 Mar 11;105(10):3939-44. Epub 2008 Feb 28.

Jovin IS, Giordano FJ. Differentiation by association: is a cell's fate determined by the company it keeps? Am J Physiol Heart Circ Physiol. 2008 Apr;294(4):H1503-4. Epub 2008 Feb 22.

Lei L, Mason S, Liu D, Huang Y, Marks C, Hickey R, Jovin IS, Pypaert M, Johnson RS, Giordano FJ. Hypoxia-inducible factor-dependent degeneration, failure, and malignant transformation of the heart in the absence of the von Hippel-Lindau protein. Mol Cell Biol. 2008 Jun;28(11):3790-803. Epub 2008 Feb 19.

Lei L, Liu D, Huang Y, Jovin I, Shai SY, Kyriakides T, Ross RS, Giordano FJ. Endothelial expression of beta1 integrin is required for embryonic vascular patterning and postnatal vascular remodeling. Mol Cell Biol. 2008 Jan;28(2):794-802. Epub 2007 Nov 5.

Daniel R. Goldstein, M.D.
Associate Professor, Department Internal Medicine

There are two main themes of the lab: the role of innate immunity in transplant rejection and tolerance and the impact of aging on immune function.

Specific Research Accomplishments in the last 12 months (7/1/07-6/30/08)

a. Determine that dendritic cells use TLR signal adaptors differentially as compared to macrophages. Specifically, Trif signaling is not essential for the upregulation of costimulatory molecules during TLR4 activation in DCs, whereas Trif is essential in macrophages. The differences are due to increased sensitivity of macrophages to type I IFNs.
b. IL-6 and TNF alpha cooperate to impair costimulatory blockade extension of allograft survival.
c. Aging impairs plasmacytoid dendritic cell function.
d. Aging augments IL-17 T cell alloimmune responses.

Publications

Shen, H., B. M. Tesar, W. E. Walker, and D. R. Goldstein. 2008. Dual Signaling of MyD88 and TRIF Is Critical for Maximal TLR4-Induced Dendritic Cell Maturation. *J Immunol* 181:1849-1858.

Shirali, A., and D. Goldstein. 2008. Tracking the Toll of Kidney Disease. *J Am Soc Nephrol* 19:1444-1450.

Richard W. Kim M.D.
Assistant Professor of Surgery (Cardiothoracic)

Our primary research interest is the therapeutic application of stem cells and their derivatives such as embryonic stem cell-derived neural crest-like cells for developmental and acquired cardiovascular disease.

Specific Research Accomplishments in the last 12 months:
We have developed significant expertise in murine fetal microsurgery and the *in utero* delivery of stem cells to embryonic hosts as early as E9 using near-microscopic ultrasonographic guidance.

Significance of Key Findings Relevant for the Mission of VBT:
We are currently engaged in active collaboration with multiple VBT investigators examining the therapeutic effects of Wnt-1-derived cardiomyocytes from human embryonic stem cells (Wu, Breuer), adenoviral-mediated gene transfer in early murine fetal hosts (Giordano), and developmental heterogeneity in TGF-B-induced vascular smooth muscle cell responses (Tellides).

Publications:

Bai Y, Ahmad U, Wang Y, Li JH, Choy JC, Kim RW, Kirkiles-Smith N, Maher SE, Karras JG, Bennett CF, Bothwell AL, Pober JS, Tellides G. Interferon-gamma Induces X-linked Inhibitor of Apoptosis-associated Factor-1 and Noxa Expression and Potentiates Human Vascular Smooth Muscle Cell Apoptosis by STAT3 Activation. *J Biol Chem*. 2008; 283:6832-6842.

Martin S. Kluger, Ph.D.
Research Scientist in the Department of Dermatology

Our new direction is to understand how tumor-derived factors such as vascular endothelial cell growth factor (VEGF) increase endothelial cell permeability to blood macromolecules and how vascular hyperpermeability contributes to cancer, particularly melanoma. Vascular hyperpermeability is characteristic of most tumor-proximal vessels. Although important during critical steps in cancer progression such as angiogenesis and metastasis, the clinical significance and mechanisms of hyperpermeability are incompletely understood. We are focusing on alterations to molecules expressed at endothelial tight junctions, their associated molecules and biochemical pathways. We find that in the context of experimental human melanoma, VEGF controls hyperpermeability through distinct families of molecules expressed at endothelial cell tight junctions. The vascular hyperpermeability response of endothelial cells stands at the crossroads of cancer and inflammation and is also triggered by that fundamental cytokine mediator of inflammation, tumor necrosis factor (TNF). The goal of our second project is analyzing how distribution of the TNF receptor (TNFR1) to caveolae, plasma membrane compartments important for TNF signaling and internalization, relies on amino acid motifs found in the cytoplasmic domain of TNFR1.

Research Accomplishments and Significance:
We developed two different experimental models for studying hyperpermeability in experimental human melanoma, one involving changes in transendothelial electrical resistance across confluent monolayers of human dermal microvascular endothelial cells, and another involving sub-cutaneous injection of human melanoma cells into human skin grafted onto immunodeficient SCID-beige mice. Our orthotopic human skin/SCID mouse model allows us to examine the tumor response of human blood vessels in vivo. Results are consistent between these models in showing that melanoma-derived VEGF is necessary, and as a recombinant protein, sufficient to generate hyperpermeability in both settings. These human cell and tissue-based models are now ready for further exploration into mechanism and are clearly aligned with the clinically oriented research goals of the Program in Vascular Biology and Therapeutics.

This year Dr. Kluger's expertise in the area of vascular biology was recognized by being named to the Editorial Board of the Journal of Investigative Dermatology and serving as scientific consultant for the International Investigative Dermatology meeting in Kyoto, Japan on the subject of Angiogenesis and Vascular Biology.

Publications:

Clark, P.R., Pober, J.S. and Kluger, M.S. Knockdown of TNFR1 by the Sense Strand of an ICAM-1 siRNA: Dissection of an Off-Target Effect. **Nucleic Acids Research,** 2008. 36(4):1081–1097.

Madge, L.A., Kluger, M.S., Orange, J.S. and May, M.J. Lymphotoxin-α1β2 and LIGHT Induce Classical and Noncanonical NF-κB-Dependent Pro-Inflammatory Gene Expression in Vascular Endothelial Cells. **Journal of Immunology,** 2008. 180: 3467–3477.

Liu, M., Kluger, M.S., D'Alessio, A., García-Cardeña, G. and Pober, J.S. Regulation of Arterial-Venous Differences in Tumor Necrosis Factor Responsiveness of Endothelial Cells by Anatomic Context. **The American Journal of Pathology,** 2008. 172(4): 1088-1099.

Diane Krause M.D., Ph.D.
Professor, Departments of Laboratory Medicine, Pathology and Cell Biology

The overall goals of my research are to characterize bone marrow (BM) derived stem/progenitor cells, and to define the mechanisms that regulate the self-renewal and differentiation of these cells with the hopes that the findings can be translated to improved therapeutics. We have 2 major foci. The first is based on our discovery that BM cells can differentiate into mature epithelial cells of the lung, liver, GI tract and skin. Projects are ongoing on the functional effects of BM transplantation and to determine the cells and mechanisms responsible for this engraftment. The second focus is the molecular mechanism(s) regulating gene expression during normal and malignant hematopoiesis. We are using murine and human hematopoietic stem cells and human embryonic stem cells to better understand Acute Megakaryoblastic Leukemia. In vitro and in vivo cell and molecular approaches are being used to lead to a better understanding of hematopoiesis and leukemogenesis.

Specific Research Accomplishments in the last 12 months

We have optimized techniques for detection of marrow derived epithelial cells in the lung, kidney and liver, and have published 4 papers on this work in the last year. In the common t(1;22) translocation of acute megakaryoblastic leukemia (AMKL), the RBM15 gene on chromosome 1 is fused to the MKL gene on chromosome 22. Studies on these proteins (RBM15 and MKL) in my laboratory have been focused not only on their roles in normal hematopoiesis, but also their roles in leukemogenesis. In order to fully understand the mechanism by which the fusion protein RBM15-MKL induces leukemia, we must first understand the normal functions of RBM15 and MKL. We have found that the function of RBM15, is, at least in part, to inhibit terminal hematopoietic differentiation whereas the fusion partner MKL actually promotes normal megakaryocytic differentiation. Loss of MKL leads to decreased numbers of fully mature megakaryocytes as well as a decreased peripheral platelet count. In contrast, overexpression of MKL enhances terminal differentiation of megakaryocytes as assessed by megakaryocyte number and ploidy. These findings have been submitted for publication.

Significance of Key Findings Relevant for the Mission of VBT

A better understanding of normal megakaryocytic differentiation and platelet production is relevant to the Mission of VBT in 2 ways. First, endothelial platelet interactions are key for vessel repair and patency and second, the smooth muscle cells lining arterial ways and megakaryocytes share common differentiation pathways.

Publications

Guo JK, Cheng EC, Wang L, Swenson ES, Ardito TA, Kashgarian M, Cantley LG, Krause DS. The commonly used beta-actin-GFP transgenic mouse strain develops a distinct type of glomerulosclerosis. Transgenic Res. 16: 829-834, 2007.

Herzog EL, Van Arnam J, Hu B, Zhang J, Chen Q, Haberman AM, Krause DS. Lung-specific nuclear reprogramming is accompanied by heterokaryon formation and Y chromosome loss following bone marrow transplantation and secondary inflammation. Faseb J 21:2592-2601, 2007.

Swenson ES, Kuwahara R, Krause DS, Theise ND. Physiological variations of stem cell factor and SDF-1 in murine models of liver injury and regeneration. Liver Int. 28:308-318, 2008

Swenson ES, Guest I, Ilic Z, Mazzeo M, Lizardi P, Hardiman C, Sell S, Krause DS. Hepatocyte Nuclear Factor-1 as marker of epithelial phenotype reveals marrow-derived hepatocytes, but not duct cells, after liver injury in mice. Stem Cells, 26: 1768-1777, 2008 PMID: 18467658

Themis R. Kyriakides, Ph.D.
Assistant Professor, Pathology

The main area of my research is the elucidation of the molecular events that dictate the course of healing and especially inflammation and angiogenesis following the implantation of biomaterials and scaffolds for tissue engineering applications. Our primary research focus is on three molecules, MCP-1, MMP-9, and TSP-2 that we have shown to be critical to various aspects of these processes. In addition, through the process of molecular dissection of cell-matrix interactions, we aim to incorporate rational design in the development of bioengineering applications such as tissue-engineered vascular grafts.

Specific Research Accomplishments in the last 12 months (7/1/07-6/30/08)

We have continued our investigation of the participation of TSP-2 in angiogenesis and for the first time demonstrated its role in arteriogenesis. Furthermore, we have discovered a link between the expression of eNOS and down-regulation of TSP2. To explore this link, we have generated double eNOS/TSP2-null mice in collaboration with Dr. Sessa and discovered that the absence of TSP2 ameliorates the phenotype of the eNOS-null mice. Specifically, we have observed that both the recovery from hindlimb ischemia and wound healing are improved in the double-null mice. We have also made progress in determining the significance of macrophage-related activities such as phagocytosis and fusion to the overall fate of biomaterials. More importantly, we have identified an association between the expression of MCP-1 and MMP-9, which explains, in part, the macrophage fusion defect in MCP-1-null macrophages. We have submitted three provisional patent applications. Within the VBT program we have continued our collaborations with the following investigators: Sessa, Giordano, Saltzman, Niklason, Breuer, and Tellides.

Significance of Key Findings Relevant for the Mission of VBT

Studies in angiogenesis, arteriogenesis, and engineering of vascular grafts are central to the mission of the VBT. In addition, our studies using ischemia and wound healing models and the link between TSP2 and eNOS, and MCP-1 and MMP-9 overlap with the research interests of several members of the VBT.

Publications

Marie M. Krady, Jianmin Zeng, Jun Yu, William C. Sessa, Themis R. Kyriakides. Thrombospondin-2 limits arteriogenesis and ischemia-induced physiologic angiogenesis. 2008 ***Am. J. Pathol*.** (in press).

Fred Cahn and Themis R. Kyriakides Generation of an artificial skin construct containing a non-degradable fiber mesh: a potential transcutaneous interface. 2008 **Biomed. Mater.** (in press).

Janson C. Sullivan, Donny D. Kakati, Elliot Carter, Amy K. Boyd, Themis R. Kyriakides, Azin Agah. Elevated expression of isopeptide bond cross-links contributes to fibrosis in scleroderma and the healing wounds of tight skin mice. 2008 **Wound Rep. Regen.** (in press).

Lei, L., Liu, D., Huang, Y., Jovin, I., Shai, S.Y., Kyriakides, T.R., Ross, R.S., Giordano, F.J. Endothelial expression of beta-1 integrin is required from embryonic vascular patterning and postnatal vascular remodeling. 2008 **Mol Cell Biol.** 28:794-802, 2008.

Roh JD, Nelson GN, Brennan MP, Mirensky TL, Yi T, Hazlett TF, Tellides G, Sinusas AJ, Pober JS, Saltzman WM, Kyriakides TR, Breuer CK. Small-diameter biodegradable scaffolds for functional vascular tissue engineering in the mouse model. 2008 **Biomaterials.** 29:1454-63.

Roh JD, Sawh-Martinez R, Brennan MP, Devine L, Jay SM, Yi T, Mirensky T, Udelsman B, Nelson GN, Hibino N, Shin'oka T, Saltzman WM, Snyder E, Kyriakides TR, Pober JS, Breuer CK Human Bone Marrow Cell Seeding Improves Outcomes of Tissue-Engineered Vascular Grafts through MCP-1 Mediated Monocyte Recruitment. **Nature Med.** (in revision)

Themis R. Kyriakides, Ph.D.

Steven M. Jay, Eleni A. Skokos, Jianmin Zeng, Kristin Knox,Themis R. Kyriakides. Engagement of macrophages in phagocytosis does not prevent fusion: examining the frustrated phagocytosis hypothesis. Submitted (**J. Leukoc. Biol.**).

Weiming Tian and Themis R. Kyriakides. MMP-9 deficiency leads to prolonged foreign body response in the brain associated with increased IL-1β levels and leakage of the blood brain barrier. Submitted (**J. Neurotrauma**).

Susan MacLauchlan, Eleni Skokos, Norman Meznarich, Dana Zhu, Sana Raoof, J. Michael Shipley, Robert M. Senior, Paul Bornstein, Themis R. Kyriakides. Abnormal foreign body response in mice that lack matrix metalloproteinase-9 associated with disordered matrix remodeling, compromised angiogenesis, and foreign body giant cell formation. Submitted (**FASEB J.**).

Shu-Ping Lin, Jia-Jin J Chen, Themis R Kyriakides. Synthesis and characterization of a degradable hydrogel as a biocompatible coating for neural implants. Submitted (**Biomaterials**)

Jan Schroers, Golden Kumar, Themis R. Kyriakides. Bulk Metallic Glass- A New Material for Biomedical Applications. Submitted (**Biomaterials**).

Erin Lavik, Sc.D.
Associate Professor, Biomedical Engineering

Our research focuses on creating environments that promote repair using three dimensional biodegradable polymer systems. These polymer systems allow us to direct the temporal and spatial patterning of growth factors, to design specific surfaces to probe ECM interactions, and to use scaffold architecture to guide cells. Our particular interest lies with developing new approaches to understanding the central nervous system (CNS) following injury with the potential for developing new treatment paradigms.

Over the last few years, in collaboration with the Madri lab, we have looked at the coculture of neural stem cells (NSCs) and endothelial cells (ECs) in these polymer environments to make stable, three dimensional vascular networks.

This year, we have focused on the development and characterization of all primary cell cocultures and the application of these networks to spinal cord injury. Implantation of our coculture system leads to a greater density of blood vessels at the injury epicenter than the controls as well as the presence of markers for the blood-spinal cord barrier, a critical component of functional vessels in the central nervous system.

We have also begun to further investigate the nature of the vessels that form as a result of the implantation of the coculture in the subcutaneous model. We are mapping the contributions of the host versus donor ECs in the vessels as well as looking at the role of the concentration of the NSCs on the nature of the vascular structures produced.

Publications:

T. F. Ng, E. Lavik, H. Keino, A. W. Taylor, R. S. Langer and M. J. Young, Creating an immune-privileged site using retinal progenitor cells and biodegradable polymers. Stem Cells, **25**(6): p. 1552-1559. 2007.

J. Bertram, S. S. Saluja, J. A. McKain and E. B. Lavik, Sustained Delivery of Timolol Maleate from Poly(lactic-co-glycolic acid)/Poly(lactic acid) Microspheres for over 3 months. Journal of Microencapsulation. accepted.

S. R. Hynes, M. F. Rauch, J. Bertram and E. B. Lavik, A library of tunable poly(ethylene glycol)/poly-L-lysine hydrogels to investigate the materials cues that influence neural stem cell differentiation. Journal of Biomedical Materials Research Part A. accepted.

M. F. Rauch, M. Michaud, H. Xu, J. A. Madri and E. B. Lavik, Coculture of primary neural progenitor and endothelial cells in a synthetic, degradable macroporous gel promotes stable vascular networks in vivo. Journal of Biomaterials Science-Polymer Edition. accepted.

S. Tzeng, M. Nkansah, A. M. Holdt and E. Lavik, Sustained delivery of CNTF from polymer nano- and microspheres for neural differentiation and neuroprotection. Biotechnology and Bioengineering. accepted.

Patty J. Lee, M.D.
Associate Professor, Dept of Medicine; Pulmonary & Critical Care Medicine

Our research goals are to define the mechanisms of tissue protection and cell survival during oxidant lung injury. Specifically, we have focused upon the lung endothelial cell as central orchestrator of injury and repair responses during oxidant injury. We have identified the stress protein heme oxygenase-1 (HO-1), its reaction products, carbon monoxide (CO), and the signaling pathway, mitogen-activated protein kinases (MAPKs), as important protective molecules in lung endothelium. Recently, we discovered that toll-like receptors (TLRs) play an essential role in the survival of lung structural cells, including endothelium, *in vivo*. In the process of dissecting these mechanisms, we successfully employed lung-targeted siRNA approaches *in vivo*.

Specific Research Accomplishments in the last 12 months:

We have defined novel mechanisms of CO-mediated cytoprotection and expanded the use of intranasal siRNA to target transcription factors in the lung (1). We also characterized tissue effects of IL-13 in the lung (3, 5) and identified novel pathways of endothelial cell apoptosis (4).

Significance of Key Findings Relevant for the Mission of VBT

We have combined our HO-1 signaling interests with the translational, pulmonary hypertension focus of Dr. Nicholas Morrell (University of Cambridge) and have defined novel molecular pathways in human myocytes (2). Our focus upon protective molecular pathways in lung endothelial cells and our expanded application of lung-targeted siRNA have resulted in potentially new therapeutic strategies.

Publications:

Chin, B.Y., Jiang G., Wegiel, B., Wang, H.J., MacDonald, T., Zhang X., Gallo, D., Cszimadia, E., Bach, F.H., **Lee P.J.**, and Otterbein, L.E. HIF1α stabilization by carbon monooxide results in cytoprotective preconditioning, *Proc Natl Acad Sci USA*, 104: 5109-5114, 2007.

Yang, X., **Lee, P.J.,** Long, L., Trembath, R.C. and Morrell, N. BMP4 Induces HO-1 via a Smad independent, p38MAPK dependent, Pathway in Pulmonary Artery Myocytes. *Am J Resp Cell Mol Biol,* 37: 579-607, 2007.

Chapoval, S.P., Al-Garawi, A., Lora, J.M., Strickland, I., Ma, B., **Lee, P.J**., Homer, R.J., Ghosh, S., Coyle, A.J., and Elias, J.A. Inhibition of NF-kappaB activation reduces the tissue effects of transgenic IL-13. *J. Immunol,* 179: 7030-7041, 2007.

Medler, T.R., Petrusca, D.N., **Lee, P.J.**, Hubbard, W.C., Berdyshev, E.V., Skirball, J., Kamocki, K., Schuchman, E., Tuder, R.M., and Petrache, I. Apoptotic sphingolipid signaling by ceramides in lung endothelial cells. *Am J Resp Cell Mol Biol,* 38: 639-640, 2008.

Manning, C.B., Sabo-Attwood, T., Robledo, R.F., MacPherson, M.B., Rincon, M., Vacek, P., Hemenway, D., Taatjes, D.J., **Lee, P.J.**, and Mossman, B.T. Targeting the MEK1 cascade in lung epithelium inhibits proliferation and fibrogenesis by asbestos. *Am J Resp Cell Mol Biol,* 38: 618-626, 2008.

Joseph A. Madri, M.D., Ph.D.
Professor of Pathology

During the past year we have continued our investigations on the roles of cell adhesion molecules (PECAM-1), proteases (MMPs) and soluble factors (VEGF, BDNF, SDF-1) in modulating vascular development and behavior. Using whole conceptus and AV cushion cultures we are investigating the roles of these molecules and their receptors in the processes of vasculogenesis, angiogenesis and epithelial to mesenchymal transformation in the murine embryo and their dysregulation in maternal diabetes. We are also investigating the interactions of neural progenitor cells and endothelial cells, comprising the neurovascular niche, during brain development and in response to chronic hypoxia. Lastly, we are investigating the roles of T-cell and endothelial cell proteinases and proteinase inhibitors in modulating T-cell transendothelial migration and their roles in initiating and maintaining the inflammatory response in murine models of autoimmune disease (multiple sclerosis) and in several tissue culture models.
A multi-disciplinary approach is used which includes the use of knockout & transgenic animals, tissue and embryo culture model systems of cell adhesion, migration, angiogenesis and neurogenesis and a variety of biophysical, biochemical, molecular and cell biological methods.

Publications:

Wu, Y., Welte, T., Michaud, M., Jiang, X., Madri, J.A., PECAM-1: A multifaceted regulator of megakaryocytopoiesis, Blood, 110(3):851-859, 2007.

Li, Q., Michaud, M., Stewart, W., Schwartz, M, Madri, J.A., Modeling the Neurovascular Niche: Murine strain differences mimic the range of responses to chronic hypoxia in the premature newborn, J. Neurosci., 86(6):1227-1242, 2008.

Seguin, C.A., Pilliar, R.M., Madri, J.A., Kandel, R.A., TNFA-stimulated activation of pro-MMP2 in nucleus pulposus cells occurs through Egr-1 mediated transcription of membrane type I matrix metalloproteinase, Spine, 33(4):356-365, 2008.

Madri, J.A., Inside Blood Capsule: Need MT1-MMP? Just say NO!, Blood, 110:2790-2791, 2008.

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Kalinowski, L., Dobrucki, W.L.,., Meoli,D.F., Dione, D.P., Sadeghi,M.M., Madri, J.A., Sinusas, A.J., Targeted Imaging of Hypoxia-Induced Integrin Activation in Myocardium Early After Infarction, J. App.l Physiol., 104(5):1504-12, 2008, Mar 20; [Epub ahead of print].

Kim, J.I., Cordova, A.C., Hirayama, Y., Madri, J.A., Sumpio, B.E., Differential Effects of Shear Stress and Cyclic Strain on Sp1 Phosphorylation by PKCz Modulates MT1-MMP in Endothelial cells, Endothelium, 15:33-42, 2008.

Ford-Rauch, M., Michaud, M., Xu, H., Madri, J.A., Lavik, E.B., Coculture of primary neural progenitor and endothelial cells in a macroporous gel promote stable vascular networks in vivo, J. Biomaterials Sci: Polymer Edn., In Press, 2008.

Chyou, S., Ekland, E.E., Carpenter, A.E., Tzeng, T-C., Michaud, M., Browning, J., Madri, J..A., Lu, T.T., Lymph node VEGF modulates homeostatic endothelial cell proliferation and is expressed by stromal cells in a lymphotoxin beta receptor-dependent manner, J. Immunol., In Press, 2008.

Wang, P., Dai, J., Bai, F., Kong, K-F., Montgomery, R., Madri, J.A., Fikrig, E., Matrix Metalloproteinase 9 Facilitates West Nile Virus Entry into the Brain, J. Virol., In Press, 2008, July 16, 2008 ; [Epub ahead of print].

Wang Min, Ph.D.
Associate Professor, Pathology

The primary goal in my laboratory is to dissect signal pathways during inflammatory responses and develop therapeutic targets for treatment of vascular diseases.

Specific accomplishments in the last year:

Mitochondrial thioredoxin and endothelial cell function: The major accomplishment in the past year is that we have published our studies on mitochondrial thioredoxin and atherosclerosis. By using endothelial cell (EC)-specific transgenesis of mitochondrial form of thioredoxin gene in mice (Trx2 TG), we show the critical roles of Trx2 in regulating endothelium functions and atherosclerosis. Our data provides the first evidence that Trx2 plays a critical role in preserving vascular EC function and prevention of atherosclerosis development. This work was contributed by several investigators (Drs. Sessa, Giordano in VBT and Dr. Shadel from Pathology), and has been published in *Am. J. Pathol* with a highlighted editorial commentary (Zhang, H et al., 2007). As a result, we have obtained an outstanding score from the NIH study section and expected to be funded from September, 2008.

Define the role of AIP1 in ER stress signaling: We have previously shown that ASK1-interacting protein 1 (AIP1) transduces TNF-induced ASK1-JNK signaling. Recently we have investigated the role of AIP1 in other stress signaling pathways. We used mouse embryonic fibroblasts (MEF) and vascular endothelial cells (EC) isolated AIP1-deficient mice (AIP1-KO) and showed that AIP1-KO cells dramatically reduced ER stress- but not oxidative stress-induced ASK1-JNK activation and cell apoptosis. We further showed that AIP1 via it PH domain facilitates IRE1 dimerization, a critical step for activation of IRE1 signaling. More importantly, AIP1-KO mice show impaired ER stress-induced IRE1-dependent signaling in vivo. We conclude that AIP1 is essential for transducing the IRE1-mediated ER stress response. This is the first study to demonstrate that a TNF signaling component can function as an upstream activator of ER stress signaling (Luo et al., 2008).

Molecular mechanism for ASK1 regulation. We have shown that AIP1 preferentially binds to dephosphorylated ASK1 to facilitate release of 14-3-3, a novel mechanism for TNF-induced ASK1 activation. This work was published in *J Clin Invest* with a highlighted editorial commentary (Zhang, R et al., 2003) with highlighted commentary. Recently, we have identified PP2A as a phosphatase in TNF-induced dephosphorylation of ASK1 pSer967. More importantly, we show that AIP1 is critical in recruiting Protein phosphates 2A (PP2A) to ASK1, leading to dephosphorylation of ASK1 at pSer967 and activation of ASK1-JNK signaling (Min, W et al., 2008).
Upon release from its inhibitors, ASK1 activation involves subsequent ASK1 oligomerization and autophosphorylation. However, the structural basis for ASK1 regulation is unknown. We have begun to address this issue by collaborating with structural biologists. Our recent crystallographic analysis showed that ASK1 forms a tight dimer interacting in a head-to-tail fashion. We found that the ASK1 autophosphorylation sites (Thr813, Thr838, Thr842) regulate ASK1 signaling (Bunkoczi et al, 2007).

Funding: Hong Chen, An Associate Research Scientist, won a prestigious National American Heart Scientist Development Grant entitled- Epsin in Ubiquitin-Mediated Endocytosis in Notch Ligand: Implication in Angiogenesis. Dennis Jones, s second year Immunobiology student, won the Anna Fuller Fellowship Award to investigate the role of Bmx in lymphangiogenesis and tumor metastasis. We also extend a Research of Contract with

Wang Min, Ph.D.

Atherogenics (PI: Min) to determine the effects of AGI-1067, a leader compound in Atherogenics, on LPS-induced ASK1-JNK activation and implications in diabetes.

Publications:

Zhang, H., Luo, Y., Zhang, W., He, H., Zhang, R., Huang, Y., Bernatchez, P., Giordano, F.J., Shadel, G., Sessa, W.C., and Min, W*. (2007) Endothelial-specific expression of mitochondrial thioredoxin improves endothelial cell function and reduces atherosclerosis. *Am J. Pathol*. **170** (3), 1108-20. (see **Editorial Comment on page 805**).

Bunkoczi, G., Salah, E., Filippakopoulos, P., Fedorov, O., Muller, S., Sobott, F., Turk, B.E., Zhang, H., Min, W. and Knapp, S. (2007) Structural and functional characterization of human protein kinase ASK1. *Structure*. **15**, 1215-1226.

Hsieh, J.T., Karam, J.A., Min, W. (2007) Genetic and biologic evidence that implicates a gene in aggressive prostate cancer. *J Natl Cancer Inst.* **99**(24):1823-4.

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Luo, D., He, Y., Zhang, H., Yu, L., Chen, H., Xu, Z., Tang, S., Urano, F., and Min, W* (2008) AIP1 is critical in transducing IRE1-mediated endoplasmic reticulum stress response. *J. Biol. Chem*. **283**(18):11905-12.

Min, W*., Lin, Y., Tang, S., Yu, L., Zhang, H., Wan, T., Luhn, T., Fu, H. and Chen, H. (2008). AIP1 recruits PP2A to ASK1 in TNF-induced ASK1-JNK activation. *Cir. Res.* **102** (7): 840-8.

Laura Elizabeth Niklason, M.D., Ph.D.
Associate Professor, Anesthesia and Biomedical Engineering

Dr. Niklason's research program focuses on cardiovascular tissue engineering, utilization of stem cells for tissue regeneration, and on mechanical characteristics of native and engineered vascular structures.

Specific research accomplishments in the last 12 months:

Recently, we've shown that human mesenchymal stem cells that are derived from bone marrow can be driven to differentiate along a smooth muscle lineage. These differentiation events likely involve serum response factor, and are potently triggered by transforming growth factor beta, as well as by specific protein substrates and by cyclic strain. Such differentiated smooth muscle cells can be utilized to form arterial walls in vitro that possess an array of contractile markers as well as the extracellular matrix molecule collagen. In the rat system, we are in the process of showing that mesenchymal stem cells also can serve as mural cells for the directed growth and stabilization of microvasculature that is cultured in vitro. In ongoing and planned studies, we will evaluate the role of bulk fluid flow and shear stress on the formation and persistence of microvessels in vitro, such microvessels being formed from differentiated endothelial cells and mesenchymal stem cells.

To gain a better understanding of the evolution of engineered tissue mechanics, we are designing novel bioreactors that will allow the precise application of 3-dimensional strains to tubular constructs, such as engineered blood vessels. These bioreactors are designed with optical windows that allow us, in collaboration with investigators from Texas A&M University, to non-invasively image the deposition of collagen matrix fibers using non-linear optical microscopy. We hypothesize that mechanical cues delivered via bioreactors, in concert with soluble factors, will drive deposition of collagen in engineered tissues. Since extracellular collagen is the principle component that controls tensile properties of all connective tissues, understanding the controlling factors and time course for collagen synthesis is critical for our fundamental understanding of regenerative medicine. Specialized bioreactors will be utilized to engineer tissues, which will be queried using non-linear optical microscopy in serial fashion during culture. Input mechanical stimuli and resultant collagen deposition will be combined with precise measurements of tissue mechanics to provide an overall theoretical framework for understanding and predicting collagen formation in vitro.

Lastly, we are conducting studies of changes in the mechanical properties of cerebral arteries that are exposed to clotted blood. These studies are designed to elucidate some fundamental mechanisms contributing to the syndrome of delayed cerebral vasospasm that follows subarachnoid hemorrhage. These modeling and in vitro studies will be supplemented with in vivo studies in large animals, using induced subarachnoid hemorrhage and quantifying vessel mechanics over the course of vasospasm.

Publications:

Dahl, S.L., Vaughn, M.E., **Niklason, L.E.,** "An ultrastructural analysis of collagen in tissue engineered arteries", Annals of Biomedical Engineering 2007; 35: 1749-1755.

Petersen, T., **Niklason, L.,** Cellular lifespan and regenerative medicine, Biomaterials 2007; 28: 3751-3756.

Gong, Z., **Niklason, LE,** Small-diameter human vessel wall engineered from bone marrow-derived mesenchymal stem cells (hMSC), FASEB Journal 2008; 22: 1635-1648.

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Herbert, K.E., Mistry, Y., Hastings, R., Poolman, T., **Niklason, L**., Williams, B., Angiotensin II-mediated oxidative DNA damage accelerates cellular senescence in cultured smooth muscle cells via telomere-dependent and independent pathways, Circulation Research 2008; 102: 201-208.

Jordan S. Pober, M.D., Ph.D.
Vice Chair, Immunobiology for Section of Human and Translational Immunology,
Professor of Immunobiology, Pathology and Dermatology

The Pober laboratory studies interactions of the immune system with the blood vascular system. Specific issues include the role of vascular cells in activating, maintaining and modulating the adaptive immune response; the mechanisms by which immune cells and mediators alter the functions of vascular cells; and therapeutic strategies to protect vascular cells from immune-mediated injury as applied to cell, organ and tissue engineered transplants.

Specific Research Accomplishments in the last 12 months: The Pober laboratory has identified IL-1 as a key signal of vascular injury that can alter the immune response to an allograft and has collaborated with Tellides laboratory to evaluate IL-1 effects on IL-17 and IFN-γ production by alloreactive human T cells in vivo. Such effects can be observed in CD4+ T cell memory populations, which the Pober lab has shown can recognize antigens presented by human endothelium. The Pober laboratory has continued its collaboration studies with the Saltzman and Breuer laboratories in the development of engineered microvessels and large vessels, respectively.

Significance of Key Findings Relevant for the Mission of VBT: The results of studies on the role of IL-1 in allograft rejection is the basis of planning a new clinical trial at Yale and Cambridge providing an example of tranlataing basic laboratory work into therapies targeted at real clinical problems.

Publications:

Wang Y, Bai Y, QinL, Zhang P, Yi T, Teesdale SA, Zhao L, Pober JS, Tellides G. Interferon-gamma induces human vascular smooth muscle cell proliferation and intimal expansion by phosphatidylinositol 3-kinase dependent mammalian target of rapamycin raptor complex 1 activation. Circ Res. 2007; 101:560-569.

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Induction of indoleamine 2,3-di oxygense in vascular smooth muscle cells by interferon-gamma contributes to medial immunoprivilege. J Immunol. 2007; 179:5246-5254.

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Súarez Y, Shepherd BR, Rao DA, Pober JS. Alloimmunity to human endothelial cells derived from cord blood progenitors. J Immunol. 2007; 179:7488-7496.

Clark PR, Pober JS, Kluger MS. Knockdown of TNFR1 by the sense strand of an ICAM-1 siRNA: dissection of an off-target effect. Nucleic Acids Res. 2008; 36:1081-1097.

Roh JD, Nelson GN, Brennan MP, Mirensky TL, Yi T, Hazlett TF, Tellides G, Sinusas AJ, Pober JS, Saltzman WM, Kyriakides TR, Breuer CK. Small-diameter biodegradable scaffolds for functional vascular tissue engineering in the mouse model. Biomaterials. 2008; 29:1454-1463.

Bai Y, Ahmad U, Wang Y, Li JH, Cho JC, Kim RW, Kirkiles-Smith N, Maher SE, Karras JG, Bennett CF, Bothwell ALI, Pober JS, Tellides G. Inteferon-gamma induces X-linkd inhibitor of apoptosis-associaed factor-1 and Noxa expression and potentiates human vascular smooth muscle apoptosis by STAT3 activation. J Biol Chem. 2008; 283:6832-6842.

Jordan S. Pober, M.D., Ph.D.

Al-Lamki RS, Wang J, Tolkovsky AM, Bradley JA, Griffin JL, Thiru S, Wang EC, Bolton E, Min W, Moore P, Pober JS, Bradley JR. TL1A both promotes and protects from renal inflammation and injury. J Am Soc Nephrol. 2008; 19:953-960.

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Jay SM, Shepherd BR, Bertram JP, Pober JS, Saltzman WM. engineering of multifunctional gels integrating highly efficient growth factor delivery with endothelial cell transplantation. FASEB J. 2008 (in press).

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Shepherd BR, Jay SM, Saltzman WM, Tellides and Pober JS. Human aortic smooth muscle cells promote arteriole formation by co-engrafted endothelial cells. Tissue Engineering. 2008 (in press).

Gerber SA, Pober JS. IFN-{alpha} Induces Transcription of Hypoxia-Inducible Factor-1{alpha} to Inhibit Proliferation of Human Endothelial Cells. J Immunol. 2008; 181:1052-62.

Nancy H. Ruddle, Ph.D.
John Rodman Paul Professor, Epidemiology and Public Health and Immunobiology

The laboratory uses several experimental models to evaluate cell trafficking in inflammation and lymphoid organ development. Several autoimmune models are under investigation. In experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis, myelin oligodendrocyte glycoprotein (MOG) induces an inflammatory, demyelinating, paralytic disease. By varying a single amino acid in MOG, we are able to change the pathogenic mechanism from one mediated by inflammatory T cells and cytokines to one mediated by antibodies. This is particularly relevant with regard to the recent observation that Rituximab, a monoclonal antibody against a B cell determinant, is proving to be efficacious in the treatment of some cases of Multiple Sclerosis. In studies of Type 1 diabetes we are studying trafficking of human T cells from diabetic patients to the pancreas of humanized mice. Our long-term goal is to understand how the vasculature of lymphoid organs -lymphatic vessels and high endothelial venules (HEVs) controls the immune response. We study HEV regulation in canonical secondary lymphoid organs and "tertiary lymphoid organs" (TLOs), ectopic lymphoid accumulations arising in situations of chronic inflammation in autoimmunity, graft rejection, and microbial infection. Visualization of HEVs and lymphatic vessels in living mice will provide a better understanding of the dynamics of their interaction in lymph nodes and TLOs with therapeutic potential in autoimmunity and malignancy.

Specific Research Accomplishments in the last 12 months:

a. We have demonstrated that regulatory T cells in EAE have an unexpected highly restricted specificity.
b. We have demonstrated an important role for B cells in expression of self antigens in the thymus.
c. We have completed our development and initial analysis of mice transgenic for an HEV restricted gene that drives expression of green fluorescent protein. We have shown that this gene is appropriately expressed in HEVs and have established their utility for in vivo imaging.
d. We have shown that lymphotoxin contributes to regulation of lymphatic vessels in several different systems: the steady state, immunization induced inflammation, and infection with *Mycobacterium pulmonis.*

Significance of Key Findings Relevant for the Mission of VBT:

Understanding the vasculature will provide insight into therapeutics in inflammtion in immunity, autoimmuity, and cancer

Publications:

Mounzer R, Shkarin P, Papademetris X, Constable T, **Ruddle NH**, Fahmy T. Dynamic imaging of lymphatic vessels and lymph nodes using a bimodal nanoparticulate contrast agent. *Lymphatic Res. Biol.* 5:151-158, 2007

Akirav E, Bergman CM, Hill M, **Ruddle NH.** Depletion of $CD4^{+}CD25^{+}$ T cells exacerbates experimental autoimmune encephalomyelitis induced by mouse but not rat antigens. *J. Neuroscience Res*. Under review. 2008

Kerry S. Russell, M.D., Ph.D.
Associate Professor of Medicine, Section of Cardiovascular Medicine, Department of Internal Medicine

Our research broadly encompasses 2 common themes in cardiovascular medicine: preservation of cardiac function and promotion of angiogenesis. We have chosen to focus on 2 major targets in the cardiovascular system that could potentially be manipulated to achieve the goals of myocyte preservation and angiogenesis. The first of these targets is the neuregulin/erbB ligand/receptor system. Evidence for the importance of this signaling system in the heart comes from clinical data showing that interruption of this system (e.g. using Herceptin in breast cancer patients) leads to depression of cardiac function, ultimately leading to heart failure in some patients. Our work over the past years has shown that activation of this signaling system can protect cardiac myocytes against injury in response to ischemia and can promote angiogenesis. The second target under investigation is the IL-6/STAT3 signaling cascade. Clinical data has revealed a paradoxical relationship between detrimental and protective effects of several "pro-inflammatory" cytokine pathways, including that of IL-6, in patients with heart failure. Our data suggests that one particular downstream target of IL-6 signaling, the STAT3 protein, may be important for the cardioprotective effects of IL-6. We hope that unraveling the details of this signaling pathway will provide novel targets to protect the heart in the setting of ischemic or inflammatory injury.

Specific Research Accomplishments in the last 12 months:
Over the past year, we have successfully developed and implemented an inducible model of endothelial selective neuregulin knockout. Using this model, we have shown that neuregulin expression in endothelium provides a crucial protective signal to cardiac myocytes. This recent data has helped us to complete an important body of in vitro and in vivo work which is being submitted for publication currently. We have also successfully implemented a core for cardiovascular imaging of murine models of cardiovascular disease using the Vevo770 system as planned. This facility has been used by multiple diverse faculty both inside and outside of VBT.

Significance of Key Findings Relevant for the Mission of VBT:
Our key findings provide important support for the signaling link between cardiac and vascular endothelium and cardiac myocytes. The role of endothelium in preservation of cardiac myocyte survival and function and the factors involved in this process continue to be of significant interest to our group and to the field of cardiovascular medicine in general.

Publications:

Kalinowski, A, Huang, Q, Plowes, NJR, Berdejo-Izquierdo, C, Russell, RR, Russell, KS. Metalloproteinase-dependent cleavage of neuregulin and autocrine stimulation of vascular endothelial cells, submitted, 2008.

Huang, Q, Kalinowski, A, Palmeri, M, Russell, RR, Russell, KS. Cardioprotective effects of endothelial-derived neuregulin-1, submitted 2008.

Mehran M. Sadeghi, M.D.
Associate Professor of Medicine (Cardiology)

The main goal of our laboratory research is to develop novel molecular imaging approaches for cardiovascular disease, with a focus on the vascular system, including vascular remodeling. Vascular remodeling is a common feature of a broad spectrum of vasculopathies, from atherosclerosis to graft arteriosclerosis. For each process studied, we identify specific imaging targets based on pathophysiology or genomic and proteomic screening, develop novel ligands for imaging or use existing radiotracers, establish various small animal models, and use a dedicated hybrid microSPECT/CT small animal imaging system to image the process in vivo. Study of the pathophysiology of vascular remodeling is an integral part of our research.

Specific accomplishments in the last year:
Over the past several years we have focused on four examples of vascular remodeling, namely injury-induced vascular remodeling, graft arteriosclerosis, aneurysm, and atherosclerosis. We have established the feasibility of matrix metalloproteinase (MMP)-targeted imaging of injury-induced vascular remodeling in vivo, and have demonstrated that MMP-targeted imaging may be used to track the remodeling process in vivo. We have extended this work to demonstrate the feasibility of imaging arterial aneurysm formation by targeting MMP activation in vivo. In parallel, we continued our work on characterization of a potentially novel target, endothelial and smooth muscle derived neuropilin-like protein (ESDN) identified in previous years through cDNA array analysis. Previous work demonstrated that ESDN is expressed in the course of vascular remodeling and is temporally and spatially linked to cell proliferation. We have demonstrated that ESDN modulates vascular smooth muscle cell phenotype, and this may be linked to modulation of growth factor signaling. Other work has focused on the interaction between growth factors, integrins and ESDN in graft arteriosclerosis, demonstrating that inhibition of VEGF reduces vascular remodeling in graft arteriosclerosis through effects on leukocyte trafficking. VEGF inhibition also reduces IFN-γ-induced vascular remodeling, suggesting that VEGF has additional effects on graft arteriosclerosis beyond the observed effects on leukocyte trafficking. These findings may potentially lead to the development of novel diagnostic and therapeutic approaches for vascular remodeling.

Publications:
Sadeghi MM, Esmailzadeh L, Zhang J, Guo X, Asadi A, Krassilnikova S, Rastegar Fassaei H, Luo G, Al-Lamki RSM, Takahashi T, Tellides G, Bender JR, Rodriguez ER. Endothelial and Smooth Muscle Cell-derived Neuropilin-Like Protein is a marker and regulator of cell proliferation in vascular remodeling. *American Journal of Transplantation*, 2007, 7(9):2098-2105.

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Zhang J, Nie L, Razavian M, Ahmed M, Dobrucki LW, Asadi A, Edwards DS, Azure M, Sinusas AJ, Sadeghi MM, Molecular imaging of activated matrix metalliproteinases in vascular remodeling, *Circulation*, 2008, in press

W. Mark Saltzman, Ph.D.
Goizueta Foundation Professor of Chemical and Biomedical Engineering/Chair of Biomedical Engineering

Our laboratory is creating new technology, based on the use of biocompatible polymeric materials, for the controlled delivery of drugs, proteins, and genes. We also develop and study new polymeric materials that influence the growth and assembly of tissues. Our research projects in the area of tissue engineering are the most relevant to the VBT program. Tissue engineering is a new field of inquiry, defined about 15 years ago. In our view tissue engineering involves the use of synthetic polymers as scaffolds for cell transplantation, in which the properties of the scaffold are tuned to encourage the formation or regeneration of tissue structure and function. Importantly, tissue engineering involves a combination of disciplines to achieve new therapies and, in some cases, entirely new approaches to therapy.

Specific accomplishments in the last year:
We have been particularly interested in developing methods for transplantation of neo-tissues—combinations of cells and synthetic materials that are assembled ex vivo. In the past year, we have made progress in four areas that are important in this overall effort. First, we have used electrospinning techniques to make synthetic polymer meshes with fibers that are much less than 1 micron and therefore mimic the natural extracellular matrix dimensions. We have developed a variety of approaches for modifying the surface of these fibers, allowing us to tune them for attach and growth of different cell populations. Second, in collaboration with Christopher Breuer, we have developed a variety of microparticle controlled release systems that are useful in tissue engineering, including systems that release parathyroid hormone, , rapamycin, and proteins such as osteopontin and MCP-1. Third, we have developed controlled release systems for VEGF and are using these in collaboration with Jordan Pober, to optimize the delivery of VEGF in the context of endothelial cell transplantation for treatment of ischemia. Fourth, we are collaborating with Al Bothwell on a project involving protein delivery systems for enhancing the function of transplanted islets for treatment of diabetes.

Publications:

Saltzman WM and Kyriakides. Cell interactions with polymers *In* Textbook of Tissue Engineering, 3rd edition, Academic Press, NY, p. 279-292 (2007).

Zhang Q, Cuartas E, Mehta N, Gilligan J, Ke D, Saltzman WM, Kotas M, Ma M, Rajan S, and Vignery A. Replacement of bone marrow by bone in rat femurs: the bone bioreactor. *Tissue Engineering* **14**:237-246 (2008).

Roh JD, Nelson GN, Brennan MP, Mirensky TL, Yi T, Hazlet TF, Tellides G, Sinusas AJ, Pober JS, Saltzman WM, Kyriakides TR, Breuer CK. Small –diameter biodegradable scaffolds for functional vascular tissue engineering in the mouse model. *Biomaterials*. **29**(10):1454-63 (2008).

Blum JS and Saltzman WM. High Loading Efficiency and Tunable Release of Plasmid DNA Encapsulated in Submicron Particles Fabricated from PLGA Conjugated with Poly-L-lysine, *Journal of Controlled Release* **129**:66-72 (2008).

Jay SM, BR Shepherd, JP Bertram, JS Pober, and WM Saltzman, Engineering of multifunctional gels integrating highly efficient growth factor delivery with endothelial cel transplantation, *FASEB Journal* **22**:2949-2956 (2008).

Kobsa S and Saltzman WM. Bioengineering approaches to controlled protein delivery, *Pediatric Research* **63**:513-519.

Woodrow KA, Wood MJ, Saucier-Sawyer JK, and Saltzman WM. Biodegradable meshes printed with extracellular matrix proteins support micropatterned hepatocyte cultures, *Tissue Engineering*, in press.

William C. Sessa, Ph.D.
Director, VBT; Vice-Chair, Department of Pharmacology

Our laboratory is very interested in endothelial cell biology, signaling and regulation of post-natal angiogenesis/ arteriogenesis in health and disease.

Specific accomplishments in the last year:

In the past year, we have made successful inroads into understanding the role of plasma membrane microdomains termed caveolae in physiology. We have developed a novel mouse model re-expressing the coat protein for caveolae, caveolin-1, back into the endothelium of mice globally deficient in caveolin-1 and have shown that the endothelial expression of this gene is critical for capacitive calcium signaling in endothelial cells by regulating the localization of TRPC channels and interaction with the IP3 receptors in the endoplasmic reticulum. In addition, endothelial cell caveolin-1 rescues the cardiac and pulmonary defects in global caveolin-1 KO mice. An additional interest of the lab is the Akt1- eNOS pathway and we have generated mutant mice that develop coronary atherosclerosis. This occurs due to a defect in eNOS phosphorylation, endothelial and macrophage apoptosis and accelerated vascular inflammation. This model is unique since mice do not typically develop coronary atheromas, the main culprit in acute coronary disease in humans.

Publications:

Murata T, Lin M.I., Huang Y., Yu J., Bauer P.M., Giordano F.J. and Sessa W.C. Reexpression of caveolin-1 in endothelium rescues the vascular, cardiac, and pulmonary defects in global caveolin-1 knockout mice. J Experimental Medicine, 204(10):2373-82 (2007).

Fernandez-Hernando, C., Ackah, E., Yu, J., Suarez, Y., Murata, T., Iwakiri, Y., Prendergast, J., Miao, R.Q., Birnbaum, M.J. and Sessa, W.C. Loss of Akt1 leads to severe atherosclerosis and occlusive coronary artery disease. Cell Metabolism, 6(6):446-57 (2007).

Pober, J.S. and Sessa, W.C. Evolving functions of endothelial cells in inflammation. Nature Reviews Immunology. 7(10):803-15 (2007).

Miao RQ, Fontana J, Fulton D, Lin MI, Harrison KD, Sessa WC. Dominant-negative Hsp90 reduces VEGF-stimulated nitric oxide release and migration in endothelial cells. Arterioscler Thromb Vasc Biol. 28(1):105-11 (2008).

Sowa G, Xie L, Xu L, Sessa WC. Serine 23 and 36 phosphorylation of caveolin-2 is differentially regulated by targeting to lipid raft/caveolae and in mitotic endothelial cells. Biochemistry. 8;47(1):101-11 (2008).

Albert J. Sinusas, M.D.
Professor of Medicine and Diagnostic Radiology

Research in the Sinusas laboratory is directed at development of noninvasive imaging approaches for the assessment of myocardial viability, angiogenesis, arteriogenesis, and post-infarction remodeling. The laboratory has been employing the 3-D modalities of single photon emission computed tomography (SPECT), positron emission tomography (PET), echocardiography, X-ray tomography, and magnetic resonance (MR) imaging for assessment of a wide range of physiological and molecular processes primarily focused in the cardiovascular system. The laboratory is currently focused on targeted molecular imaging, developing non-invasive nuclear imaging strategies for identifying the hypoxic stimulus for angiogenesis, and targeted imaging of selected integrins previously established to modulate the angiogenic process, and the interrelationship of angiogenesis and arteriogenesis. These studies involve the use of rodent models of myocardial ischemia as well as hindlimb ischemia.

Publications:

Sinusas AJ. Cardiovascular molecular imaging: promoting utilization and outreach. J Nucl Med. 2008 Jun;49(6):60N-63N.

Kalinowski L, Dobrucki LW, Meoli DF, Dione DP, Sadeghi MM, Madri JA, Sinusas AJ. Targeted imaging of hypoxia-induced integrin activation in myocardium early after infarction. J Appl Physiol. 2008 May;104(5):1504-12. Epub 2008 Mar 20.

Edward L. Snyder, MD
Professor Laboratory Medicine
Director, Apheresis/Cell Processing VBT Core Facility (Core D)

The Apheresis/Cell Processing Core Facility plays a critical role in the Vascular Biology and Transplantation Program. The Cell Processing Core Laboratory is designed to support the needs of the VBT Program users who are performing basic science and clinical research involving mononuclear and other cell types, by providing five specific functions. First, the Apheresis section of the Cell Processing Core Laboratory will procure and provide both patient and normal donor specimens in support of research projects. These samples, obtained under IRB approved protocols from fresh specimens, will be available to VBT membership. Second the Elutriation section of the VBT Core Laboratory can provide cell purification services in the form of elutriation technology using closed system devices to ensure safety and sterility of the cellular product. Third, the main Cell Processing Core provides large-scale processing capabilities in support of specific research studies involving human MNCs as well as CD34 positive and other cell types. Included within this section is the development of new cell selection and culturing techniques to support the novel cell therapy protocols, as well as the pre-clinical validation of research procedures. The VBT Core resource provides the critical instrumentation and technical expertise in cell processing and cryopreservation, needed for the in vitro use of cells, or infusion of cells into animals. Fourth, the Core will provide any needed G-CSF injections and collections of MNC and CD34+ cells from G-CSF stimulated donors. Fifth, the Apheresis/ Elutriation/Cell Processing VBT Core Facility will maintain compliance with institutional, NIH, FDA, AABB and FACT guidelines, and will ensure that the protocols can be safely and effectively applied. Included with this objective will be training new investigators in compliance and Quality Control issues. Thus, this resource provides access to cell collection, selection, purification, processing and culturing technologies, as well as services and scientific consultation to enhance the productivity of the VBT members. This technically sophisticated resource is critical to the Vascular Biology and Transplantation Section's research progress.

Specific Accomplishments in the last 12 months:
In 2007, Core D performed 20 MNC apheresis collections for Program Leaders' research

Publications:

Slichter SJ, Baril L, Corda T, Dincecco D, Bolgiano D, MK Jones, Christoffel T, Corson J,.Mantha S, Eisenbarth S, Champion M, Snyder EL. Logistics of Platelet Concentrates. Vox Sang. 2007:92:180-181

Cancelas J, Bandarenko N, Snyder E, et al. Cryopreservation of AS-5 Collected RBCs in PVC or EVA bags, by the Closed ACP-215 System Allows Extended Storage for 14 Days Post Thawing. Transfusion 2007;47:680-6

Bing Su, Ph.D.
Associate Professor, Department of Immunobiology

The overall goal (s) of the research program of the laboratory is to understand the biology of signal transduction mediated by the mitogen-activated protein kinase (MAPK) pathways, and by the mammalian target of rapamycin (mTOR) pathway. We use mice with targeted deletion of genes that encode key components of these pathways, and combine with the biochemistry and molecular biology approaches, to investigate the biology and regulation of these pathways. In the past 12 months, we have investigated the role of the MEKK3, a key MAPK activator, in T lymphocyte development and function. We also study its role in endothelial cell function. Both these studies utilize MEKK3 conditional knockout mice with specific MEKK3 deletion in T cells and endothelial cells respectively. We also investigated the role of SIN1, an essential component of mTOR complex 2 and discovered that SIN1 is crucial for embryonic angiogenesis. At the molecular level, we reveled a novel function of SIN1/mTORC2 in regulating the protein stability of Akt and PKC through phosphorylation of a conserved threonine residue. More recently, we revealed a critical role of SIN1 in B cell development. Our findings from studying both the MEKK3 and the SIN1-mTORC2-Akt pathway are relevant for the mission of VBT. Since both the MAPK pathways and the mTOR pathway control numerous physiological and pathologic processes ranging from cell growth, stress-responses, aging, survival, to diabetes, autoimmunity and cancer, it is important to understand their roles in these processes, especially by focusing on their roles in the vascular system and in immune responses.

Publications:

Yao J, Kim T, Qin J, Jiang Z, Qian Y, Xiao H, Lu Y, Wen Q, Gulen MF, Sizemore N, Didonato J, Sato S, Akira S, **Su B**, Li X: IL-1-induced TAK1- versus MEKK3-dependent NFkB activation pathways bifurcate at IRAK modification. J Biol Chem. 282(9):6075-89, 2007.

Kihwan Kim, Omar Duramad, Xiaofeng Qin and **Su B**. MEKK3 Is Essential for Lipopolysaccharide-induced IL-6 and GM-CSF Production in Macrophages. Immunology 120:242-250, 2007.

Blonska M, Pappu BP, Matsumoto R, Li H, **Su B**, Wang D, Lin X: The CARMA1-Bcl10 Signaling Complex Selectively Regulates JNK2 Kinase in the T Cell Receptor-Signaling Pathway. Immunity 26: 55-66, 2007.

Lande R, Gregorio J, Facchinetti V, Chatterjee B, Wang YH, Homey B, Cao W, Wang YH, **Su B,** Nestle FO, Zal T, Mellman I, Schroder JM, Liu YJ, Gilliet M. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. Nature. 449:564-9, 2007.

Deng Y, Yang J, McCarty M, **Su B**. MEKK3 is required for endothelium function but is not essential for tumor growth and angiogenesis. Am J Physiol Cell Physiol. 293(4):1404-11, 2007.

Mongan, M., Z. Tan, L. Chen, Z. Peng, M. Dietsch, **Su B.**, G. Leikauf, and Y. Xia. Mitogen-activated protein kinase kinase kinase 1 protects against nickel-induced acute lung injury. Toxicol Sci. 2008.

Valeria Facchinetti, Weiming Ouyang, Hua Wei, Nelyn Soto, Adam Lazorchak, Christine Gould, Carolyn Lowry, Alexandra C. Newton, Yuxin Mao, Robert Q. Miao, William C. Sessa, Jun Qin, Pumin Zhang, **Su B***, and Estela Jacinto* (*corresponding authors). The mammalian target of rapamycin complex 2 controls folding and stability of Akt and protein kinase C. EMBO J. 27:193–943. 2008.

George Tellides, M.D., Ph.D.
Professor of Surgery

Our primary research interest is immune-mediated vascular remodeling focusing on the effects of T cells and their products on vascular smooth muscle cells and on the regulation of artery-infiltrating T cell immune responses by vascular smooth muscle cells.

Specific Research Accomplishments in the Last Year :
We have described: STAT3 signaling by interferon-γ in vascular smooth muscle cells resulting in XAF1- and Noxa-mediated apoptosis, mTOR/S6K1-dependent proliferation of vascular smooth muscle cells by interferon-γ, IDO-dependent immune regulation of T cell alloresponses by vascular smooth muscle cells, MyD88- and CXCR3-dependent inflammation in flow-dependent vascular remodeling, and IL-17 production and effects in atherosclerosis and graft arteriosclerosis.

Significance of Key Findings Relevant for the Mission of VBT:
We have contributed to a successful VBT Program Project, we have participated in the development of new cardiac surgeon-scientist faculty, and we have assisted in the administrative re-organization of the clinical cardiac transplantation service.

Publications:

Wang Y, Bai Y, Qin L, Zhang P, Yi T, Teesdale SA, Zhao L, Pober JS, Tellides G. Interferon-gamma Induces Human Vascular Smooth Muscle Cell Proliferation and Intimal Expansion by Phosphatidylinositol 3-Kinase Dependent Mammalian Target of Rapamycin Raptor Complex 1 Activation. *Circ Res*. 2007;101:560-569.

Sadeghi MM, Esmailzadeh L, Zhang J, Guo X, Asadi A, Krassilnikova S, Fassaei HR, Luo G, Al-Lamki RS, Takahashi T, Tellides G, Bender JR, Rodriguez ER. ESDN Is a Marker of Vascular Remodeling and Regulator of Cell Proliferation in Graft Arteriosclerosis. *Am J Transplant*. 2007;7:2098-2105.

Cuffy MC, Silverio AM, Qin L, Wang Y, Eid R, Brandacher G, Lakkis FG, Fuchs D, Pober JS, Tellides G. Induction of Indoleamine 2,3-Dioxygenase in Vascular Smooth Muscle Cells by Interferon-γ Contributes to Medial Immunoprivilege. *J Immunol*. 2007;179:5246-5254.

Pimiento JM, Maloney SP, Tang PC, Muto A, Westvik TS, Fitzgerald TN, Fancher TT, Tellides G, Dardik A. Endothelial Nitric Oxide Synthase Stimulates Aneurysm Growth in Aged Mice. *J Vasc Res*. 2008;45:251-258.

Roh JD, Nelson GN, Brennan MP, Mirensky TL, Yi T, Hazlett TF, Tellides G, Sinusas AJ, Pober JS, Saltzman WM, Kyriakides TR, Breuer CK. Small-diameter biodegradable scaffolds for functional vascular tissue engineering in the mouse model. *Biomaterials*. 2008;29:1454-1463.

Bai Y, Ahmad U, Wang Y, Li JH, Choy JC, Kim RW, Kirkiles-Smith N, Maher SE, Karras JG, Bennett CF, Bothwell AL, Pober JS, Tellides G. Interferon-gamma Induces X-linked Inhibitor of Apoptosis-associated Factor-1 and Noxa Expression and Potentiates Human Vascular Smooth Muscle Cell Apoptosis by STAT3 Activation. *J Biol Chem*. 2008;283:6832-6842.

Shepherd BR, Jay SM, Saltzman WM, Tellides G, Pober JS. Human Aortic Smooth Muscle Cells Promote Arteriole Formation by Coengrafted Endothelial Cells. Tissue Eng. In press.

Yi T, Rao DA, Tang PCY, Wang Y, Cuchara LA, Bothwell ALM, Colangelo CM, Tellides G, Pober JS, Lorber MI. Amelioration of Human Allograft Arterial Injury by Atorvastatin or Simvastatin Correlates with Reduction of Interferon-γ Production by Infiltrating T Cells. *Transplantation*, In press.

Nelson GN, Roh JD, Shkarin P, Shapiro EM, Mirensky TL, Wang Y, Yi T, Tellides G, Pober JS, Saltzman WM, Papademetris X, Fahmy TM, Breuer CK. Initial evaluation of the use of USPIO cell labeling and noninvasive MR monitoring of human tissue-engineered vascular grafts in vivo. *FASEB J*, In press.

Agnès Vignery, DDS, Ph.D.
Associate Professor of Orthopaedics and Rehabilitation

Research in our laboratory focuses on osteoporosis, a disease that is at the cross road of the immune, vascular and nervous system. Our main line of investigation regards the differentiation of osteoclasts, which resorb bone, and giant cells, which resorb foreign bodies, with particular emphasis on the molecular mechanisms of fusion of their mononucleate precursor cells that belong to the monocyte-macrophage lineage. Macrophages seed all tissues and can fuse with themselves to differentiate into multinucleate osteoclasts, in bone, or giant cells, in chronic inflammatory reactions and cancer. Although osteoclasts and giant cells play a central role in these diseases, the molecular mechanisms that are responsible for the fusion of macrophages remain poorly understood.

Most recently, we have initiated a new line of investigation, which focuses on the targeted induction of new bone to specific sites of the skeleton. We have published our data and filed three new patents on the methodology and concept of targeted formation of new bone.

Specific Research Accomplishments in the last 12 months:
Conferences:
July 1-6, 2007, I organized and co-chaired with Diana Myles from UC Davis a new Gordon Research Conference (GRC) entitled " Cell-Cell Fusion":
http://www.grc.uri.edu/programs/2007/cellcell.htm. The success of that first conference secured the second one, which will take place in 2009:
http://www.grc.org/programs.aspx?year=2009&program=cellcell
Patents:

a. Use soluble recombinant extracellular domain of CD200R or antibodies that block CD200R to prevent and/or repair bone loss. Agnès Vignery, Jun Li and Juan Ke, Inventors. Filed jointly with Boehringer Ingelheim, Yale # 20070111
b. Regulate MKP-1 expression or activity to control bone mass. Agnès Vignery, Anton Bennett, Fatih Mercan and Jodi Carlson, Inventors. Provisional filing, Yale OCR 00004956, 2008
c. Targeted delivery of stem cells to repair/regenerate tissues and organs. Agnès Vignery, Inventor. Provisional filing, Yale OCR 00004586, 2008

Significance of Key Findings Relevant for the Mission of VBT:
The formation of intramembranous bone, in contrast with endochondral bone, requires extensive vascularization. Our recently published discovery that new bone forms at targeted sites in the skeleton as a result of marrow ablation and daily treatment with parathyroid hormone has revealed an essential link between the formation of new bone and vascularization.

Publications:

Chen E, Grote E, Mohler W, **Vignery A**. Membrane Exchange Special Issue: Cell-Cell Fusion. Invited Minireview. FEBS Lett. 581:2181-2193, 2007. Epub 2007 Mar 21

Zhang Q, Cuartas E, Mehta N, Gilligan J, Ke H-Z, Saltzman W M, Kotas M, Ma M, Rajan S, Chalouni C, Carlson J, **Vignery A**. PTH promotes the formation of lamellar bone at targeted skeletal sites after marrow ablation in rats. Tissue Engineering, 14: 237-246, 2008

Cui W, Cuartas E, Ke J, Zhang Q, Einarsson HB, Sedgwick JD, Li J, **Vignery A**. CD200 and its receptor, CD200R, modulate bone mass via the differentiation of osteoclasts. Proc Natl Acad Sci USA, 2007 Sep 4;104:14436-14441; 2007 Epub 2007 August 28

Vignery, A. Macrophage fusion: molecular mechanisms. Methods in Molecular Biology "Cell Fusion". Invited review*, in press*

Vignery, A. Methods to fuse macrophages. Methods in Molecular Biology "Cell Fusion"'. Invited review*, in press*

Dianqing Wu, Ph.D.
Professor, Department of Pharmacology

The overall objective of our research activities is to understand the mechanisms and functions of signal transduction activated by chemoattractants and Wnts.

The key research accomplishment includes: 1) Investigation of three GPCR signaling pathways for their role in atherosclerosis. These are PI3Kγ–, P-Rex1- and PLC β-linked pathways. We found that PLC β3-linked pathway has a significant role in regulating macrophage survival in atherosclerotic lesions and that its deficiency significantly reduces lesion formation. PI3Kγ-deficiency has a mild effect, while P-Rex1-deficiency has little effect, on atherogenesis. 2) Characterization of a LRP6 mutation for its relation with Wnt activity. This LRP6 mutation is associated with early coronary heart disease, diabetic onset, and metabolic abnormalities. 3) Resolving the structure of a Wnt antagonist Dkk2 and identification of motifs for its interaction with LRP5/6 and Kremen.

Publications:

Chang JD, Sukhova GK, Libby P, Schvartz E, Lichtenstein AH, Field SJ, Kennedy C, Madhavarapu S, Luo J, Wu D, Cantley LC. (2007) Deletion of the phosphoinositide 3-kinase p110gamma gene attenuates murine atherosclerosis. ***Proc Natl Acad Sci U S A***. 104:8077-82.

Nomura S, Fukaya M, Tsujioka T, Wu D, Watanabe M. (2007) Phospholipase Cbeta3 is distributed in both somatodendritic and axonal compartments and localized around perisynapse and smooth endoplasmic reticulum in mouse Purkinje cell subsets. ***Eur J Neurosci.*** 25:659-72.

Zhao T, Nalbant P, Hoshino M, Dong X, Wu D, Bokoch GM. (2007) Signaling requirements for translocation of P-Rex1, a key Rac2 exchange factor involved in chemoattractant-stimulated human neutrophil function.
J Leukoc Biol. 81:1127-36.

Mani, A., Radhakrishnan, J., Wang, H., Mani, A., Mani, M-A., Nelson-Williams, C., Carew, K. S., Mane, M., Najmabadi, H., Wu, D., and Lifton, R. P. (2007) LRP6 Mutation in a Family with Early Coronary Disease and Metabolic Risk Factors. ***Science*** 315, 1278-1282

Tang, L.Y., Deng, N., Wang, L.S., Dai, J., Wang, Z.L., Jiang, X.S., Li, S.J., Li, L., Sheng, Q.H., Wu, D.Q.*, et al.* (2007). Quantitative phosphoproteome profiling of Wnt3a mediated signaling network: indicating the involvement of ribonucleoside-diphosphate reductase M2 subunit phosphorylation at residue serine-20 in canonical Wnt signal transduction. ***Mol Cell Proteomics.*** 6, 1952-1967.

Wang, Z., Liu, B., Wang, P., Dong, X., Fernandez-Hernando, C., Li, Z., Hla, T., Claffey, K., Smith, J.D., and Wu, D. (2007). Phospholipase C beta3 deficiency leads to macrophage hypersensitivity to apoptotic induction and reduction of atherosclerosis in mice. ***J Clin Invest.*** 118(1): p. 195-204.

Wang, Z.L., Dong, X.M., Smith, J.D., and DWu, D., Lack of a significant role of P-Rex1, a major regulator of macrophage Rac1 activation and chemotaxis, in atherogenesis. ***Prostaglandins Other Lipid Media***t 2008. 10.1016/j.prostaglandins.2008.04.001

Wang, K., Zhang, Y., Li, X., Chen, L., Zheng, J.*, and Wu, D.* (2008). Characterization of the Kremen-binding site on Dkk1 and elucidation of the role of Kremen in Dkk-mediated Wnt antagonism. ***J Biol Chem*** 10.1074/jbc.M802376200 (*co-corresponding authors)

Chen, L., Wang, K., Shao, Y., Huang, J., Li, X., Shan, J., Wu, D.*, and Zheng, J.J.*, Structural insight into the mechanisms of Wnt signaling antagonism by Dkk. ***J Biol Chem***, 2008. 10.1074/jbc.M802375200 (*co-corresponding authors)

INTERACTIONS WITH INDUSTRY

VBT continued its role as the primary partner for the Yale-Boehringer-Ingelheim Pharmaceuticals Inc Research Alliance in the fields of Cardiovascular Diseases and Immunology. This non-exclusive relationship has led to four new pilot projects (1 yr) and two full projects (2 yrs). VBT continues to explore additional opportunities for university-corporate partnerships, especially in translational applications of VBT scientific discoveries.

FUND RAISING AND DEVELOPMENT

The Medical School is an active participant in the current Yale University fund raising campaign, and the Amistad research building has been identified as a major target for the medical school. VBT has not engaged in separate fund raising activities this past year, although Boehringer-Ingelheim Pharmaceuticals Inc has provided an unrestricted gift to help equip the Amistad Building and to support our annual retreat

Appendix 1

The Seventh Annual VBT Retreat

The seventh annual retreat
held in conjunction
with
Yale University School of Medicine's Vascular Biology and Therapeutics Program
with
Human and Translational Immunology Program
and
Yale Stem Cell Center

Saturday, October 6, 2007
8:00 A.M. – 1:30 P.M.
The Anlyan Center for Medical Research & Education (TAC)
Auditorium N107

Supported by an Unrestricted Gift from Boehringer Ingelheim Pharmaceuticals, Inc.

Program In Vascular Biology and Therapeutics

Human and Translational Immunology Program

Stem Cell Center

8:00 - 8:30	**Registration** Continental Breakfast
8:30 - 9:00	**William C. Sessa, Ph.D.** “Novel mechanisms controlling angiogenic signaling”
9:00 - 9:10	Questions/Discussion
9:10 - 9:40	**Natalia Ivanova, Ph.D.** “Dissecting self-renewal in stem cells with RNA interference”
9:40 - 9:50	Questions/Discussion
9:50 - 10:10	**Break**
10:10 - 10:40	**Jordan S. Pober, M.D., Ph.D.** "Therapeutic potential and immunological peril of progenitor cell-derived endothelium“
10:40 - 10:50	Questions/Discussion
10:50 – 11:20	**Kevan Herold, M.D.** “Moving forward with immunotherapy of Type 1 diabetes”
11:20-11:30	Questions/Discussion
11:30-1:30	**Lunch and Poster Session**
1:30	**Announcement of Poster Awards**

Appendix 2

The 6th Annual Meeting of the Joint Cambridge-Yale Program in Cardiovascular Research

Cambridge-Yale Cardiovascular Research Program

23 – 25 September 2007

Amistad Research Building
Yale University School of Medicine

Meeting report

Agenda

Monday, September 24

9:00	Welcome & Introduction Jordan Pober & John Bradley
Session 1	**Tissue engineering, stem cells, vascular remodelling** **Session Chair – John Wallwork**
9:15	Sanjay Sinha “Development of smooth muscle cells from human embryonic stem cells”
9:30	Agnes Vignery “Bioengineering new bone *in vivo*”
9:45	Themis Kyriakides “A matrix-based anti-thrombotic and pro-angiogenic coating for vascular grafts
10:00	Roger Pedersen “Pluripotency and differentiation of mammalian embryonic stem cells”
10:15	Laura Niklason "Engineered Human Vessels -Cells or No Cells?
10:30-11:00	Break
11:00	Steve Charnock Jones “Regulatory gene networks and endothelial cell function”
11:15	Cathy Shanahan “The nuclear envelope and VSMC ageing”
11:30	Martin Bennett “Surprising effects of chronic cell death in the vasculature”
11:45	Frank Giordano “Recruitment of progenitor cells"
12:00-1:00	Lunch
Session II	**Inflammation and Immunity** **Session Chair - Patrick Sissons**
1:00	Dan Wu “PLC signaling in macrophage apoptosis and atherogenesis”
1:15	George Tellides “Mechanisms of Vascular Remodeling”
1:30	Patty Lee “Innate Immune Mechanisms in Emphysema”
1:45	Jordan Pober “Allogeneic responses to progenitor cell-derived endothelium”

2:00	Andrew Bradley “Mechanisms of cardiac allograft rejection”
2:15	Chris Watson “Rituximab induction in renal transplantation”
2:30-3:00	**Break**
3:00	Su Metcalfe “Evidence for Foxp3-mediated suppression of SOCS-3 transcription”
3:15	John Bradley “Signaling diverse effects of TNF in the heart”
3:30	David Rothstein "Tolerance Through Regulation of T cells by CD45"
3:45	Jack Elias “Regulation of VEGF effects in the lung”
4:00	Kevan Herold “Moving forward with immunotherapy of Type 1 diabetes”
4:15	Craig Taylor “The effect of genotype on PECAM function”
6:00	Dinner

Tuesday, September 25

Session 3	**Atherothrombosis angiogenesis and vascularisation** **Session Chair - William Sessa**
9:00	Michael Gaunt “Remote Ischaemic preconditioning”
9:15	Willem Ouwehand “The role of platelet systems biology in unraveling the genetic architecture of atherothrombosis”
9:30	Alan Dardik - “Vessel identity during vein graft adaptation”
9:45	Albert Sinusas “Targeted imaging of angiogenesis”
10:00	Martin Kluger “Melanoma-induced Vascular Hyperpermeability”
10:15	Nancy Ruddle “Visualization of the Vascualture in Secondary and Tertiary Lymphoid Organs”
10:30-11:00	Break
11:00	Chris Breuer “Tissue engineering vascular grafts”

11:15	Jane Smith “Endothelial dysfunction in kidney transplantation”
11:30	Joseph Madri “Effects of strain differences and sensorimotor enrichment on endothelial and neural progenitor survival and proliferation in the neurovascular niche”
11:45	Lunch
1:00-2:30 3:00	Wrap Up Depart to JFK

Session 1. Tissue engineering, stem cells, vascular remodeling

Development of Smooth Muscle Cells from Human Embryonic Stem cells

Sanjay Sinha
Although there are a variety of studies that have investigated smooth muscle cell (SMC) development in animal models, there is little information specific to humans SMC development. Human embryonic stem cells (ESC) can be induced to differentiate into a wide range of cell types. Sanjay Sinha described conditions in which contractile human SMCs develop from huESCs. This system may be used to investigate underlying developmental mechanisms and data will be presented suggesting that myocardin is a key regulator of human SMC development from ESCs. A key limitation in applying the ESC-based system to tissue engineering is the heterogenous nature of the differentiated cell population. Various strategies to isolate pure SMC or progenitor populations using cell specific promoters are currently being investigated.

Bioengineering new bone *in vivo*: rapid site-specific bone growth by a combination of bone marrow ablation, PTH and bisphosphonate therapy

Agnès Vignery
During development and repair of bone, two distinct yet complementary mechanisms, intramembranous and endochondral, mediate new bone formation via osteoblasts. Mechanical bone marrow ablation leads to differentiation of osteoblasts, and formation of cancellous bone. The intramembranous bone-formation phase that follows bone marrow ablation is complete by day 7 at which time osteoclasts differentiate in synchrony and resorb the newly formed bone to re-create the marrow cavity into which bone marrow cells return. This transient induction of bone formation in response to marrow ablation has been used as an *in vivo* model to discover new genes, and to investigate the role of known genes, in the process of bone formation. Agnès Vignery described work, which questioned whether the calcium regulating hormone parathyroid hormone (PTH) could promote the formation of new bone that occurs after marrow ablation. The left femurs of rats were subjected to marrow ablation, and the animals were injected daily with the PTH analog PTH(1-34)NH2 for one, two or three weeks. Both femurs from each rat were analyzed by soft X-ray, pQCT, microCT and histology, and serum osteocalcin concentration was determined. Bone progressively filled the marrow cavity of the ablated femoral shafts in animals treated with PTH. The newly formed bone endowed femoral shafts with improved biomechanical properties when compared to those of contra lateral femurs as well as left femurs from control, sham operated and vehicle-treated rats. To determine whether this new bone could be maintained for extended times, the left femurs of rats were subjected to marrow ablation, and the animals were injected daily with PTH(1-34)NH2 for three weeks followed by treatment with the bisphononate alendronate or PBS for two months. Both femurs from each rat were analyzed. The bone that had formed in response to marrow ablation and daily PTH for 3 weeks was resorbed after two months in the PBS treated controls. By contrast, bone was maintained in the ablated marrow cavity following daily alendronate treatment for two months. These findings might potentially be useful for investigations on the role of marrow neovascularization in intramembranous bone formation, and for preferential site-directed bone growth in areas of high bone loss, for fracture repair, and for reinforcing the implantation of prosthetic devices.
Discussion centred on how the findings might provide useful insights into the role of neovascularisation in intramembranouse bone formation, and the relevance to vascularisation of other tissues. The role of TNF and TNF receptors in the process provided a potential area for collaborative investigation.

A matrix-based anti-thrombotic and pro-angiogenic coating for vascular grafts

Themis Kyriakides

Themis Kyriakides described the dual focus of his research group on vascular biology and biomedical engineering. The specific research focus is on elucidating the mechanism through which the endogenous inhibitor of angiogenesis thrombospondin (TSP)-2 limits angiogenesis and arteriogenesis. Themis Kyriakides described utilization of an in vitro, TSP-2-sensitive, three-dimensional angiogenesis assay and to investigate the effects of TSP-2 on endothelial cells, with the aim of identifying the signaling pathways that mediate the anti-angiogenic effect of TSP2. Recent experiments have shown that TSP2 is critical for the recovery of blood flow in an experimental model of hindlimb ischemia, predominantly through enhanced arteriogenesis in the upper limb and increased angiogenesis in the lower limb. Finally, work is in progress to translate the basic findings regarding TSP2 function into a strategy for the generation of matrix-coated synthetic vascular grafts. Based on the assumption that a TSP2-null-derived matrix could confer anti-thrombotic and pro-angiogenic properties to the luminal surface of synthetic vessels, several in vitro and in vivo studies have been pursued in order to provide proof-of-principle for this hypothesis. Specifically, TSP2-null-derived matrix is more permissive for endothelial cell migration and compromised in its ability to bind von Willebrand factor. Furthermore, transplantation of denuded aortic segments from TSP2-null to wild type mice indicates that they are resistant to thrombosis and, unlike transplanted wild type segments, remain patent in the long term.

In discussion the potential role of collagen and thrombin were considered; there is no known relationship between matrix derived from TSP2-null mice and thrombin. Further unresolved issues included identifying the potential source of endothelial cells, either from migration or circulating progenitors, and determining whether grafts that resist thrombosis also resist development of intimal hyperplasia.

Pluripotency and differentiation of mammalian embryonic stem cells

Roger Pedersen

Roger Pedersen described how his research has focused on understanding fundamental mechanisms that govern human embryonic stem cells (hESC) pluripotency and differentiation as the foundation for future progression to their possible therapeutic use. This work has revealed that signalling through the Activin/Nodal pathway is critical for maintenance of hESC pluripotency, thereby providing insight into this key property of hESCs. In addition, analysis of the expression of imprinted genes and their regulation in hESCs, has shown that they have a generally stable epigenetic status, contrasting with the relative epigenetic instability of mouse ESCs. The findings of distinct pluripotency mechanisms and epigenetic status in human and mouse embryonic stem cells led Roger Pedersen's group to determine whether these were species or developmental differences. The recent discovery that human ES-like cells could be derived from the late epiblast layer of mouse and rat embryos provides a developmental explanation for the unique properties of human ES cells and adds a strategically-placed system for gaining insight into the mechanisms governing their development into mature tissues. Finally, Roger Pedersen described work to address the task of matching the immune identity of recipients of cell-based therapies by estimating the number of human embryo-derived stem cell lines needed for a bank aimed at meeting the needs of the UK population through allograft transplantation. This estimate indicates that a moderate number of randomly selected lines, or a small number of highly selected lines would maximize any benefits of matching the donor and host major histocompatibility loci. The effectiveness of the highly selected lines relates to their homozygous status for HLA haplotypes that are prevalent in the recipient population. These could potentially be obtained from parthenogenetically activated oocytes, and we have demonstrated that the putative SCNT- hESC-1 line is the first example of a parthenogenetic human ESC.

Engineered human vessels – cells or no cells

Laura Niklason

Regenerative medicine strategies often rely on cells to repair or replace damaged tissues. But the capacity of differentiated cells to regenerate functional tissues is adversely affected by aging. Laura Niklason described work in arterial regeneration that has exploited cells derived from young animals, which could be used to "grow" new arteries that are functional in these same animals. These studies have shown that limitations in cellular replicative lifespan are an important limitation for tissue engineering in the elderly, but that this roadblock can be partially overcome by gene therapies to increase telomere length However, increases in cell lifespan do not de facto reverse all of the consequences of cellular aging. If autologous cells are to be used to re-grow tissues, it is likely that, in many cases, a "younger" cell source will have to be identified. This has led to investigations aimed at defining the functional utility of adult stem cells for vascular reconstruction. Bone marrow is likely to be populated with vascular precursor cells, but differentiation of these cells needs to be followed by proliferation and expansion as a precursor to utilisation for tissue generation. These cells may be resistant to age-induced phenotypic changes, and create tissues that are more facile at maintenance/repair. Preliminary results concur with those of others that EC and SMC phenotypes can be derived from human bone marrow, but the extent of functional differentiation in terms of anti-coagulant phenotype, contractility, matrix synthesis is unclear. Overall, engineering of human vessels from bone marrow-derived cells is feasible, but the impact of donor age on vascular cell differentiation, function, and tissue reconstitution remain open questions.

The presentations from Roger Pedersen and Laura Niklason highlighted the complementary strengths in stem cell biology and tissue engineering that form the basis of the application to Fondation Leducq to develop a trans-Atlantic network to develop cellular engineering of vascularised tissues.

Regulatory gene networks and endothelial function

Stephen Charnock-Jones

Steve Jones highlighted the many complex and overlapping regulatory networks within a cell that control and regulate cell function, one of which operates at the level of the transcriptome (ie it describes the relationship between RNA transcripts). The identification and characterization of such gene regulatory networks offers an insight into cell function, and a way of manipulating this experimentally or therapeutically. Steve Jones described work that set out to generate regulatory gene networks in endothelial cells as these cells are critically involved in numerous physiological and pathological processes. This involved specifically knocking down approximately 400 individual transcripts in human umbilical endothelial vein endothelial cells and performing gene array analysis on each of these. Combining this data with a time course data of endothelial cells treated with TNF has allowed generation of a regulatory network. One of the striking features of this network is that there are several so called '*hub'* genes, which are highly connected and appear to have regulatory relationships with a large number of transcripts. Specifically, knocking down two of these hubs, which are previously uncharacterised EST's reveals that one of them is involved in the sensitivity of endothelial cells to TNF induced apoptosis and the other has a role in TNF regulated chemokine production by endothelial cells. Both of these processes are important for endothelial function and this network technology allows the identification of functionally important transcripts within this specific cell type.

A key element of the discussion, and potential focus for future work, was whether model diseases such as myocardial infarction could be studied, looking for sequence variation in key regulatory genes.

The role of the nuclear envelope in vascular ageing.

Cathy Shanahan

The nuclear envelope is a specialized organelle with roles in nuclear organisation, maintenance of cellular architecture and cell signalling. Mutations in the *LMNA* gene, which encodes A-type lamins, intermediate filament proteins of the nuclear envelope result in a variety of tissue specific disorders known as laminopathies which include Emery-Dreifuss muscular dystrophy, dilated cardiomyopathy, lipodystrophies and progerias. In Hutchinson-Gilford Progeria Syndrome (HGPS) death occurs before the second decade due to severe premature arteriosclerosis characterized by vascular smooth muscle cell (VSMC) calcification, lipid accumulation and attrition. Studies in HGPS patients have demonstrated that progerin, a toxic mutant lamin A protein, produced by a splicing defect in *LMNA* preferentially accumulates in VSMCs.
Cathy Shanahan described work to determine if defects in lamin A are associated with normal vascular ageing. Specifically, the profile of lamin A/C expression in normal and aged VSMCs both *in vitro* and *in vivo* was examined. Progerin was not detected however the accumulation of prelamin A was found to occur coincidently with nuclear morphology defects, with both nuclear and cytoplasmic accumulation evident in senescent cells. Prelamin A accumulation correlated with downregulation of the metalloproteinase FACE1 the enzyme responsible for the cleavage of farnesyl groups from prelamin A to produce mature lamin A. Importantly, siRNA knockdown of FACE1 in VSMCs induced nuclear morphology defects, proliferation and mitotic failure. Moreover, in response to oxidative stress, FACE1 expression was reduced leading to the accumulation of prelamin A and the induction of cell cycle arrest. Importantly, farnesyl transferase inhibitors and atorvastatin reduced nuclear morphology defects and blocked the effects of stress induced accumulation of prelamin A in VSMCs suggesting that these drugs may be effective in protecting against 'ageing', a potent risk factor for arteriosclerosis previously thought of as non-modifiable.

Discussion focused on the toxicity of pre-lamin A, demonstrated by its effects on nuclear organization, and the ability of statins to reverse defects in nuclear morphology in vascular smooth muscle cells.

Martin Bennett

Vascular smooth muscle cell accumulation has long been implicated in plaque development. In contrast VSMC apoptosis is implicated in plaque rupture, coagulation, vessel remodeling, medial atrophy, aneurysm formation, and clacificaiton. Although VSMC apoptosis accompanies multiple pathologies there is little proof of driect causality, particularly with the low levels of VSMC apoptosis seen in vivo. Using a mouse model of inducible VSMC-specific apoptosis, Martin Bennett described work, which has demonstrated that low-level VSMC apoptosis during either atherogenesis or within established plaques of ApoE-/- mice accelerated plaque growth by two-fold, associated with features of plaque vulnerability including a thin fibrous cap and expanded necrotic core. Chronic VSMC apoptosis induced development of calcified plaques in younger animals and promoted calcification within established plaques. In addition, VSMC apoptosis induced extensive medial infiltration and expansion, associated with increased elastic lamina breaks, and abnormal matrix deposition reminiscent of cystic medial necrosis in humans. VSMC apoptosis prevented outward remodelling associated with atherosclerosis, promoting marked vessel stenosis. These changes occurred in the absence of both local and systemic inflammation, or accumulation of apoptotic bodies. We conclude that VSMC apoptosis is sufficient to accelerate atherosclerosis, promote plaque calcification and medial degeneration, prevent expansive remodelling and promote stenosis in atherosclerosis.

Discussion focused on the role of macrophages and endothelial cells in the model; the phenomenon appears to be macrophage independent, and endothelial cell function depends on the ApoE background. George Tellides commented that in aortic graft transplants smooth muscle cells die, but grafts do not develop pathology.

Allogeneic Responses to Progenitor Cell-Derived Endothelium

Jordan S. Pober
Progenitor cells constitute a potential source of robust cells for use in tissue repair or tissue engineering. Jordan Pober described strategies to differentiate and expand human progenitors *in vitro* to generate large numbers of mature vascular cells. Such cells can be evaluated both for their capacities to form microvessels and for their ability to stimulate an alloimmune response. In comparisons of human umbilical cord blood-derived endothelial cells (HCBEC) with human umbilical vein endothelial cells (HUVEC) from the same donor, both cell types display a similar pattern of surface-expressed, secreted and intracellular molecules relevant for alloimmune responses. They are also comparable in their ability to stimulate cytokine production and proliferation by allogeneic T cell subsets in vitro and elicit comparable in vivo responses from adoptively transferred allogeneic T cells in immunodeficient mouse hosts. Preliminary studies are underway to compare vascular smooth muscle cells (VSMC) differentiated from bone marrow-derived mesenchymal stem cells with both VSMC isolated from human aorta and with pericytes isolated from human placenta.

In discussion the possibility that mesenchymal stem cells could be derived from peripheral blood was considered, and the adhesive properties of EC progenitor cells was confirmed, adhesion being the first step in isolation.

Session 2. Inflammation and Immunity

PLC signaling in macrophage apoptosis and atherogenesis

Dianqing (Dan) Wu
Phospholipase C (PLC) is known to be regulated by G protein-coupled ligands in macrophages. However, its roles in regulation of macrophage functions are not known. Dan Wu described work that studied macrophages from mice lacking PLC β2 PLC β3, and showed that PLCβ3 is the major functional PLCβ isoform in mouse macrophages. Although PLCβ3 deficiency did not affect migration, adhesion, or phagocytosis, it resulted in macrophage hypersensitivity to 25-OHC-, oxLDL-, and LPS-induced apoptosis. PLC β3 regulates this sensitivity via PKC-dependent-upregulation of Bcl-XL. The *in vivo* significance of PLC β signaling was examined using a mouse atherosclerosis model. PLC β3-deficiency is associated with an increase in macrophage apoptosis in the atherosclerotic lesions and a reduction in the lesion size. The study highlighted a novel function of PLC signaling in macrophages and identifies a potential target for treating atherosclerosis.

In discussion Dan Wu confirmed that although increased macrophage death was found in atherosclerotic plaques, there was no increase in apoptosis in circulating monocytes.

Mechanisms of vascular remodeling

George Tellides
George Tellides described cross sectional area of a vessel can be altered by both inward and outward vascular remodeling, and how luminal area is generally maintained in transplant coronary arteries during the initial transplant year despite significant intimal hyperplasia due to positive remodeling. George Tellides described his experimental model of human atherosclerosis involving transplantation human coronary artery into SCID mice, which receive human peripheral blood mononuclear cells and interferon-gamma. This model has been used to show that interferon-gamma plays a nonredundant role in mediating T cell-dependent outward vascular remodeling of allogeneic human coronary arteries, and that alloimmune-mediated vascular remodeling of human coronary artery grafts in immunodeficient mouse recipients is independent of preexisting atherosclerosis. In addition, IFN-γ elicits intimal expansion and outward vascular remodeling, and rapamycin inhibits IFN-γ-induced intimal expansion, but not outward remodeling.

Recent investigations have focused on the roles of iNOS, which is required for alloimmune-mediated intimal expansion, but not outward vascular remodeling; IL-17, which has no role in alloimmune-mediated vascular remodeling; extracellular matrix, in particular fibrillin-1 mutation, and elastin deficiency; and embryology, including genetic differences between ascending vs. descending aortic VSMCs. Studies using murine outflow ligation models of flow-mediated vascular remodeling have shown that
MyD88 is required for inward vascular remodeling, not medial thickening. Chronic flow reduction results in a transient leucocytic infiltrate. Ongoing studies are investigating the roles of vascular vs. leucocyte MyD88, using bone marrow chimeras, and conditional KO's, the link between vascular cytokine production and macrophage recruitment, and the link between flow disturbance and MyD88-dependent inflammation.

The potential role of IL12 was raised in discussion; MyD88 has roles in IL1 and IL18 signalling, but there is no evidence for involvement of IL12 from TLR null mice.

Innate immune mechanisms in emphysema

Patty Lee
Toll-like receptor 4 (TLR4) has been studied extensively in the context of pathogen challenges, yet its role in the context of non-infectious challenges in the lung and endothelium remain unclear. Patty Lee described work that has shown that *TLR4*$^{-/-}$ mice develop spontaneous emphysema after 2 months of age. Pulmonary emphysema is a major manifestation of chronic obstructive pulmonary disease (COPD) and is defined anatomically as the destruction of the distal lung parenchyma and enlargement of the airspaces. By 2020, COPD is predicted to become the third most common cause of death in the world due to the lack of specific therapies. Recently, the lung vasculature has been found to play an important role in the pathogenesis of emphysema. TLR4 deficiency induces what is believed to be a novel NADPH oxidase (Nox), Nox3, in lung endothelial cells resulting in excessive oxidant generation. Treatment of *TLR4*$^{-/-}$ mice or lung endothelial cells with Nox inhibitors or Nox3 siRNA reversed the phenotype. Generation of bone marrow chimeras has shown that TLR4 expression on non-hematopoetic cells is required to maintain normal lung architecture. Recent work has identified the induction of a poorly understood cathepsin, Cathepsin E, in *TLR4*$^{-/-}$ lungs. Previously, Cathepsin E had been only described in the context of immune cells and tumors. Patty Lee has shown that Cathepsin E expression is regulated by TLR4 and possesses elastolytic activity in the lung. Thus, the simultaneous inactivation of endogenous lung anti-proteases by Nox3-induced oxidants and the unopposed protease activity of Cathepsin E in *TLR4*$^{-/-}$ mice are important underlying mechanisms of emphysema. The process of dissecting the molecular mechanisms of emphysema in lung endothelial cells, has demonstrated for the first time the utility of intranasal RNA interference approaches in modulating lung endothelial responses. These findings have potentially significant implications for the therapeutic applications of RNAi targeting a variety of vascular lung diseases.

The effects of intrinsic and extrinsic factors were discussed. Specifically, alpha-1 antitrypsin levels are normal and smoking has no effect in the short term.

Akt1 deficiency – a model for coronary atherosclerosis

Bill Sessa
Bill Sessa described how Akt signaling is activated by various factors, including growth factors and shear stress, mediating various effects through PIP3. Three Akt isofroms are expressed in blood vessels; 1 > 2 > 3. Genetic loss of Akt1 reduces VEGF mediated angiogenesis and permeability, and loss of Akt1 retards arteriogenesis, reduces ischaemia induced EPC mobilization and markedly reduces basal phosphorylation of S1176 eNOS and NO production. Studies using Akt and Apo E null mice have shown that loss of Akt1 on and ApoE null background increases lesion progression in mice fed a Western diet, with exaggerated aortic

root, coronary ostia and mural vessel atherosclerosis. The coronary ostial lesion shows increased cellularity, with macrophage infiltration and lipid laden cells. Akt1 null / Apo E null mice undergo spontaneous death with micro-infarcts throughout the myocardium and angiographic evidence of stenosis and post-stenotic dilation of coronary arteries. Lipid profiles are identical in the two strains and LDL binding and uptake and foam cell formation are similar. Apoptosis of endothelial and smooth muscle cells is enhanced in ApoE / Akt1 null cells. Akt1 appears to be important for macrophage survival and necessary for endothelial cell survival in vessels; loss of Akt triggers early apoptosis *in vivo*. Bone marrow transfer of cells lacking ApoE / Akt1 into ApoE null hosts does not worsen atherosclerosis. Aortic extracts from Apo E / Akt1 null mice show increased inflammation and reduced eNOS phosphorylation. Overall, Akt1 is the first kinase associated with coronary lesions in mice. Additional work is needed to define how loss of Akt1 promotes coronary lesions.

Differences between Akt1 and Akt2 were discussed. It is possible that eNOS is more of an Akt1 substrate.

Autoantibody production in chronic allograft rejection

J Andrew Bradley

Andrew Bradley described a heterotopic mouse heart transplant model, using bm12 mice as donors and B6 as recipients. An important feature of this model is that the donor and recipient differ only minimally by 3 amino acids at the IA MHC class II antigen, and they are identical at Class I and also minor antigens. This limited antigen disparity, results in slow rejection of bm12 hearts, with approximately half the grafts still beating at day 100. To explore the mechanisms of rejection, transplanted hearts were harvested at day 50 for histological examination. Two striking features were found; bm12 allografts showed both myointimal proliferation and vascular fibrinoid necrosis, which are hallmark features of antibody-mediated rejection. The contribution of antibody to rejection was confirmed by positive complement C4d staining, which bound very strongly to allograft endothelium. In comparison, there was no staining in syngeneic grafts. Since the indirect alloantigen recognition pathway is thought to be most important for the development of chronic rejection, the hypothesis that indirectly activated CD4 T cells are responsible for giving help to autoreactive B cells was tested. A group of B6 animals were immunized with a synthetic bm12 allopeptide designed to incorporate the 3 differing amino acids. Because peptide was administered, T-cell activation was limited to the indirect pathway only. Animals were given bm12 hearts 2 weeks after priming, and a further group of B6 animals which were class II KO, CD4 T cell deficient, were transplanted. Class II KO, CD4 T cell deficient recipients did not reject their heart grafts, whereas peptide primed mice rejected grafts more rapidly than unprimed animals. To explore the possibility that CD4 T cells present in the donor heart migrate from the graft and recognize allogeneic MHC class II of recipient B cells, CD4T depleted mice were used as heart donors. Depletion of CD4 T cells from the donor resulted in abrogation of the autoantibody response. In conclusion, graft-vs-host recognition of alloMHC on recipient B cells by donor CD4 T cells may provide help for autoantibody production, which may contribute to chronic rejection.

Rituximab induction in renal transplantation

Chris Watson

Chris Watson described a randomised study comparing rituximab vs. dacluzimab induction in renal transplantation. The rationale is based on studies suggesting a key role for B cells in acute rejection. Biopsies from patients with acute renal transplant rejection that are indistinguishable on conventional histological analysis reveal extensive differences in microarray gene expression, which are associated with differences in immunologic and cellular features and clinical course. In particular, dense clusters of B cells are strongly associated with severe graft rejection, suggesting a pivotal role of infiltrating B cells in acute rejection.

To test the hypothesis that B cells may initiate rejection by acting as antigen presenting cells patients receiving renal transplants were randomised to receive rituximab or dacluzimab at induction. Composite endpoints were rejection, death, graft loss and loss to follow up. The study was stopped after recruitment of

13 patients, when analysis of results showed that rituximab caused profound B cell depletion, but acute rejection occurred in 5/6 rituximab patients but only 1/7 dacluzimab patients. Use of rituximab was associated with increased acute rejection and poorer renal function, but no excess infection.

Potential mechanisms were discussed, including disturbance of B cell mediated regulation of T cells and stimulation of cytokine release.

Evidence for Foxp3-mediated suppression of SOCS-3 transcription

Su Metcalfe
Su Metcalfe described ongoing work with Al Bothwell, which has arisen through the collaborative programme. Immune self tolerance is controlled by a subset of T lymphocytes that are regulatory (Treg) and epigenetically programmed to suppress auot-reactive immune effector cells in vivo. Treg require expression of Foxp3, a transcription factor that not only represses the IL2 gene promoter, but also sequesters key mediators of T cell signal transduction by complexing with cytoplasmic NFAT and NFkB. Su Metcalfe described collaborative studies that have shown that expression of Foxp3 is linked to two stem cell-related factors, namely leukaemia inhibitory factor (LIF) and axotrophin. Since both LIF and axotrophin each influence Foxp3, studies were designed to ask if reciprocal cross talk occurs, ie does Foxp3 in turn influence LIF or axotrophin? The effect of wt-Foxp3, versus mutant ΔE251-Foxp3 that lacks transcriptional activity, on transcript levels of axotrophin, LIF, and SOCS-3 (suppressor of cytokine signalling-3, a feedback inihibitor of LIF) in the JURKAT human T cell line. Unexpectedly, a 50 fold increase in SOCS-3 transcripts occurred in the ΔE251-Foxp3 cells, co-incident with a dramatic decrease in LIF transcription. This implies that, either directly or indirectly, transcription of SOCS-3 is negatively regulated by wt-Foxp3. Suppression of SOCS-3 by Foxp3 would support a model wherein Foxp3 promotes LIF signalling in Treg and is further evidence of reciprocity between Foxp3, LIF and axotrophin.

The potential role of IL6, which inhibits Treg and induces SOCS-3 was discussed

Signalling the diverse effects of TNF in the heart

John Bradley
Experimental evidence indicates that tumor necrosis factor (TNF) plays a key role in mediating diverse cardiovascular processes ranging from myocardial infarction, ischemia reperfusion injury, and congestive heart failure to transplant rejection, but anti-TNF therapoies have proved ineffective in cardiovascular disease. TNF exerts its effects by binding to two specific receptors: TNF receptor type 1 (TNFR1) and type 2 (TNFR2). Previous collaborative work has demonstrated that TNFRs are highly regulated in renal allograft rejection, when TNFR1 mediates cell death via apoptotic signaling kinases (Ask1) whilst TNFR2 mediates tubular cell proliferation via endothelial/epithelial non-receptor kinases (Etk). In normal human heart TNFR1 is constitutively expressed in myocytes and vascular endothelium. TNFR1 expression is lost in cardiac allograft rejection, whereas TNFR2 mRNA and portein is upregulated in myocytes, EC and cell infiltrates, where it co-localizes with Etkp and Ask1Thr845. Immunolocalisation by confocal and immunogold microscopy and immunoblotting of fractionated cells demonstrate both ASK1 and a ~50 kd protein recognised by anti-TNFR2 antibodies in mitochnodria, raising the possibility that TNF may activate mitochondrial ASK1 through interaction with a mitochondrial TNF receptor.

Anti-CD45RB induces Treg by both de novo conversion and promotion of homeostatic proliferation

David Rothstein
Transplant tolerance is often associated with regulatory T cells (Tregs) which arise in vivo over time in the setting of Ag-exposure. While various tolerogenic agents reportedly induce Treg, the mechanisms involved

in their induction remains unclear. Anti-CD45RB mAb is a potent tolerogenic agent that acts through the induction of CTLA-4 expression in the absence of CD4 cell depletion or activation. David Rothstein described work, which has shown that anti-CD45RB treatment of naïve mice also causes a 1.8-fold increase in both % and number of Foxp3+ CD25+ CTLA-4+ CD4 cells in spleen and LN by 7-10d. Thus, anti-CD45RB acutely induces Tregs in the absence of Ag exposure. To identify the mechanisms involved, we adoptively transferred CFSE-labeled congenic CD4 cells into naïve wt mice +/- anti-CD45RB therapy. On d10, anti-CD45RB increased the number of transferred cells expressing Foxp3+ in both proliferating and non-proliferating populations. 40% of cells newly expressing Foxp3 had not proliferated, and must have arisen by de novo conversion from Foxp3- cells. Yet, 60% of Foxp3+ cells induced by anti-CD45RB had proliferated. Few Foxp3- cells underwent proliferation regardless of anti-CD45RB. To more clearly define these events, unique Foxp3–red fluorescent protein reporter mice were utilized. Sorted highly pure Foxp3+ or Foxp3- CD4 cells from these mice were CFSE labeled and transferred into wt congenic mice. Transferred Foxp3- cells underwent little proliferation (10%). In striking contrast, 70% of Foxp3+ cells underwent “homeostatic proliferation” (HP) in these naïve wt hosts. Surprisingly, anti-CD45RB markedly enhanced HP of Foxp3+ cells resulting in a 10X increase in cell number. anti-CD45RB induced Foxp3 in transferred Foxp3- cells, and many of these cells proliferated (suggesting conversion and HP). These results have important implications: 1) Foxp3 cells undergo a previously unappreciated level of HP. 2) anti-CD45RB can induce *de novo* conversion of Treg in the absence of antigen. 3) anti-CD45RB also alters normal controls and significantly increases the number of Foxp3 cells undergoing HP. Understanding the signals involved in altered regulation of HP and *de novo* induction of Tregs may reveal new therapeutic targets for induction of Treg.

Regulation of VEGF effects in the lung

Jack Elias

Jack Elias described work, which has shown that targeted pulmonary expression of IL-13 causes a mononuclear and eosinophilic inflammatory response, mucus cell metaplasia, and airway fibrosis and obstruction, suggesting that IL-13 may be an important effector molecule at sites of Th2 inflammation. Using lung-targeted VEGF(165) transgenic mice, VEGF was found to be a mediator of pulmonary vascular and extravascular remodelling and inflammation that enhances antigen sensitization and is crucial in adaptive Th2 inflammation. VEGF induces pulmonary alterations via NO-dependent and -independent mechanisms with angiogenesis, edema, mucus metaplasia, airway hyperresponsiveness, lymphocyte accumulation, dendritic cell hyperplasia and S-nitrosoglutathione reductase stimulation being NO-dependent.
Jack Elias went on to describe a phenomenon whereby the lungs of VEGF transgenic mice get better when infected with Poly (I:C), a viral mimic, raising the possibility that the VEGF response is regulated by anti-viral responses. Treatment with Poly (I:C) inhibited VEGF-induced hyperplasia, permeability, bleeding and angiogenesis, and reduces eNOS and iNOS and Akt-1 phosphyorylation.

The possibility that the effect of Poly (I:C) could be mimicked by INF-γ was discussed; studies to explore this using INF-γ null mice are ongoing.

Mechanisms of anti-CD3 monoclonal antibody in patients with type 1 diabetes

Kevan Herold

Kevan Harold described studies to elucidate the mechanisms of immune regulation in patients with Type 1 diabetes who have been treated with an anti-CD3 monoclonal antibody. The overall goal is to identify the mechanisms of immune regulation so that the therapies for the disease can be improved by making them more potent, longer lasting, and specific. Kevan Harold described work that has looked at changes in the relative number of diabetes antigen specific T cells using Class I MHC tetramers. Studies to date indicate that while there is a decrease in the relative number of cells of some specificities, others increase after anti-CD3 mAb treatment. Ongoing studies will identify the function of these cells. In addition, previous studies have shown that treatment with anti-CD3 mAb results in an

increase in the number of circulating CD8+ T cells in the peripheral blood in the clinical responders. In vitro, the antibody induces a subpopulation of CD8+ T cells that function as regulatory T cells. They are not antigen specific since regulatory cells from one donor can inhibit responder cells from another. Interestingly, their induction with anti-CD3 monoclonal antibody is dependent on TNF-α.

The effect of genotype on PECAM-1 function

Craig Taylor
Platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31) plays an important role in leukocyte-endothelial cell adhesion and transmigration. Single nucleotide polymorphisms of PECAM-1 encoding amino acid substitutions at positions 98 (L/V), 536 (S/N) and 643 (R/G) occur in strong genetic linkage resulting in two common haplotypes (LSR and VNG). These PECAM-1 polymorphisms are associated with graft versus host disease following haematopoietic stem cell transplantation, and with cardiovascular disease, but whether they influence PECAM-1 function is unknown. Craig taylor described work that investigated the effect of homozygous and heterozygous expression of the PECAM-1 LSR and VNG genotypes on the adhesive interactions of peripheral blood monocytes and activated endothelial cell monolayers under shear stress forces in a flow based cell adhesion assay. There was no difference in monocyte adhesion between the two homozygous genotypes of PECAM-1 but when monocytes expressed both alleles in heterozygous form, firm adhesion of monocytes to endothelial cells was markedly increased. PECAM-1 polymorphism expressed in homozygous or heterozygous form by endothelial cells did not influence monocyte adhesion. This is the first demonstration that PECAM-1 genotype can alter the level of monocyte binding to endothelial cells and a demonstration that heterozygous expression of a polymorphic protein may lead to altered function.

Potential explanations were discussed, including the possibility that heterozygotes are less able to dimerise. A key issue to be taken forward in future experiments is to study transmigration, rather than adhesion, as a PECAM-1 dependent process.

Session 3. Atherothrombosis angiogenesis and vascularisation

Remote ischemic preconditioning

Michael Gaunt
Myocardial injury, as defined by cardiac troponin elevation, is a recognised cause of perioperative morbidity and mortality after abdominal aortic aneurysm (AAA) repair. Remote ischemic preconditioning is a phenomenon whereby brief periods of ischemia followed by reperfusion prior to prolonged ischemic events may provide protection from cellular injury in distant organs. Michael Gaunt described a randomized trial to investigate whether remote preconditioning could prevent myocardial injury in patients undergoing elective open AAA repair. In total 82 patients were randomized to AAA repair with remote preconditioning or conventional AAA repair. Myocardial injury was assessed by cardiac troponin I and myocardial infarction according to the revised ACC/AHA definition. Remote preconditioning significantly reduced the incidence of myocardial injury and myocardial infarction. Furthermore, in the remote preconditioning group the relative risk of renal impairment was lower. In conclusion, remote preconditioning reduces myocardial injury, myocardial infarction and postoperative renal impairment in patients undergoing elective open AAA repair.

Discussion focused on mechanism, and the possibility of performing proteomic analysis of plasma, neutrophils and platelets to try and elucidate mediators. The possibility that alterations in systemic vascular resistance might play a key role was also considered.

The role of platelet systems biology in unravelling the genetic architecture of atherothrombosis

Willem Ouwehand

Willen Ouwehand presented work on behalf of the Bloodomics and Wellcome Trust Case Control Consortia. Platelets play a pivotal role in atherothrombosis after coronary artery plaque rupture. The extent of response of platelets to such an event varies between individuals and in part determines whether thrombus formation is occlusive and myocardial infarction follows. The variation in platelet responsiveness is for a large extent genetically controlled. A comprehensive study of sequence variation, which modifies the platelet response to agonists is however lacking. The consortia set out to discover the regulatory nodes of platelet function by an integrated systems biology approach. The high density genotyping of 100 genes in a cohort of 506 individuals, in whom the platelet response to ADP and collagen-related peptide was determined, allowed the definition of the first set of regulatory nodes, demonstrating the effect size of the GP6 locus. Microarray studies on platelets from individuals with a so called “Extreme End” response phenotype provided further insight in key regulators of platelet function with a surprising over-representation of genes encoding proteins involved in transcription.

In addition, a recently completed genome-wide association study (GWAS) in 14,000 case samples from seven diseases and 3000 shared controls with the Affymetrix 500,000 SNPs genotyping array showed robust association signals for all seven diseases, with four regions observed in the MI cohort. These have been recently replicated in further genotyping studies. However, many weaker association signals were observed in the same MI genome scan and novel strategies are now required to separate the true signals from false ones. Integration of the information gleaned from platelet systems biology with the results of the GWAS has identified 16 genes for which sequence variation showed correlation with both the platelet functional response with an increased risk of MI. Some of these genes were hitherto unknown to be of relevance to platelet biology. Gene silencing in *Danio rerio* by morpholinos has been used to study the effect of the proteins encoded by these genes on thrombus formation.

In conclusion the integration of the results from a systems biology study with those of a GWAS enhances our understanding of the mechanisms underlying common conditions such as atherothrombosis and places and pointers to novel cellular mechanisms and pathways.

Vessel identity during vein graft adaptation

Alan Dardik

Since vein grafts are placed in elderly patients, Alan Dardik’s group has established a rat model of vein graft adaptation to determine the effect of aging on this process. Specifically, the regulation of Eph-B4, a venous marker, and Ephrin-B2, an arterial marker, was examined during vein graft adaptation in humans and aged rats. Eph-B4 transcripts and immunodetectable protein were downregulated in endothelial and smooth muscle cells of patent vein grafts in both humans and in aged animals, whereas Ephrin-B2 transcripts and protein were not strongly induced. Other markers of arterial identity, including dll4 and notch-4, were also not induced during vein graft adaptation. These results suggest that venous identity is preserved in the veins of aged animals, but is lost during adaptation to the arterial circulation; arterial markers are not induced. Therefore, markers of vessel identity are plastic in adults and their selective regulation may mediate vein graft adaptation to the arterial environment in aged animals and humans. To further define the mechanism of this process, a mouse model of vein graft adaptation has been established that recapitulates the human and rat.

Targeted imaging of angiogenesis

Albert J. Sinusas

Angiogenesis represents the formation of new capillaries by cellular outgrowth from existing microvessels and plays a critical role in the response to ischemia associated with peripheral arterial

disease and myocardial infarction. Imaging of angiogenesis would be valuable in risk stratification of patients with arterial occlusive disease. The progress in noninvasive imaging strategies to assess angiogenesis has been made possible with the availability of many technological advances which include dedicated hybrid SPECT-CT and PET-CT systems and agents targeted at molecular markers of the angiogenic process, involving both receptor-probe interactions and reporter gene technology. VEGF receptors could serve as targets for imaging of angiogenesis, by imparting physiologic information on hypoxic stress within viable tissue. The $\alpha v\beta 3$ integrin appears to be another important target for imaging of angiogenesis. Several radiolabeled ligands targeted at the $\alpha v\beta 3$ integrin have already proved useful for tracking angiogenesis in both experimental models of myocardial ischemia and hindlimb ischemia. This targeted molecular imaging should include imaging of both endothelial cell and non-endothelial cell markers, along with markers of the extracellular matrix and intercellular adhesions, including integrins, involved with the angiogenic process. The optimal application of any of the targeted imaging approaches will require registration with physiological images, and will play a crucial role for evaluation of therapeutic interventions to promote angiogenesis.

Models of Melanoma-Induced Vascular Hyperpermeability

Martin S. Kluger

Human melanoma cells secrete vascular endothelial growth factor-A (VEGF-A, and other vasoactive factors) that induce vascular hyperpermeability of blood macromolecules, leading to formation of a provisional extracellular matrix. It has been suggested that tumor cell and endothelial cell migration through such a provisional matrix can promote metastasis followed by angiogenesis that promotes tumor growth. A better understanding of the mechanisms and structures involved in the endothelial cell response to such factors could benefit therapies aimed at both cancer and ischemic heart disease. Martin Kluger described work, which has shown that human umbilical vein endothelial cells (HUVEC) forming leak-resistant monolayers in culture reduced their transendothelial electrical resistance (TEER; an index of barrier function) 30-40% in response to melanoma cell-conditioned medium in a manner largely inhibitable by humanized anti-VEGF antibody (Avastin, bevacizumab; two independent experiments). Depletion of VEGF from melanoma cell-conditioned culture supernatants also prevented loss of barrier function from human dermal microvascular cells (HDMEC), but not completely, suggesting production of other tumor-derived factors may also underlie the EC leak response. These observations have been valdiated in vivo by measuring a VEGF-dependent induction of microvessel permeability in mouse skin and in human skin grafts using a new human skin graft/SCID mouse model available for ongoing studies. Ongoing studies are now investigating the structural bases of these HDMEC and HUVEC responses, focusing on junctional adhesion molecules such as claudin-5 and VE-cadherin using biochemical and immunocytochemical assays.

Visualization of the Vasculature in Secondary and Tertiary Lymphoid Organs

Nancy Ruddle

Nancy Ruddle described how cytokines of the lymphotoxin/ tumor necrosis factor family (LT/TNF) contribute to inflammation, and secondary lymphoid organ (eg lymph node and spleen) development. They also contribute to chronic inflammation giving rise to “ectopic” or “tertiary lymphoid organs” through the process of lymphoid neogenesis. High endothelial venules (HEVs) and lymphatic vessels are key to the normal function or lymph nodes and are present in tertiary lymphoid organs. Nancy Ruddle described work to elucidate LT regulation of HEVs. The pCLASPER technique was used to isolate an HEV unique gene, HEC-6ST, to drive expression of reported genes, β-galactosidase and enhanced green fluorescent protein (GFP) in transgenic mice. Since, the transgenes recapitulate expression of the endogenous gene, *hec6stegfp* mice will be useful for in vivo analysis of HEVs.

Lymphatic vessels are being visualized by fluorescent microscopy and magnetic resonance imaging with dendrimers conjugated to fluorescein and gadolinium. New constructs will be prepared that use lymphatic vessel unique genes to drive expression of fluorescent proteins.

The potential for developing strategies to manipulate lymphatics as a treatment for lymphoedema was discussed.

Tissue engineering vascular grafts

Chris Breuer

Chris Breuer described the Total CavoPulmonary Connection (TCPC) Fontan operation to correct complex cardiac abnormalities in children. The operation requires a vascular graft between the inferior vena cava and pulmonary artery. The most commonly used graft is Gortex, but this has a tendency to thrombose. Tissue engineered grafts in which autologous cells are seeded onto a biodegradable scaffold provide an alternative that has been piloted in paediatric surgery in Japan. Chris Breuer described strategies to miniaturise biodegradable scaffolds to use in SCID mouse models. Use of PGA + PGA-P scaffolds creates functional grafts, which are limited by ischemic graft failure. Seeding with bone marrow derived mononuclear cells yields excellent graft patency without wall thickening. Seeded cells appear essential for patency, although occasionally (~ 10%) unseeded grafts function well. Host immune cells appear to play an integral role in the neo-tissue; seeded grafts show an inflammatory response around day 7. Experiments using MCP1 KO mice showed no significant difference in patency after 10 weeks.
The technology is being developed to allow drug delivery through nanotechnology and imaging using paramagnetic iron labeling of cells and MR.

The potential for mouse endothelium to grow into the graft was considered in discussion.

Endothelial dysfunction in kidney transplantation.

Jane Smith

As short term survival of organ transplants has improved, chronic problems have emerged as limitations to long term success. Late loss of transplants, often attributed to remodelling of blood vessels within the transplant (sometimes called graft arteriosclerosis or graft vasculopathy or chronic rejection) and excess cardiovascular mortality compared to the general population, are important factors following kidney transplantation. One of the earliest manifestations of vascular disease is endothelial dysfunction (ED), a failure to mediate vasodilatation in response to blood flow. Hypertension, hyperlipidaemia, diabetes and renal dysfunction, all potentially exacerbated by immunosuppressive agents, and systemic inflammation leading to excess production of pro-inflammatory molecules including TNF and interferon-gamma, may all be important causes of ED in kidney transplant recipients. Jane Smith described preliminary results of a Cambridge-Yale collaborative prospective longitudinal study of recipients of kidney transplant recipients. To date, 20 patients have been recruited and followed up at 3 months. Aortic pulse wave velocity was increased at 1 week post transplant but by 3 months had returned to pre transplant levels. In contrast, the augmentation index was significantly reduced at 3 months. Venous distensibility did not alter significantly at 3 months. Early findings from the endothelial function studies suggest that FMD had improved at 3 months. Biochemical markers are currently being processed. The results suggest that renal transplantation does not improve aortic stiffness, but may improve indices relating to wave reflections and endothelial function within 3 months post transplantation. However, in the longer term arterial properties may return to baseline levels, or indeed deteriorate compared to pre transplant levels.

Modeling the neurovascular niche: murine strain differences mimic the range of responses to chronic hypoxia in the premature newborn

Jo Madri

While preterm birth results in significant cognitive and motor disabilities, recent evidence suggests that there is variable recovery over time. One possibility that may explain this variable recovery entails variable neurogenic responses in the subventricular zone (SVZ) following the period of chronic hypoxia experienced by these neonates. Jo Madri described work, which has characterized the responses to chronic hypoxia of two mouse strains that represent a wide range of susceptibility to chronic hypoxia. C57BL/6 pups and neural progenitor cells (NPCs) derived from them exhibit a blunted response to hypoxic insult compared to CD-1 pups and NPCs. Specifically, C57BL/6 pups and NPCs exhibited blunted in vivo and in vitro proliferative and increased apoptotic responses to hypoxic insult. Additionally, C57BL/6 NPCs exhibited lower baseline levels and hypoxia-induced levels of selected transcription factors, growth factors and receptors (including HIF-1α, PHD2, BDNF, VEGF, SDF-1, TrkB, Nrp-1, CXCR4 and NO) that determine, in part, the responsiveness to chronic hypoxic insult compared to CD-1 pups and NPCs, providing insight into this important and timely problem in perinatology.

Publications and patents arising from the collaborative programme

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Patent No. PCT/GB2006/001313 Selective modulation of tumour necrosis factor receptors in therapy.

Appendix 3

Cardiovascular Disease in Williams-Beuren Syndrome

Cardiovascular Disease in Williams-Beuren Syndrome: Understanding Pathophysiology to Pioneer Treatment

May 7, 2008 – May 9, 2008
The Amistad Building, 10 Amistad Street, Room 112
Yale University School of Medicine
New Haven, CT 06519

Sponsored by The Vascular Biology and Therapeutics (VBT) Program of the Yale University School of Medicine and The Chloe's Quest Foundation

PROGRAM

Wednesday May 7, 2008

6:00pm – 7:05 pm	Cocktail reception
7:10 pm – 7:15pm	Welcome Remarks: William Sessa, Yale University
7:15 pm – 8:00 pm	***Keynote Speaker: George Yancopoulos*** **(Regeneron Inc*)*** "Growth factors and cytokines as therapeutic targets in vascular disease"

Thursday May 8, 2008

7:30am – 8:00am	Continental breakfast
8:00 am – 8:10am	Welcoming remarks Robert Alpern, M.D, Dean and Ensign Professor of Medicine
8:10 – 8:20	Opening remarks Marshall & Johanna Kiev Chloe's Quest Foundation
8:20am – 1:00pm	**Session 1: Overview of Williams-Beuren Syndrome** ***Moderator: Barbara Pober***
8:25am – 8:45am	Setting the stage: clinical overview and key research issues Barbara Pober (Harvard Medical School, Boston, MA)
9:00am – 9:40am	Molecular genetics of WBS and gene interactions/modifier genes Lucy Osborne (University of Toronto, Toronto, Canada) and Luis Perez-Jurado (Universitat Pompeu Fabra, Barcelona, Spain)
9:55am – 10:15am	Natural history and treatment of cardiovascular problems in WBS Leslie Smoot (Harvard Medical School, Boston, MA)
10:30am – 10:50am	Coffee break
10:50am – 11:10am	Vascular pathology of WBS Steven Schwartz (University of Washington, Seattle, WA)

11:25am – 11:45am	Elastin: normal biology and pathology in mouse models and in WBS Robert Mecham (Washington University School of Medicine, St Louis, MO)
12:00pm – 1:00pm	Lunch and continued discussion
1:00pm – 3:00pm	**Session 2: Biology of the Arterial Wall** ***Moderator: George Tellides***
1:10pm – 1:30pm	Development of the blood vessel wall Mark W Majesky (University of North Carolina, Chapel Hill, NC)
1:45pm – 2:05pm	Large vessel remodeling William Sessa (Yale School of Medicine, New Haven, CT)
2:20pm – 2:40pm	Pulmonary vascular development and remodeling; pulmonary hypertension Marlene Rabinovich (Stanford University, Stanford, CA)
2:55pm – 3:25pm	Coffee break
3:25pm – 6:00pm	**Session 3: Arterial Wall Matrix** ***Moderator: Robert Mecham***
3:35pm – 3:55pm	Matrix turnover Marie-Paule Jacob (Hopital Bichat, Paris, France)
4:10pm – 4:30pm	Arterial wall stiffness Ian Wilkinson (Addenbrooke's Hospital, Cambridge, England)
4:45pm – 5:05pm	Role of matrix in regulating growth factor availability Luisa Iruela-Arispe (University of California, Los Angeles, CA)
5:20pm – 6:00pm	Discussion

Friday May 9, 2008

7:45am – 8:20am	Continental breakfast
8:20am – 1:00pm	**Session 4: Potential Treatment Modalities for Cardiovascular Disease in WBS** ***Moderator: Frank Giordano***
8:30am – 8:50am	Targets for gene therapy Frank Giordano (Yale School of Medicine, New Haven, CT)
9:05am – 9:25am	Pharmacologic approaches to block vascular smooth muscle cell proliferation Bradford Berk (University of Rochester, Rochester, NY)
9:40am – 10:20am	Coffee break

10:20am – 10:40am	Pharmacotherapy for pulmonary hypertension Terence Trow (Yale School of Medicine, New Haven, CT)
10:55am – 11:40am	Wrap-up discussion to identify therapies, treatment targets, and funding opportunities Brian Annex (Duke University, Durham, NC) and Todd Rosengart (Stony Brook University Medical Center, Stony Brook, NY)
12:00pm – 1:00pm	Lunch

Invited Speakers and Expert Discussants:

Alpern, Robert	Yale University School of Medicine	Robert.alpern@yale.edu
Annex, Brian	Duke University	Annex001@mc.duke.edu
Berk, Bradford	University of Rochester	Bradford_berk@urmc.rochester.edu
Blaustein, David	Sutton Brook Capital	davidb@suttonbrook.com
Breuer, Christopher	Yale University School of Medicine	Chrostopher.breuer@yale.edu
Brueckner, Martina	Yale University School of Medicine	Martina.brueckner@yale.edu
Calderwood, David	Yale University School of Medicine	David.calderwood@yale.edu
Coller, Barry	Rockefeller University	Collerb@rockefeller.edu
Cotts, William	Williams Syndrome Association	Wcotts@nmff.org
Davis, Elaine	McGill University	Elaine.davis@mcgill.ca
Gelb, Bruce	Mt Sinai School of Medicine	Bruce.gelb@mssm.edu
Giordano, Frank	Yale School of Medicine	Frank.giordano@yale.edu
Iruela-Arispe, Luisa	UCLA	Arispe@mbi.ucla.edu
Jacob, Marie-Paule	Hopital Bichat	Jacob@bichat.inserm.fr
Kiev, Johanna	Chloe's Quest Foundation	jkiev@rockwoodcap.com
Kiev, Marshall	Chloe's Quest Foundation	Mkiev1@gmail.com
Kim, Richard	Yale University School of Medicine	Richard.kim@yale.edu
Kyriakides, Themis	Yale University School of Medicine	Themis.kyriakides@yale.edu
Levinson, Marty	Williams Syndrome Association	Drmarty6@aol.com
Liddicoat, John	Medtronics	John.liddicoat@medtronics.com
Majesky, Mark	UNC Chapel Hill	Mmajesky@med.unc.edu
Marion, Robert W.	Albert Einstein College of Medicine	rmarion@aecom.yu.edu
Mecham, Robert	Washington University in St Louis	Bmecham@wustl.edu
Mervis, Carolyn	University of Louisville	Cbmervis@louisville.edu
Milewicz, Dianna	University of Texas Medical School	Diana.m.milewicz@uth.tmc.edu
Monkaba, Terry	Williams Syndrome Association	monkaba@williams-syndrome.org
Morris, Colleen	University of Nevada	cmorris@medicine.nevada.edu
Morrow, Bernice	Albert Einstein College of Medicine	morrow@aecom.yu.edu
Niklason, Laura	Yale University School of Medicine	Laura.niklason@yale.edu
Osborne, Lucy	University of Toronto	Lucy.osborne@utoronto.ca
Perez-Jurado, Luis	Universitat Pompeu Fabra	Luis.perez@upf.edu
Pober, Barbara	Harvard Medical School	pober.barbara@mgh.harvard.edu
Pober, Jordan	Yale University School of Medicine	Jordan.pober@yale.edu
Rabinovich, Marlene	Stanford University	Marlener@stanford.edu
Rosengart, Todd	Stony Brook	Trosengart@notes.cc.sunysb.edu
Ruddle, Nancy	Yale University School of Medicine	Nancy.ruddle@yale.edu
Saltzman, Mark	Yale University School of Medicine	Mark.saltzman@yale.edu
Samanich, Joy	Children's Hospital at Montefiore	jsamanic@montefiore.org
Schwartz, Steven	University of Washington Seattle	Steves@u.washington.edu
Sessa, William	Yale School of Medicine	William.sessa@yale.edu
Smoot, Leslie	Harvard Medical School	Leslie.smoot@cardio.chboston.org
Su, Bing	Yale University School of Medicine	Bing.su@yale.edu
Tellides, George	Yale University School of Medicine	George.tellides@yale.edu
Trow, Terence	Yale School of Medicine	Terence.trow@yale.edu
Urban, Zsolt	Washington University in St Louis	Urban_z@kids.wustl.edu
Vignery, Agnes	Yale University School of Medicine	Agnes.vignery@yale.edu
Wilkinson, Ian	Addenbrooke's Hospital	Ibw20@cam.ac.uk
Yancopoulos, George	Regeneron Inc	Christina.wells@regeneron.com