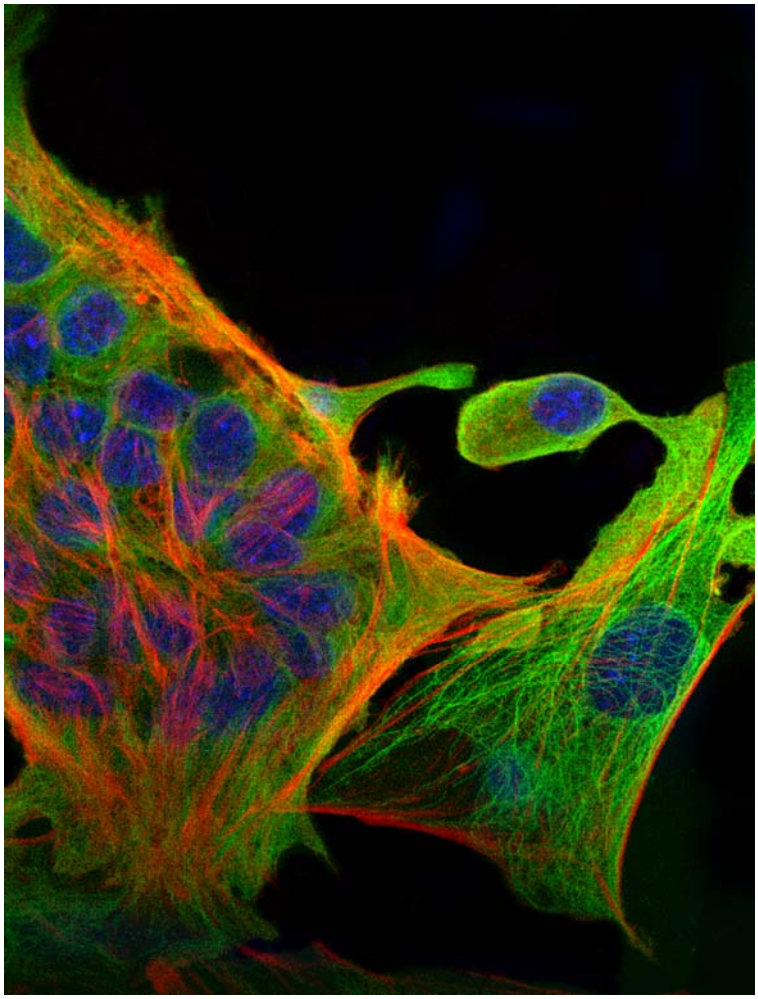
YALE UNIVERSITY SCHOOL OF MEDICINE

Vascular Biology and Therapeutics Program



Annual Report 2009-2010

Vascular biology
& therapeutics

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On the Cover:
On the Cover: Confocal analysis of primary murine magakaryocyte stained for polymerized actin (red phalloidin) and tubulin (green). DAPI stainign of nuclei (blue) reveals high cell ploidy.
Photograph courtesy Yuan Gao PhD, laboratory of Diane Krause MD PhD

Vascular Biology and Therapeutics Program

Annual Report 2009 – 2010

Message from the Director

The past academic year has been filled with exciting scientific developments. For example, several VBT faculty have published breakthrough papers in top notch journals such as Science, Developmental Cell, EMBO J, Science Signaling, Cell Metabolism, PNAS, J Experimental Medicine and J Clinical Investigation. This is a testimony to the excellence and high quality of work here at Yale within the VBT program. Financially, VBT is in good shape and we are continually trying to develop corporate relationships to allow for expansion of research opportunities.

Table I. VBT Steering Committee

Jeffrey R. Bender, M.D., Professor of Internal Medicine (Cardiovascular Medicine) and Immunobiology
Alfred L.M. Bothwell, Ph.D., Professor of Immunobiology
Jack A. Elias, M.D., Waldmar von Zedwitz Professor of Medicine, Professor of Immunobiology, Chairman of Internal Medicine
Frank Giordano, M.D., Associate Professor of Internal Medicine (Cardiology)
Joseph A. Madri, M.D., Ph.D., Professor of Pathology and Molecular, Cellular and Developmental Biology
Laura Niklason, M.D., Ph.D., Professor and Vice Chair of Anesthesia and Professor 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 Vascular Biology & Therapeutics, Professor and Vice Chair of Pharmacology
Michael Simons, M.D., RW Berliner Professor of Medicine & Cell Biology, Chief Section of Cardiovascular Medicine
George Tellides, M.D., Ph.D., Professor of Surgery (Cardiothoracic) and Chief, Cardiothoracic Surgery

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

Ms. Carol Muzzey serves as the Program Manager and is assisted by Ms. Diane Strumpf. The program is served by the Central Administration Business Office.

Program Faculty Membership

All faculties at Yale with a significant interest in vascular biology and/or therapeutics are eligible to join VBT. VBT members in academic year 2009-2010 are:

Table 2 VBT Membership

Jeffrey R. Bender, M.D., Professor of Internal Medicine (Cardiovascular Medicine) and Immunobiology	Joseph A. Madri, M.D., Ph.D., Professor of Pathology and Molecular, Cellular and Developmental Biology
Anton Bennett, Ph.D., Associate Professor of Pharmacology	Kathleen A. Martin, Ph.D., Associate Professor of Medicine (Cardiovascular Medicine) and Pharmacology
Alfred L.M. Bothwell, Ph.D., Professor of Immunobiology	Laura R. Ment, M.D., Professor of Pediatrics (Neurology)
Christopher Breuer, M.D., Associate Professor of Surgery (Pediatrics)	Wang Min, Ph.D., Associate Professor with Tenure, Pathology
David Calderwood, Ph.D., Associate Professor of Pharmacology	Laura Niklason, M.D., Ph.D., Professor & Vice Chair of Anesthesia; Professor Biomedical Engineering
Hyung J Chun, M.D., Assistant Professor of Medicine, Cardiovascular Medicine	Jordan S. Pober, M.D., Ph.D., Vice Chair, Section of Human and Translational Immunology, Department of Immunobiology, Professor of Immunobiology, Pathology and Dermatology
Alan Dardik, Ph.D., M.D., Associate Professor of Surgery (Vascular Surgery)	David M. Rothstein, M.D., Associate Professor of Internal Medicine (Nephrology)
Jack A. Elias, M.D., Waldmar von Zedwitz Professor of Medicine, Professor of Immunobiology, Chairman of Internal Medicine	Nancy H. Ruddle, Ph.D., John Rodman Paul Professor Emerita & Senior Research Scientist, Departments of Epidemiology and Public Health and Immunobiology
Tarek Fahmy, Ph.D., Associate Professor of Biomedical Engineering and Chemical Engineering	Kerry S. Russell, M.D., Ph.D., Associate Professor of Medicine, Cardiovascular Medicine
Richard Flavell, Ph.D., FRS, Sterling Professor and Chairman of Immunobiology, Investigator of Howard Hughes Medical Institute	Mehran M. Sadeghi, M.D., Associate Research Scientist of Internal Medicine (Cardiovascular Medicine)
Arnar Geirsson, M.D., Assistant Professor of Surgery	W. Mark Saltzman, Ph.D., Goizueta Foundation Professor of Chemical and Biomedical Engineering
Frank J. Giordano, M.D., Associate Professor Internal Medicine (Cardiovascular Medicine)	William C. Sessa, Ph.D., Director Vascular Biology & Therapeutics and Professor and Vice Chair of Pharmacology
Daniel R. Goldstein, M.D., Associate Professor Internal Medicine (Cardiovascular Medicine)	Michael Simons, M.D., RW Berliner Professor of Medicine & Cell Biology, Chief Section of Cardiovascular Medicine
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
John Hwa, M.D., Ph.D., Associate Professor of Medicine, Cardiovascular Medicine	Jeffrey Sklar, M.D., Ph.D., Professor of Pathology and Laboratory Medicine
Richard W. Kim, M.D., Assistant Professor of Surgery	Edward Snyder, M.D., Professor Laboratory Medicine, Director, Apheresis/Cell Processing VBT Core Facility
Martin Kluger, Ph.D., Research Scientist, Department of Dermatology	Bing Su, Ph.D., Associate Professor of Immunobiology
Diane Krause, M.D., Ph.D., Professor of Laboratory Medicine	George Tellides, M.D., Ph.D., Professor of Surgery (Cardiothoracic)
Sanjay Kulkarni, M.D., Associate Professor of Surgery (Transplantation & Immunology)	Agnes Vignery, DDS, Ph.D., Associate Professor of Orthopaedics and Rehabilitation
Themis Kyriakides, Ph.D., Associate Professor of Pathology and Biomedical Engineering	Dianqing (Dan) Wu, Ph.D., Professor of Pharmacology
Patty J. Lee, M.D., Associate Professor of Internal Medicine, Pulmonary & Critical Care	

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 2009-2010 Seminar Series
SEPTEMBER 2009
Eli Keshet Ph.D., Professor, Hebrew University, Hadassah Medical Center, Woll Brother and Sisters, Chair for Cardiovascular Research, "VEGF: more than just an angiogenic factor"
OCTOBER 2009
Karen Hirschi, Ph.D., Professor, Department Pediatrics, Adjunct in Departments Molecular &Cellular Biology & Molecular Physics & Biophysics, BCM and Department of Bioengineering, Rice University, "Vascular Morphogenesis and Regeneration"
Ken Bloch, M.D., William T.G. Morton Prof. of Anesthesia,Prof. of Medicine, Anesthesia Center for Critical Care Research, "Understanding BMP signal transduction using novel small molecule BMP inhibitors"
Maria Rupnick, M.D., Instructor in Medicine, Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School, "Emerging Lessons in Cancer, Cardiovascular Homeostasis & Metebolism"
NOVEMBER 2009
Dashzeveg Bayasihan, Ph.D., Assistant Professor, Center for Regenerative Medicine and Skeletal Development, Department of Reconstructive Sciences, University of Connecticut Health Center, "Role of Williams Syndrome Associated TFII-I Genes in Development"
David Harrison, MD, Bernard Marcus Prof. of Medicine, Director of Cardiology, Emory University, "Regulation of endothelial cell tetrahydrobiopterin"
Xuri Li, Chief, Unit on Retinal Vascular Neurobiology, NIH/NEI, Maryland, "VEGF-B in Vascular biology: New insights of an old VEGF family member"
Richard N. Kitsis, M.D., Professor of Medicine & Cell Biology, The Gerald & Myra Dorros Chair in Cardiovascular Diseases, Director, Einstein-Montefiore Center for Cardiovascular Research, "Towards a Unified Model of Cell Death and its Role in Human Disease"
DECEMBER 2009
Tarek Fahmy, Ph.D., Associate Professor of Biomedical Engineering & Chemical Engineering, Yale University, "Biomimetic approaches to modulating immuno responses with biodegradable polymers"
JANUARY 2010
Michael Paidas, M.D., Associate Professor & Co-Director Yale Women & Children's Center for Blood Disorder, Co-Director, National Hemophilia Foundation-Baxter Clinical Fellowship at Yale Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, "Placenta mediated complications; maternal health consequences and recurrence risk"
FEBRUARY 2010
Paul Pilch, Ph.D., Professor of Biochemistry and Medicine, Boston University Medical Center, "Caveolae and adipocyte membrane function"
Dr. Charles E. Murry, M.D., Ph.D., Arra & Eva Woods Professor of Pathology & Bioengineering, Director, Center for Cardiovascular Biology, Co-Director, Institute for Stem Cell and Regenerative Medicine, University of Washington, "Stem Cells and Cardiovascular Repair"
Charles C. Hong, Assistant Professor of Cardiovascular Medicine, Co-director, Center for Inherited Heart Disease, Vanderbilt University School of Medicine, "Chemical Genetics of Vertebrate Development & Stem Cell Differentiation"
MARCH 2010
Murat Gunel, M.D., Associate Professor of Neurosurgery, Yale University School of Medicine, "Aneurysms and Cavernomas of the brain: tale of two vascular disorders"
Carol Parent, Ph.D., Laboratory of Cellular & Molecular Biology, Senior Investigator, National Cancer Institute, National Institute of Health, "cAMP and TORC2: two highly conserved signals that regulate chemotaxis in Dictyostelium and neutrophils"
Suk-Won Jin, Ph.D., Assistant Professor, Department of Cell & Molecular Physiology, Carolina Cardiovascular Biology Center, Member Curriculums in Genetics and Molecular Biology, University of North Carolina at Chapel Hill, "BMP signaling functions as a selective proangiogenic factor for venous endothelial cells during developmental vascular morphogenesis"
Christophe Depre, M.D., Ph.D., Associate Professor of Cell Biology and Molecular Medicine, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, "Pre-emptive conditioning of the ischemic heart"
APRIL 2010
Jesse Rinehart. Ph.D., Associate Research Scientist Genetics, Yale University School of Medicine, "New mechanisms controlling cell volume, neuronal excitability, & blood pressure revealed via quantitative phosphoproteomics"
Michael Parmacek, M.D., Herbert C. Rorer Professor of Medical Sciences, Director, Penn Cardiovascular Institute, Chief, Division of Cardiovascular Medicine, University of Pennsylvania School of Medicine, "Myocardin a Coactivator Poised at the Precipice of Death or Differentiation"
Arya Mani, M.D., Associate Professor of Medicine and Cardiology, Section of Cardiovascular Medicine, Yale University, "Coronary artery disease and its metabolic risk factors linked by a single mutation"
MAY 2010
Dan Berkowitz, M.D., Professor Department of Anesthesiology and Critical Care Medicine, Professor of Biomedical Engineering, John Hopkins University, "Arginase and Atherosclerosis: Location[3]"
Cecilia Giachelli, Ph.D., Professor Biomaterials & Tissue Engineering, Molecular Engineering, University of Washington, "Mechanisms of Vascular Calcification"
Giuseppo Cirino, Ph.D., FBPharmacols, Professor Pharmacology, Dean Faculty of Pharmacology, "Is hydrogen sulphide an endogenous inhibitor of phosphodiesterase?"
JUNE 2010
Satish N. Nadig, M.D., PH.D., Administrative Chief Surgical Resident, Harvard University, "Immunoregulation of Transplant Arteriosclerosis"

Retreat

The annual retreat continues to be an extremely popular activity, bringing together over one hundred fifty scientists from the laboratories of VBT faculty members. This past year, the retreat was held on November 7, 2009 at the Grace Murray Hopper Auditorium, West Campus. The retreat continued the poster session competition with prizes for the best posters by a graduate student and by a post-doctoral fellow.

The Keynote Address at this years retreat was William Aird, M.D., Beth Israel Deaconess Medical Center, Harvard University.

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

Yale-Cambridge Program in Cardiovascular Disease

The research alliance with Cambridge has continued as an important activity, with 24 faculty from Cambridge visiting Yale in September 2009 for a two day scientific meeting. The program for this retreat is listed in Appendix 2. A visit by Yale members to Cambridge is scheduled for September 2010.

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

1. Overall Goal(s) of the Research Program of the Laboratory:
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.

2. Specific Research Accomplishments in the last 12 months: (1) through proteomics analysis, we determined that leukocyte integrin engagement leads to a series of kinase activation events, resulting in the dissociation of 2 nuclear proteins, HuR and RNP-C, ultimately affecting stabilization of mRNA transcripts encoding pro-inflammatory cytokines and angiogenic factors; (2) macrophage-specific deletion of HuR results in impaired neovascular responses in the murine hindlimb ischemia model; (3) through TIRF microscopy, we have documented that an estrogen receptor α splice form, ER46, has transmembrane spanning and ectodomains in endothelial cells and; (4) through the generation of ceramide, free fatty acids promote a state of profound golgi dysfunction, leading to impaired endothelial responses to angiogenic growth factors. These findings have major mechanistic implications in allograft rejection, atherosclerosis and angiogenesis, all major clinical targets for the VBT Program.

3. Publications:

Kim KH, Bender JR. Membrane-initiated actions of estrogen on the endothelium. Mol Cell Endocrinol. 2009 Sep 24;308(1-2):3-8. Epub 2009 Apr 9. Review. PubMed PMID: 19549586; PubMed Central PMCID: PMC2701909.

Guo X, Nie L, Esmailzadeh L, Zhang J, Bender JR, Sadeghi MM. Endothelial and smooth muscle-derived neuropilin-like protein regulates platelet-derived growth factor signaling in human vascular smooth muscle cells by modulating receptor ubiquitination. J Biol Chem. 2009 Oct 23;284(43):29376-82. Epub 2009 Aug 19. PubMed PMID: 19696027; PubMed Central PMCID: PMC2785569.

Zhang J, Silva T, Yarovinsky T, Manes TD, Tavakoli S, Nie L, Tellides G, Pober JS, Bender JR, Sadeghi MM. VEGF blockade inhibits lymphocyte recruitment and ameliorates immune-mediated vascular remodeling. Circ Res. 2010 Aug 6;107(3):408-17. Epub 2010 Jun 10. PubMed PMID: 20538685; PubMed Central PMCID: PMC2929975.

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

1. Overall Goal (s) of the Research Program of the Laboratory:
The broad research interest of this laboratory is to define the molecular mechanisms, physiological and pathophysiological roles of the protein tyrosine phosphatase (PTP) family of enzymes. PTPs are involved in controlling cellular signaling pathways that are regulated by tyrosine phosphorylation. We are currently pursuing the physiological and pathophysiological roles of the dual-specificity phosphatases known as the MAP kinase phosphatases (MKPs) which inactivate the MAP kinases. We have discovered that the MKPs play essential roles in a number of physiological processes that include metabolism, innate immunity, skeletal muscle regeneration and the maintenance of bone mass.

2. Specific Research Accomplishments in the last 12 months:
During the course of the year we have discovered that MKP-1 plays an essential roles in MAP kinase-dependent signaling to genes involved in mitochondrial biogenesis, fatty acid metabolism and lipid droplet formation. We have also found that MKP-1 plays an important role in osteoclast activation to control RANK-L-dependent signaling in the maintenance of bone mass. Finally, we showed that MKP-1 exhibits spatio-temporal regulation of JNK activity to control cytoskeletal function and axonal guidance.

3. Significance of Key Findings Relevant for the Mission of VBT:
Our findings that MKP-1 regulates a MAP kinase-dependent pathway that couples to the control of lipid homeostasis may provide further insight into the molecular mechanisms of diseases of the vasculature, such as atherosclerosis.

4. Publications:

Soulsby, M. and Bennett, A.M. (2009) Physiological Signaling Specificity by Protein Tyrosine Phosphatases, Physiology, 24:281-9.

Roth, R.J., Le, A., Zhang, L., Samuel, V.T., Shulman, J., and Bennett, A.M., (2009) MAPK phosphatase-1 facilitates the loss of oxidative myofibers associated with obesity in mice. J. Clin. Invest., 119:3817-29.

Carlson, J., Bennett, A.M. and Vignery, A. (2009) Deletion of mitogen activated protein kinase phosphatase-1 (MKP-1) modifies the response to mechanical bone marrow ablation in a mouse model, Comparative Medicine, 59:221-6.

Carlson, J, Cui, W., Zhang, Q., Mercan, F., and Vignery, A.[§] and Bennett, A. M.[§], (2009) Role of MKP-1 in osteoclasts and bone homeostasis, Amer. J. Pathol. 175:1564-1573.

Hao, S., Boadu, E., Mercan, F., Le, A.M., Roth, R.J., Zhang, L., and Bennett, A.M. (2010) MAP Kinase Phosphatase-1 Deficiency Impairs Skeletal Muscle Regeneration and Exacerbates Duchenne Muscular Dystrophy, FASEB J., 24:2985-97.

Roth, R, and Bennett, A.M. (2010) MAP kinase phosphatase-1 - a new player at the nexus between sarcopenia and metabolic disease, Aging, 2: 170-166.

Roth, R. and Bennett, A.M. (2010) MKP-1 as a candidate for Nuclear-targeted MAP kinase therapies in Obesity, Expert Opinion On Therapeutic Targets, in press.

Jeanneteau, F., Deinhardt, K., Miyoshi, G., Bennett, A.M., and Chao, M.V. (2010) The MAP kinase phosphatase, MKP-1, regulates BDNF-induced axonal guidance, Nature Neuroscience, in press.

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

1. Overall Goals of our Research Program:
The research goals of the lab are to characterize the development and function of regulatory T cells and characterize mechanisms that affect autoimmunity, inflammation, cancer 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. This includes work to develop a model of type 1 diabetes in humanized mice.

2. Specific accomplishments in the 12 months:
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), and ciglitazone and pioglitazone on vascular remodeling of human artery induced by alloreactive PBMC and the IFN-γ model. The data indicate that 15-d-PGJ_2 and pioglitazone significantly inhibit human GA in our *in vivo* human arterial graft model in immunodeficient mice.
Type 1 diabetes model: To develop a model we need to generate human T cell lines and clones derived from patients. We are doing this for several well characterized CD4 and CD8 T cell epitopes and then immortalizing the cells with an hTERT retrovirus. Currently mice that are transgenic for HLA-A2 and HLA-DR4 on the NOD.scid γc KO background are being injected with human lymphocytes. In addition, we are attempting 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.
Inflammation and Colon Cancer: The APC/Min mouse is a highly studied model of intestinal tumorigenesis. During the last year we have shown that APC/Min mice that are deficient in the proinflammatory cytokine IL-17A have a 90% reduction in small intestinal polyps. We hope to define both the source of the IL-17A that is critical as well as the cell type that is the target of this cytokine. Pilot studies have been initiated to develop a model of breast cancer in humanized mice.

3. Significance of Key Findings Relevant for the Mission of VBT:
Our studies are to characterize the immunologic properties of human lymphocytes in immunodeficient mice to understand vascular disease, autoimmune disease and cancer.

4. Publications:
Lepus, C.M., Gibson, T., Kawikova, I., Gerber, S.A., Kirlikes-Smith, N., Szczepanik, M., Bothwell, A.L.M., Pober, J.S. and Harding, M.J. (2009). Characterization of human fetal liver, umbilical cord blood, and adult hematopoietic stem cell ingraftment in C.B.17-scid/bg, Balb/c-Rag2-/-γc-/- and NOD-scid/γc-/- immunodeficient mice. Human Immunol. 70:790-802. PMID: 19524633.

Choi, J.-M., Shin, J.-H., Sohn, M.-H., Harding, M.J., Park, J.-H., Tobiasova, Z., Kim, D.-Y., Maher, S.E., Chae, W.-J., Park, S.-H., Lee, C.-G., Lee, S.-K. and Bothwell, A.L.M. Cell permeable Foxp3 protein alleviates autoimmune disease associated with IBD and allergic airway inflammation. Submitted.

Tobiasova, Z., Zhang, L., Yi, T., Qin, L., Kulkarni, S., Rodriguez, F.C., Choi, J.-M., Pober, J.S., Tellides, G., Kawikova, I., and Bothwell, A.L.M. PPARγ ligands prevent *in vivo* development of graft arteriosclerosis of human artery by alloreactive T cells. submitted.

Christopher Breuer, M.D.
Associate Professor of Surgery

1. Overall Goal (s) of the Research Program of the Laboratory:
The overall goal of my research program is to use tissue-engineering techniques to create autologous cardiovascular neotissues that can be used for reconstructive surgical applications such as bypass grafting.

2. Specific Research Accomplishments in the last 12 months:
In the past year I have received my first R01 research grant entitled, " Investigating the cellular and molecular mechanisms of neotissue formation in tissue engineered vascular grafts"

3. Significance of Key Findings Relevant for the Mission of VBT:
The key finding related to the mission of VBT has been our demonstration that neovessel formation is critically dependent on the host inflammatory response.

4. Publications:

Mirensky TL, Hibino N, Sawh-Martinez RF, Yi T, Villalona G, Shinoka T, Breuer CK. Tissue-engineered vascular grafts: does cell seeding matter? J Pediatr Surg. 2010 Jun;45(6):1299-305. PubMed PMID: 20620335.

Roh JD, Sawh-Martinez R, Brennan MP, Jay SM, Devine L, Rao DA, Yi T, Mirensky TL, Nalbandian A, Udelsman B, Hibino N, Shinoka T, Saltzman WM, Snyder E, Kyriakides TR, Pober JS, Breuer CK. Tissue-engineered vascular grafts transform into mature blood vessels via an inflammation-mediated process of vascular remodeling. Proc Natl Acad Sci U S A. 2010 Mar 9;107(10):4669-74. Epub 2010 Mar 5. PubMed PMID: 20207947; PubMed Central PMCID: PMC2842056.

Villalona GA, Udelsman B, Duncan DR, McGillicuddy E, Sawh-Martinez RF, Hibino N, Painter C, Mirensky T, Erickson B, Shinoka T, Breuer CK. Cell-seeding techniques in vascular tissue engineering. Tissue Eng Part B Rev. 2010 Jun;16(3):341-50. Review. PubMed PMID: 20085439.

Mirensky TL, Nelson GN, Brennan MP, Roh JD, Hibino N, Yi T, Shinoka T, Breuer CK. Tissue-engineered arterial grafts: long-term results after implantation in a small animal model. J Pediatr Surg. 2009 Jun;44(6):1127-32; discussion 1132-3.PubMed PMID: 19524728.

Gui L, Chan SA, Breuer CK, Niklason LE. Novel utilization of serum in tissue decellularization. Tissue Eng Part C Methods. 2010 Apr;16(2):173-84. PubMed PMID: 19419244.

Gui L, Muto A, Chan SA, Breuer CK, Niklason LE. Development of decellularized human umbilical arteries as small-diameter vascular grafts. Tissue Eng Part A. 2009 Sep;15(9):2665-76. PubMed PMID: 19207043; PubMed Central PMCID: PMC2735599.

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

1. Overall Goal (s) of the Research Program of the Laboratory:
The goal of my lab is to understand integrin adhesion receptors and their links to the actin cytoskeleton. Control of cell adhesion, spreading and migration requires the integrin cytoplasmic tails. These short tails bind intracellular ligands and connect integrins to signaling and cytoskeletal networks. Thus, integrins provide a link for the bidirectional transmission of mechanical force and biochemical signals across the plasma membrane.

2. Specific Research Accomplishments in the last 12 months:
Our investigations into integrin activation have revealed a central role for the cytoskeletal protein talin and ongoing work indicates that additional co-activators cooperate with talin to activate subsets of integrins. The first class of co-activators identified are the kindlins and our work and that of others has shown important roles for the kindlin family of integrin β tail binding proteins. However, the molecular basis for this effect is unclear. We have revealed similarities between talin and kindlins at a structural and functional level and in collaboration with structural biologists we seek a molecular understanding for their activities. In related work we have identified other co-activators and seek to characterize their activities and mechanisms of action.

Continuing our investigation into the roles of filamins in integrin signaling and mechanosensing we find that filamins are important for cell spreading and initiation of migration and integrin-filamin interactions are important for matrix density sensing during epithelial morphogenesis. In ongoing work we are characterizing the role of ASB2, an E3 ubiquitin ligase specificity subunit that targets filamins for proteosomal degradation.

3. Significance of Key Findings Relevant for the Mission of VBT:
We seek to understand the molecular basis of integrin activation and signaling which is critical for platelet aggregation, angiogenesis and leukocyte trafficking. We also seek to better understand the links between integrins and the actin cytoskeleton, with a particular interest in filamins, and how they are involved in morphogenesis, differentiation and mechanosensing.

4. Publications:
Gehler S., Baldassarre M., Lad Y., Leight J. L., Wozniak M.A., Riching K.M., Eliceiri K.W., Weaver V.M., Calderwood D.A. and Keely P.J. (2009) Filamin A-β1 Integrin Complex Tunes Epithelial Cell Response to Matrix Tension. Mol. Biol. Cell. 20: 3224-38

Goult B.T., Bouaouina M., Harburger D.S., Bate N., Patel B., Anthis N.J., Campbell I.D., Calderwood D.A., Barsukov, I.L., Roberts, G.C., and Critchley, D.R. (2009) The structure of the N-terminus of kindlin-1: a domain important for αIIbβ3 integrin activation J. Mol. Biol. 394: 944-56

Baldassarre M., Razinia Z., Burande C.F., Lamsoul I., Lutz P.G., and Calderwood D.A. (2009) Filamins regulate cell spreading and initiation of cell migration. PLoS One, 4: e7830

Chiswell B.P., Stiegler A.L., Razinia Z., Nalibotski E., Boggon T.J., and Calderwood D. A. (2009) Structural basis of competition between PINCH1 and PINCH2 for binding to the ankyrin repeat domain of integrin-linked kinase. J Struct Biol. 170, 157-63.

Goult B.T, Bouaouina M., Elliott P.R., Bate N, Patel B, Gingras AR, Grossmann JG, Roberts GC, Calderwood D.A., Critchley DR, and Barsukov IL. (2010) Structure of a double ubiquitin-like domain in the talin head: a role in integrin activation. EMBO J. 29(6):1069-80.

Bouaouina M., Harburger D.S., and Calderwood D.A. (In press) Talin and signaling through integrins. Methods in Mol. Biol.

Hyung J. Chun, M.D.
Assistant Professor of Medicine, Cardiovascular Medicine

1. **Overall Goal (s) of the Research Program:**
The main interest of my lab is to characterize the signaling pathways important in vascular homeostasis and disease processes such as atherosclerosis and pulmonary arterial hypertension. Our recent characterization of the apelin-APJ GPCR signaling pathway has shed new light on crosstalk and downstream signaling mechanisms that are important in animal disease models, and we will continue to elucidate how this and other related signal pathways are relevant in both animal models and in the clinical setting of vascular diseases.
We recently found that augmenting apelin signaling pathway is highly protective against a rodent model of atherosclerosis. This effect was secondary to direct antagonism of the angiotensin II signaling pathway, as well as in upregulating nitric oxide production. In addition, we found that apelin signaling also plays an important role in pulmonary arterial hypertension. Apelin deficient mice demonstrated greater susceptibility to hypoxia induced pulmonary hypertension. This was in the context of apelin deficient mice having decreased nitric oxide levels, secondary to decreased expression of endothelial nitric oxide synthase.
Currently ongoing work in the lab is focused on further characterization of the mechanisms of apelin signaling in the disease models where its signaling appears to be dysregulated. We will continue to pursue the characterization of the role of apelin signaling in pulmonary arterial hypertension. We've recently found that apelin deficient mice have defective nitric oxide signaling secondary to decreased eNOS expression. We are actively working on defining the intermediaries that regulate eNOS expression through which apelin exerts its effects in endothelial cells.
In addition, via the use of microRNA microarray screening assays, we plan on identifying and characterizing additional pathways that play relevant roles in both of these disease processes, both as a downstream target of the apelin-APJ signaling axis, and independently.

2. **Specific Research Accomplishments in the last 12 months:**
We have identified AMP activated kinase and KLF2 as downstream targets of apelin-APJ signaling in the pulmonary artery endothelial cells. We have also identified five microRNAs whose expression is highly regulated by apelin-APJ signaling and are presently characterizing their role in endothelial homeostasis.

3. **Significance of Key Findings Relevant for the Mission of VBT:**
The key findings relevant to the mission of VBT is the characterization of downstream targets of apelin-APJ signaling pathway that are critical for maintenance of endothelial/vascular homeostasis.

4. **Publications:**

Charo, D.N., Ho, M., Fajardo, G., Kawana, M., Kundu, R.K., Sheikh, A.Y., Finsterbach, T.P., Leeper, N.J., Ernst, K.V., Chen, M.M., Ho, Y.D., Chun, H.J., Bernstein, D., Ashley, E.A., Quertermous, T., Endogenous Regulation of Cardiovascular Function by Apelin-APJ, *AJP: Heart and Circulatory Physiology;* 297(5):H1904-13, 2009

Kojima Y., Kundu R.K., Cox C.M., Leeper N.J., Anderson J.A., Chun H.J., Ali Z.A., Ashley E.A., Krieg P.A., Quertermous T., Upregulation of the apelin-APJ signaling pathway promotes neointima formation in the carotid ligation model in mouse. *Cardiovascular Research*, 87(1):156-65, 2010

Chandra, S., Razavi, H., Agrawal, R., Kundu, R., de Jesus Perez, V., Zamanian, R., Quertermous, T., Chun, H.J., Loss of Apelin Signaling Leads to Pulmonary Arterial Hypertension Mediated by Disruption of Nitric Oxide Synthesis, submitted.

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

1. Overall Goal (s) of the Research Program of the Laboratory:
The Dardik laboratory continues to study the healing and function of blood vessels as used in patients having vascular bypass surgery. We are currently 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.

2. Specific Research Accomplishments in the last 12 months:
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. Based on this work we hypothesize that Eph-B4 is a critical negative regulator of multiple SMC layer development. 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. We have recently confirmed our findings that stimulation of Eph-B4 signaling, either through ligand stimulation using Ephrin-B2/Fc, or through an adenovirus that expresses a constitutively-active Eph-B4 receptor, prevents graft thickening. As such we have prevented vein graft disease in mice. This year we have also found that Nogo-B is active during vein graft adaptation and likely signals via its alternate receptor PirB, not via its classical Nogo-receptor. We continue to collaborate with the Sessa laboratory to test the importance of caveolin-1 binding and phosphorylation, as well as Nogo-B function, in vein graft adaptation. We are collaborating with the Breuer laboratory to test the importance of Eph-B4 and Ephrin-B2 in the development of tissue engineered vascular grafts.

3. 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.

4. Publications:

Bazan H, Lu Y, Thoppil D, Fitzgerald T, Hong S, Dardik A. Diminished Omega-3 Fatty Acids are Associated with Carotid Plaques from Neurologically Symptomatic Patients: Implications for Carotid Interventions. *Vascular Pharmacology* 51(5-6):331-336 (2009).

Muto A, Nishibe T, Miyauchi Y, Kondo Y, Yamamoto Y, Dardik A, Shigematsu H. Prostaglandin receptors EP2 and IP are detectable in atherosclerotic arteries and plaques. *International Angiology* 29(Suppl. 1):38-43 (2010).

Fitzgerald TN, Muto A, Fancher TT, Brown PB, Martin KA, Muhs BE, Rothman DL, Constable RT, Sampath S, Dardik A. Surgically implantable magnetic resonance angiography coils improve resolution to allow visualization of blood flow dynamics. *Annals of Vascular Surgery* 24(2):242-253 (2010).

Leite JO, Deogburn R, Ratliff J, Su R, Smyth JA, Volek JS, McGrane MM, Dardik A, Fernandez ML. Low-carbohydrate diets reduce lipid accumulation and arterial inflammation in guinea pigs fed a high-cholesterol diet. *Atherosclerosis* 209(2):442-448 (2010).

Petersen TH, Hitchcock T, Muto A, Calle EA, Zhao L, Gong Z, Gui L, Dardik A, Bowles DE, Counter CM, Niklason LE. Utility of telomerase-pot1 fusion protein in vascular tissue engineering. *Cell Transplantation* 19(1):79-87 (2010).

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

1. Overall Goal (s) of the Research Program of the Laboratory:
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.

2. Publications:

Hartl D, Lee CG, Da Silva CA, Chupp GL, Elias JA. Novel biomarkers in asthma: chemokines and chitinase-like proteins. Curr Opin Allergy Clin Immunol. 2009 Feb;9(1):60-6. Review. PubMed PMID: 19532094.

Chapoval SP, Lee CG, Tang C, Keegan AD, Cohn L, Bottomly K, Elias JA. Lung vascular endothelial growth factor expression induces local myeloid dendritic cell activation. Clin Immunol. 2009 Sep;132(3):371-84. Epub 2009 Jun 24. PubMed PMID: 19553159; PubMed Central PMCID: PMC2780370.

Lee CG, Elias JA. Role of breast regression protein-39/YKL-40 in asthma and allergic responses. Allergy Asthma Immunol Res. 2010 Jan;2(1):20-7. Epub 2009 Dec 30. PubMed PMID: 20224674; PubMed Central PMCID: PMC2831605.

Da Silva CA, Pochard P, Lee CG, Elias JA. Chitin Particles are Multifaceted Immune Adjuvants. Am J Respir Crit Care Med. 2010 Jul 23. [Epub ahead of print] PubMed PMID: 20656945.

Matsuura H, Hartl D, Kang MJ, Dela Cruz CS, Koller B, Chupp GL, Homer RJ, Zhou Y, Cho WK, Elias JA, Lee CG. Role of Breast Regression Protein (BRP)-39 in the Pathogenesis of Cigarette Smoke-Induced Inflammation and Emphysema. Am J Respir Cell Mol Biol. 2010 Jul 23. [Epub ahead of print] PubMed PMID: 20656949**.**

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

1. Overall Goal (s) of the Research Program of the Laboratory:

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.

2. Publications:

Dementó SL, Bonafé N, Cui W, Kaech SM, Caplan MJ, Fikrig E, Ledizet M, Fahmy TM. TLR9-targeted biodegradable nanoparticles as immunization vectors protect against West Nile encephalitis. J Immunol. 2010 Sep 1;185(5):2989-97. Epub 2010 Jul 26. PubMed PMID: 20660705.

Stern E, Jay SM, Dementó SL, Murelli RP, Reed MA, Malinski T, Spiegel DA, Mooney DJ, Fahmy TM. Spatiotemporal control over molecular delivery and cellular encapsulation from electropolymerized micro- and nanopatterned surfaces. Adv Funct Mater. 2009 Jul 13;19(18):2888-2895. PubMed PMID: 20445826; PubMed Central PMCID: PMC2863321.

Stern E, Mooney DJ, Fahmy TM. A biomimetic approach for the creation of two-dimensional microscale surface patterns: creation of isolated immunological synapses. Int J Biomater. 2009;2009:821308. Epub 2009 Jun 17. PubMed PMID:20130805; PubMed Central PMCID: PMC2814121.

Stern E, Vacic A, Li C, Ishikawa FN, Zhou C, Reed MA, Fahmy TM. Nanoelectronic ELISA: A Nanoelectronic Enzyme-Linked Immunosorbent Assay for Detection of Proteins in Physiological Solutions Small 2/2010. Small. 2010 Jan 14;6(2). [Epub ahead of print] PubMed PMID: 20077518.

Stern E, Vacic A, Rajan NK, Criscione JM, Park J, Ilic BR, Mooney DJ, Reed MA, Fahmy TM. Label-free biomarker detection from whole blood. Nat Nanotechnol. 2010 Feb;5(2):138-42. Epub 2009 Dec 13. PubMed PMID: 20010825; PubMed Central PMCID:PMC2818341.

Almería B, Deng W, Fahmy TM, Gomez A. Controlling the morphology of electrospray-generated PLGA microparticles for drug delivery. J Colloid Interface Sci. 2010 Mar 1;343(1):125-33. Epub 2009 Oct 24. PubMed PMID: 20022337.

Richard A. Flavell, Ph.D., FRS
Sterling Professor and Chairman of Immunobiology, Investigator of Howard Hughes Medical Institute

1. Overall Goal (s) of the Research Program of the Laboratory
Our research is focused on regulatory mechanisms related to adaptive immunity including the cytokines and the innate immune receptors involved.

2. Specific Research Accomplishments in the last 12 months:
We have used transgenic, gene knockout and cytokine reporter mice to investigate the roles of cytokines in controlling the adaptive immune response. In particular, using mice defective in TGF-beta signaling we have shown how TGF-beta controls adaptive CD8 immune responses. We found, after Listeria infection, plasma TGF-beta titers increased concomitant with the expansion of effector CD8 T cells. Blocking TGF-beta signaling did not affect effector function of CD8 T cells. However, TGF-beta controlled effector cell number by lowering Bcl-2 amounts and selectively promoting apoptosis of short-lived effector cells. TGF-beta mediated apoptosis of the effector sub-population occurred during clonal expansion and contraction, whereas IL-15 promoted their survival only during contraction. We demonstrated that the number of effector CD8 T cells is tightly controlled by multiple extrinsic signals through effector differentiation.

3. Significance of Key Findings Relevant for the Mission of VBT:
The idea that atherosclerosis is an inflammatory disease is no longer controversial. Instead, much of the current research is now focused at understanding how this inflammation is regulated. T cells are of particular interest, both due to their secretion of mediators that influence plaque development, and since their activity depends on the triggering of specific antigens found within the disease site.

4. Publications:
Watanabe A, Sohail MA, Gomes DA, Hashmi A, Nagata J, Sutterwala FS, Mahmood S, Jhandier MN, Shi Y, Flavell RA, Mehal WZ. Inflammasome-mediated activation of hepatic stellate cells. Am. J. Physiol. Gastrointest Liver Physiol. 296:G1248-G1257 (2009). PMC2697939

Sanjabi S, Mosaheb MM, Flavell RA. Opposing effects of TGF-□ and IL-15 cytokines control the number of short-lived effector CD8+ T cells. Immunity 31:131-144 (2009). PMC2765785

Kriegel MA, Rathinam C, Flavell RA. E3 ubiquitin ligase GRAIL controls primary T cell activation and oral tolerance. Proc. Natl.Acad. Sci. USA 106:16770-16775 (2009). PMC2757842

Marks BR, Nowyhed HN, Choi J-Y, Poholek A, Odegard J, Flavell RA, Craft J. Thymic self-reactivity selects for Th17 cells that regulate peripheral inflammation. Nat. Immunol. 10:1125-1132 (2008). PMC2751862

Bhowmick S. Singh A. Flavell RA, Clark RB, O'Rourke J, Cone RE. The sympathetic nervous system modulates CD4+FoxP3+ regulatory T cells via a TGF-β dependent mechanism. J. Leukocyte Biol. 86:1275-1283 (2009). PMC2780915 [Available on 2010/12/1]

Williams A, Flavell RA, Eisenbarth SC. The role of NOD-like receptors in shaping adaptive immunity. Curr. Opin. Immunol. 22:34-40 (2010).

Rathinam C, Flavell RA. c-Cbl deficiency leads to diminished lymphocyte development and functions in an age-dependent manner. Proc. Natl. Acad. Sci. USA 107:8316-8321 (2010).

Arnar Geirsson, M.D.
Assistant Professor of Surgery

1. Overall Goals of the Research Program of the Laboratory:
The laboratory pursues two main research projects. The first project involves assessment of the role of miRNAs in both cardiac remodeling following ischemic injury and in maintenance of normal cardiac function. Second project involves analyzing TGF-□ signaling in mitral regurgitation and degenerative mitral valve disease.

2. Specific Research Accomplishments in the Last Year:
Significant progress has been made in the miRNA project over the last year. Inducible cardiac specific Dicer knock-out mouse model demonstrated a dramatic rapid decline in cardiac function coinciding with a reduction in miR-1 levels. Potential biological target has been identified as Sorcin a ryanodine receptor 2 modulator that provides a mechanistic explanation of our phenotype. Significant dysregulation of calcium signaling in cultured cardiomyocytes was confirmed as well as similar finding were noted in wild type mouse model treated with antagomir-1. Reciprocal findings are observed in clinical speciment of heart failure. The result have been submitted for publication. Analysis of TGF-□ pathways in degenerative mitral valve disease has identified several targets that are up-regulated in myxomatous mitral valves. TGF-□ pathway and SMAD 2/3 are activated in myxomatous mitral valve disease and can be modulated in tissue culture experiments. Immunohistochemistry and immunofluorescence have further delineated specific cell types and activation of valve interstitial cells in myxomatous mitral valves. Isolation and culturing of valve interstitial cells are now underway to further explore specific perturbation of the TGF-□ pathway.

3. Significance of Key Findings Relevant for the Mission of VBT:
Both projects remain with significant interdepartmental collaboration in all aspects of the research projects.

4. Publications and Abstracts:

Usman Ahmad, Rahmat Ali, Amir Lebastchi, Lingfeng Qin, Sheng-fu L. Lo, Alexander O. Yakimov, Salman F. Khan, Jonathan C. Choy, Arnar Geirsson, Jordan S. Pober, George Tellides. Interferon-γ Primes Intact Human Coronary Arteries and Cultured Coronary Smooth Muscle Cells to dsRNA and self-RNA Induced Inflammatory Responses by Upregulating TLR3 and MDA5. *Journal of Immunology*. 185;1283-1294, 2010.

Rahmat Ali, Richard W. Kim, George Tellides, Arnar Geirsson. Acute Myocardial Dysfunction following Cardiac-Specific Dicer Knockout Mediated by Sorcin Dysregulation. American Heart Association Scientific Session. Orlando, Florida. November, 2009.

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

1. Overall Goals of the Research Program of the Laboratory:

Our program combines basic science and clinical/translational research. Our basic science efforts target the mechanistic basis of angiogenesis, arteriogenesis, and vascular remodeling, and to understand the specific role of oxygen sensing in defining these processes. Hypertension, for example, is a prominent risk factor for atherosclerosis, arteriolosclerosis, and additional vascular pathologies, but the mechanistic basis of these relationships remains unclear. We are studying the role played by oxygen sensing and metabolic shifts in the vessel wall as biomechanical transducer pathways in vascular pathologies associated with hypertension. We also have a specific focus on transcription, epigenetics, and the translational application of transcription factor engineering. Our translational 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. Our current Yale-based clinical trials include a mesenchymal stem cell study for post-infarction cardiac repair.

2. Publications:

Tirziu D, Giordano FJ, Simons M. Cell communications in the heart. Circulation. 2010 Aug 31;122(9):928-37. PubMed PMID: 20805439.

Lanahan AA, Hermans K, Claes F, Kerley-Hamilton JS, Zhuang ZW, Giordano FJ, Carmeliet P, Simons M. VEGF receptor 2 endocytic trafficking regulates arterial morphogenesis. Dev Cell. 2010 May 18;18(5):713-24. Epub 2010 May 6. PubMed PMID: 20434959; PubMed Central PMCID: PMC2875289.

Giordano FJ. Oxygen: both a passenger and a biological determinant in the vasculature. Arterioscler Thromb Vasc Biol. 2010 Apr;30(4):641-2. PubMed PMID:20237326.

Tang YD, Hasan F, Giordano FJ, Pfau S, Rinder HM, Katz SD. Effects of recombinant human erythropoietin on platelet activation in acute myocardial infarction: results of a double-blind, placebo-controlled, randomized trial. Am Heart J. 2009 Dec;158(6):941-7. PubMed PMID: 19958860; PubMed Central PMCID:PMC2838229.

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

1. Overall Goal(s) of the Research Program of the Laboratory:

a. Understand how the innate immune system impacts transplant tolerance
b. Understand how aging impacts innate immunity during viral infections

2. Specific Research Accomplishments in the last 12 months:

a. Established that synergy between IL-6 and TNFα induces transplant tolerance resistance
b. Discover a new pathway by which aging induces lethality during viral infection. In contrast to the current paradigm, we found that exaggerated immunity with aging induces lethality during viral infection.

3. Significance of Key Findings Relevant for the Mission of VBT:

a. Our work indicates that inhibiting IL-6 and TNF may facilitate transplant tolerance
b. Our findings demonstrate that anti-inflammatory therapy may be helpful in older people infected with viruses.

4. Publications:

Shen, H., and D. R. Goldstein. 2009. IL-6 and TNF-{alpha} Synergistically Inhibit Allograft Acceptance. *J Am Soc Nephrol* 5:1032-1040.

Shen, H., B. M. Tesar, W. Du, and D. R. Goldstein. 2009. Aging Impairs Recipient T Cell Intrinsic and Extrinsic Factors in Response to Transplantation. *PLoS ONE* 4:e4097.

Stout-Delago HW, W. Du, A. Shirali, C. Booth, and G. DR. 2009. Aging promotes neutrophil-induced mortality by augmenting IL-17 production during viral infection. *Cell Host Microbe* 6:446-456.

Tesar, B. M., Du W, Shirali AC, Walker WE, Shen H, and G. DR. 2009. Aging Augments IL-17 T-Cell Alloimmune Responses. *American Journal of Transplantation* 9:54-63.

Walker, W. E., and D. R. Goldstein. 2009. B Cells Are Dispensable for Neonatal Transplant Tolerance Induction. *Transplantation* 88:874-878 810.1097/TP.1090b1013e3181b1096eca1092.

Walker, W. E., C. J. Booth, and D. R. Goldstein. 2010. TLR9 and IRF3 Cooperate to Induce a Systemic Inflammatory Response in Mice Injected With Liposome:DNA. *Mol Ther* 18:775-784.

John Hwa, M.D., Ph.D.
Associate Professor of Medicine, Cardiovascular Medicine

1. Overall Goal (s) of the Research Program of the Laboratory

Our central hypothesis for our studies is, "Suppression of PGI_2 activity by either COX-2 inhibition or through genetically defective human prostacyclin receptor (hIP) results in accelerated atherothrombosis. In contrast, suppression of thromboxane activity by either COX-1 inhibition or through genetically defective human thromboxane receptor (hTP) results in reduced atherothrombosis". To test our hypothesis we study human patients and human tissues at multiple levels (clinical, pathophysiology, pharmacology, cell biology, molecular biology, and bioinformatics). Our goals are to identify patients at increased risk for atherothrombosis, and to develop prostacyclin and thromboxane based therapies against atherothrombosis.

2. Specific Research Accomplishments in the last 12 months:

We have sequenced the coding regions of the human prostacyclin receptor (hIP) in 2,430 patients and have discovered 39 distinct genetic variants. We recently demonstrated that at least one of the 39 detected hIP variants (an R212C missense mutation in the critical third intracellular loop) is associated with increased coronary artery disease (atherosclerosis) burden on coronary angiography and increased cardiovascular events. In search for the mechanisms for the functional defects we have determined that for heterozygote patients, the dysfunctional R212C is able to heterodimerize with wild type receptor, exerting a dominant negative effect. We also demonstrated that reduced hIP function can lead to VSMC proliferation and dedifferentiation, in part through reduced prostacyclin-induced prostacyclin release from human VSMC. We are currently assessing cAMP/PKA-dependent and novel PKA-dependent and independent pathways for prostacyclin signaling. Such studies in addition to identifying patients at increased risk of developing atherothrombosis, will provide important insights into critical components in the human prostacyclin receptor structure that are required for binding ligand and activation. We anticipate that these studies may also provide structural insights in the development of prostacyclin based drugs in combating atherothrombosis

We are concurrently discovering and characterizing human thromboxane receptor genetic variants (30 discovered from sequencing 950 patients) for receptor structure function, signaling and protection against cardiovascular disease.

3. Significance of Key Findings Relevant for the Mission of VBT

Identification of novel mechanisms that modulate atherothrombosis; Identification of patients that are at increased risk (hIP variants), or protected from (hTP variants), atherothrombosis and Identification of potential novel therapeutic targets

4. Publications in the last 12 months, July 2009-June 2010.

Gleim S., Kasza Z., Martin K.A., Hwa J. Prostacyclin receptor/thromboxane receptor interactions and cellular responses in human atherothrombotic disease. Current Atherosclerosis Reports 11(3), 227-235 2009

Kasza Z., Fetalvero K.M., Ding M., Wagner R.J., Acs K., Douville K.L. Rzucidlo E.M., Powell R.J., Hwa J. and Martin K.A. A novel PKA, Akt1 and ERK dependent prostacyclin-induced prostacyclin release propagates differentiation signaling in vascular smooth muscle cells. J Mol Cell Card 46(5),682-694 2009

Arehart E., Gleim S, White W, Hwa J., and Moore J. Multifactor Dimensionality Reduction Analysis identifies specific nucleotide patterns promoting genetic polymorphisms. BioDataMining 2(1), 2 2009

Gleim S., Stojanovic A., Arehart E., Douville K., Byington D., Hwa J.. Conserved rhodopsin intradiscal structural motifs mediate stabilization: Effects of zinc Biochemistry 8;48(8):1793-1800 2009

Martin K.A., Gleim S, Elderon L, Fetalvero K, Hwa J.. The human prostacyclin receptor; from structure function to disease. *Prog Mol Biol Transl Sci* 89C:133-166 2009

Ibrahim S., Tetruashvily M., Frey A.J., Wilson S.J., Stitham J., Hwa J., Smyth E.M.. Domninant negative actions of human prostacyclin receptor variant through dimerization: implications for cardiovascular disease. Arteriosclerosis, Thrombosis and Vascular Biology 2010 (in press)

Stitham J., Arehart E., Elderon L., Gleim S., Douville K., Martin K., Hwa J.. Comprehensive biochemical analysis of rare prostacyclin receptor variants is useful as a disease biomarker. (2010 in revision)

Garcia Rodriguez L.A., Gonazalez-Perez A., Bueno H., Hwa J.. NSAID use selectively increases the risk of non-fatal myocardial infarction (2010 submitted)

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

1. Overall Goals of the Research Program of the Laboratory:

Our primary research goal is to define how the inflammatory cytokine tumor necrosis factor (TNF) destabilizes blood vessel barrier function by increasing the paracellular permeability of endothelial cells (EC) to macromolecules. A related goal of our research is to define how EC interaction with accessory cells (pericytes) contributes to angiogenesis and may help stabilize nascent microvessels. In order to study how transmembrane adhesion molecules found at EC-EC junctions (such as claudin-5, JAM-A, occludin and VE-cadherin) regulate the paracellular pathway of permeability, we knock down and overexpress these genes in cultured primary human EC. We explore the molecular mechanisms of how cultured EC and pericytes form tubular microvessels in 3-D collagen/fibronectin matrices.

2. Specific Research Accomplishments in the Last 12 Months:

I identified a membrane-proximal sequence of the TNF receptor 1 (TNFR1) targeting this receptor to caveolae/lipid rafts that interacts with caveolin-1 protein (D'Alessio et al, 2010). I advocated and organized purchase of an automated real-time Electrical Cell Impedance Sensor (ECIS; Applied BioPhysics) available to all VBT investigators that has been utilized for generating data incorporated into several recent VBT publications. Current VBT collaborations are with Jordan Pober, Wang Min, Laura Niklasson, Mark Saltzman, and Bing Su.

3. Significance of Key Findings Relevant for the Mission of VBT:

Signaling receptor compartmentalization regulates how cytokine and growth factors activate endothelial cells in processes such as tissue re-vascularization and angiogenesis. Our finding that a membrane-proximal sequence of TNFR1 controls compartmentalization is relevant to the mechanics of these processes.

4. Publications:

*****D'Alessio, A., *****Kluger, M.S. (**Co-First Author*), Li, J.H., Al-Lamki, R., Bradley, J.R., and Pober, J.S. Targeting of Tumor Necrosis Factor Receptor 1 to Low Density Plasma Membrane Domains in Human Endothelial Cells. Journal of Biological Chemistry, 2010. 285(31) 23868-23879.

Clark, P.R., Jensen,T., Kluger, M.S., Morelock, M., Hanidu, A., Qi, Z., Revati, T. and Pober, J.S. MEK5 is Activated by Shear Stress, Activates ERK5 and Induces KLF4 to Modulate TNF Responses in Human Dermal Microvascular Endothelial Cells. (Submitted for publication).

Jones, D., Xu, Z., Zhang, H., He, Y., Li, S., Kluger, M.S., Chen, H. and Min, W.A novel role for Bmx in adult lymphangiogenesis. (Submitted for publication).

Kluger M.S. and Colegio, O. Lymphangiogenesis Arising from Macrophage-derived VEGF-C: A Therapeutic Opportunity at the Cross-Roads of Cancer and Inflammation. Journal of Investigative Dermatology (Invited commentary in press).

Diane Krause, M.D., Ph.D.
Professor, Department of Laboratory Medicine

1. Overall Goal (s) of the Research Program of the Laboratory

The overall goals of my research are to characterize bone marrow derived stem and progenitor cells, and to define the molecular mechanisms (signal transduction, biomechanical, and epigenetic) that regulate the self-renewal and differentiation of these cells. Our recent emphasis has been on megakaryocyte development and megakaryoblastic leukemia. We are studying the roles of G-proteins, the SRF signal transduction pathway, RNA binding proteins in order to better understand and treat hematopoietic diseases including myelodysplasia, myeloproliferative disease and leukemia. Projects include work with embryonic stem cells as well as hematopoietic stem cells from mice and humans. Our work provides insights not only into normal blood cell development, but also to the pathogenesis of myeloid leukemia. Studies on epithelial engraftment of bone marrow derived cells, which is also referred to 'adult stem cell plasticity,' are focused on the mechanisms of repair in response to tissue injury and disease in the lung.

2. Specific Research Accomplishments in the last 12 months:

We have made progress on multiple fronts in the past year. In our studies on megakaryocytic development and differentiation, we showed for the first time that the transcription factor SRF (Serum Response Factor) plays a critical role in the maturation and polyploidization of megakaryocytes, and our current work is focused on the role of SRF in myelopoiesis as well.

3. Significance of Key Findings Relevant for the Mission of VBT

Because platelets play a key role in atherosclerosis and inflammation and perhaps in development of lymphatic endothelial lining cells as well, the means by which they are produced, and the transcriptional regulation of the genes expressed by platelets are highly relevant to vascular biology.

4. Publications:

Mahajan MC, Karmakar S, Newburger PE, Krause DS, Weissman SM. Dynamics of alpha-globin locus chromatin structure and gene expression during erythroid differentiation of human CD34(+) cells in culture. Exp Hematol.37:1143-1156, 2009.

Halene S, Gaines P, Sun H, Zibello T, Lin S, Khanna-Gupta A, Williams SC, Perkins A, Krause DS, Berliner N. C/EBPepsilon directs granulocytic versus monocytic lineage determination and Hlx restores chemotactic function but not full maturation to C/EBPε Knockout Cells. Exp. Hem. 2009

Zhi G, Wilson JB, Chen X, Krause DS, Xiao Y, Jones NJ, Kupfer GM. Fanconi anemia complementation group FANCD2 protein serine 331 phosphorylation is important for fanconi anemia pathway function and BRCA2 interaction. Cancer Res.69:8775-8783, 2009.

Kassmer SH, Krause DS. Detection of bone marrow-derived lung epithelial cells. Exp Hematol.38:564-573, 2010

Yu L, Ji W, Zhang H, Renda MJ, He Y, Lin S, Cheng EC, Chen H, Krause DS, Min W. SENP1-mediated GATA1 deSUMOylation is critical for definitive erythropoiesis. J Exp Med.207:1183-1195, 2010.

Halene S, Gao Y, Hahn K, Massaro S, Italiano JE, Lin S, Kupfer GM, Krause DS. Serum Response Factor is an essential transcription factor in megakaryocytic maturation. Blood (In press, 2010).

Themis R. Kyriakides Ph.D.
Associate Professor, Pathology and Biomedical Engineering

1. Overall Goal (s) of the Research Program of the Laboratory:
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 ischemia, injury and the implantation of biomaterials and scaffolds for tissue engineering applications. Our primary research focus is on two molecules, MCP-1 and TSP-2 that we have shown to be critical to various aspects of these processes. In a new research effort we are exploring the relationship between the activity of eNOS and Akt with the expression of TSP2. and 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.

2. Specific Research Accomplishments in the last 12 months:
We have continued our investigation of the participation of TSP-2 in angiogenesis and arteriogenesis. Furthermore, we have explored the link between the expression of eNOS and Akt and down-regulation of TSP2. To explore this link, we have generated double eNOS/TSP2-null mice and Akt1/TSP2-null mice in collaboration with Dr. Sessa. We have discovered that the absence of TSP2 ameliorates the phenotype of the eNOS-null mice and we are currently investigating the phenotype of Akt1/TSP2-null mice. Our in vivo and vitro studies have shown that nitric oxide suppresses TSP2 expression at the transcriptional level. Within the VBT program we have continued our collaborations with the following investigators: Sessa, Giordano, Saltzman, Niklason, Breuer, and Tellides.

3. 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 investigating the link between TSP2, Akt1, and eNOS are of importance to many processes in vascular biology.

4. Publications:
Kyriakides TR, Wulsin D., Skokos E.A., Fleckman P., Pirrone A., Shipley J.M., Senior R.M., Bornstein P. Mice that lack matrix metalloproteinase-9 display delayed wound healing associated with delayed reepithelization and disordered collagen fibrillogenesis. Matrix Biology 28: 65-73, 2009.

W. Tian, T.R. Kyriakides Matrix metalloproteinase-9 deficiency leads to prolonged foreign body response in the brain associated with increased IL1-β levels and leakage of the blood brain barrier. Matrix Biology 28: 148-59, 2009.

Lin S.-P., Kyriakides TR, Chen J.-J. Characterization of a three-dimensional matrix on microelectrode arrays for on-line cell growth observation. Biomaterials 30:3110-7, 2009.

Jay S.M., Skokos, E., Zeng, J., Knox, K., Kyriakides, T.R. Macrophage fusion leading to foreign body giant cell formation persists under phagocytic stimulation by microspheres in vitro and in vivo in mouse models. J. Biomed. Mat. Res. Part A 93: 189-99, 2009

Schroers J., Kumar G., Hodges T.M., Chan S., Kyriakides T.R. Bulk metallic glasses for biomedical applications. J.O.M. 61: 21-29, 2009.

Lynn A.D., Kyriakides T.R., Bryant S.J. Characterization of the In Vitro Macrophage Response and In Vivo Host Response to Poly(ethylene glycol) based Hydrogels. J. Biomed. Mat. Res. Part A 93: 941-53, 2009.

Yu J., Fernandez-Henando C., Suarez Y., Schleicher M., Hao Z., Wright P., Kyriakides T.R., Sessa W. Reticulon 4B (Nogo-B) is necessary for macrophage infiltration and inflammatory tissue repair. P.N.A.S. 106:17511-6, 2009.

Kyriakides TR, Maclauchlan S. The role of thrombospondins in wound healing, ischemia, and the foreign body reaction. J Cell Commun Signal. 3: 215-25, 2009.

Roh J.D., Sawh-Martinez R., Brennan M.P., Devine L., Jay S.M., Yi T., Mirensky T., Udelsman B., Nelson G.N., Hibino N., Shin'oka T., Saltzman W.M., Snyder E., Kyriakides T.R., Pober J.S., Breuer C.K. Tissue-engineered vascular grafts transform into mature blood vessels via an inflammation-mediated process of vascular remodeling. P.N.A.S. 107:4669-74, 2010.

Jay SM, Shepherd BR, Andrejecsk JW, Kyriakides TR, Pober JS, Saltzman WM. Dual delivery of VEGF and MCP-1 to support endothelial cell transplantation for therapeutic vascularization. Biomaterials. 31: 3054-62, 2010.

Roh JD, Sawh-Martinez R, Brennan MP, Jay SM, Devine L, Rao DA, Yi T, Mirensky TL, Nalbandian A, Udelsman B, Hibino N, Shinoka T, Saltzman WM, Snyder E, Kyriakides TR, Pober JS, Breuer CK. Tissue-engineered vascular grafts transform into mature blood vessels via an inflammation-mediated process of vascular remodeling. P.N.A.S. 107: 4669-74, 2010.

Zhou J, Tang PC, Qin L, Gayed PM, Li W, Skokos EA, Kyriakides TR, Pober JS, Tellides G. CXCR3-dependent accumulation and activation of perivascular macrophages is necessary for homeostatic arterial remodeling to hemodynamic stresses. J Exp Med. (accepted, 2010).

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

1. Overall Goal (s) of the Research Program of the Laboratory

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 a 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. We have also 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*.

2. Specific Research Accomplishments in the last 12 months:

Based upon our findings that TLR4 is required to suppress the expression of a novel oxidant moIecule NADPH oxidase 3 (Nox3) and a protease cathepsin E (CatE), we proceeded to develop lung-targeted Nox3 and CatE transgenic mice. This past year we have successfully expanded both transgenic lines and have tested the physiologic consequence of lung-targeted Nox3 and CatE overexpression and found that they mimic the lung phenotype of TLR4 null mice (emphysema). We are exploring the regulation of Nox3 and CatE by TLR4 in lung endothelial cells. We have also collected 160 human blood samples to investigate the role of smoking and age on TLR4 function and Nox3/CatE in people. We also successfully created endothelial-targeted TLR4 transgenic mice and developed endothelial-targeted silencing RNA constructs that are effective via intranasal delivery.

3. Significance of Key Findings Relevant for the Mission of VBT:

We have identified that TLR4 regulates the expression of two novel molecules, Nox3 and CatE, in lung endothelial cells that may serve as therapeutic targets in oxidant-mediated lung diseases such as emphysema. We have also collected human blood specimens that will be analyzed for TLR4, Nox3 and CatE, which will result in broader clinical applicability. We have also developed new scientific tools to study to the role of the endothelium and vasculature by successfully generating endothelial-targeted TLR4 transgenic mice and an endothelial-targeted silencing RNA vector.

4. Publications:

Griffith, B., Pendyala, S., Hecker, L., Lee, P.J., Natarajan, V. and Thannickal, V.J. NOX Enzymes and Pulmonary Disease, Forum Issue: NADPH oxidase*, Antioxidants and Redox Signaling*, 11: 2505-2516.

Raval, C.M. and Lee, P.J. Heme oxygenase-1 in Lung Disease, *Current Drug Targets*, In Press.

Joseph A. Madri, M.D., Ph.D.
Professor, Dept. of Pathology and Molecular, Cellular and Developmental Biology

1. Overall Goal(s) of the Research Program of the Laboratory:
During the past year we have continued our investigations into neural stem cell-endothelial cell interactions and their dynamic interactions during neurovascular development and in response to CNS injury and recovery, specifically investigating GSK-3β as a signaling node and VEGF, BDNF and SDF-1 as biomarkers. We also investigated the ligands and receptors (VEGF, SEMA3A and their receptors) involved in sympathetic innervation of vessels and T cell trafficking, focusing on CD44. Our approaches focused on developing tools to better diagnose which patients are at risk for developing severe neurodevelopmental handicaps.

2. Specific Research Accomplishments in the last 12 months:
Our animal and tissue culture models have provided us with a series of target proteins that are being used to develop ELISA- and SNP-based assays for the earlier diagnosis of neurodevelopmental handicaps in the newborn and osteoporosis in the "at risk" adult populations.

3. Significance of Key Findings Relevant for the Mission of VBT:
Our findings impact both the basic and translational missions of the VBT in that we have advanced our understanding of the roles of the vasculature in the development, maintenance and responsiveness of neurovascular niches in the CNS.

4. Publications:

Buhimuschi, C.S., Baumbusch, M.A., Dulay, A.T., Oliver, E.A., Lee, S., Zhao, G., Bhandari, V., Ehrenkranz, R.A., Weiner, C.P., Madri, J.A., Buhimuschi, I.A., Characterization of rage, HMGB1 AND S100β in inflammation induced preterm birth and fetal tissue injury, Amer J Pathol., 175(3):958-75, 2009. Epub 2009 Aug 13.

Li, Q., Liu, J., Michaud, M., Schwartz, M., Madri, J.A., Modeling the Neurovascular Niche: Strain differences in behavioral and cellular responses to perinatal hypoxia, and relationships to neural stem cell survival and self-renewal, Amer. J. Pathology, 175(5):2133-2145, 2009. Epub 2009 Oct 12. (Cover Illustration)

Long, J.B., Jay, S.M., Segal, S.S., Madri J.A., VEGF-A and Semaphorin3A: Modulators of vascular sympathetic innervation, Developmental Biology, 334(1):119-32, 2009. Epub 2009 Jul 23.

Madri J.A., Modeling the Neurovascular niche: Implications for recovery from CNS injury, J. Physiol & Pharmacol., 60, Suppl 4, 95-104, 2009.

Harding, M.J., Lepus, C.M., Gibson, T., Paturzo, F., Shepard, B.R., Gerber, S.A., Graham M., Rahner, C., Madri, J., Bothwell, A., Lindenbach, B., Pober, J.S. Human fetal hepatoblasts implanted within a vascularized extrahepatic matrix survive long-term and support hepatitis C virus infection following engraftment in immunodeficient mice. PLOS ONE, 5(4), e9987, 2010. PMID: 20376322. doi:10.1371/journal.pone.0009987.

Buhimuschi, C.S., Madri, J.A., Sora, N., Zhao, G., Buhimuschi, I.A., Myometrial Wound Healing Post-Cesarean Delivery in the MRL/MpJ Mouse Model of Uterine Scarring, Amer. J Pathol., 177(1):197-207, 2010.

Lavik, E., Madri J.A., "Angiogenesis, the neurovascular niche and neuronal reintegration after Injury", in "Molecular mechanisms and targeted clinical approaches for the treatment of angiogenic disease", Slevin, M. Editor, Springer, In Press, 2010.

Wu, Y., Madri J.A., Insights into Monocyte-Driven Osteoclastogenesis and Its Link with Hematopoiesis: regulatory roles of PECAM-1 and SHP-1, Critical Reviews in Immunology, in Press, 2010.

Kathleen A. Martin, Ph.D.
Associate Professor of Medicine (Section of Cardiovascular Medicine) and Pharmacology

1. Overall Goal (s) of the Research Program of the Laboratory:
Our studies are aimed at understanding the molecular mechanisms that regulate vascular smooth muscle cell (VSMC) phenotype. Mature SMC retain the ability to de-differentiate and re-enter the cell cycle. This is essential for such processes as angiogenesis, but also contributes to the pathogenesis of intimal hyperplasia. Intimal hyperplasia is a complication that contributes to the failure of angioplasty, stenting, and bypass surgery via restenosis, as well as long term failure of heart transplants due to diffuse graft arteriosclerosis. Understanding the mechanisms that drive this process may generate new therapeutic targets for treatment and prevention.

2. Specific Research Accomplishments in the last 12 months:
Our efforts in the past year have been focused on understanding the role of the mTORC1 pathway and its key effectors in VSMC differentiation. We have previously found that rapamycin, an mTORC1 inhibitor commonly used on drug-eluting stents, not only inhibits VSMC proliferation and migration but actively promotes a transcriptional program that induces VSMC differentiation. We have identified Akt2 as a key intermediate signal, and have recently implicated transcriptional regulators including GATA-6, as downstream mTORC1 targets that drive these responses. We are working to understand the mechanism of GATA-6 regulation as this may lead to new VSMC-specific therapeutic targets that would spare the endothelium from adverse effects of rapamycin that may contribute to late stent thrombosis.

We have recently demonstrated that the fat-derived cardioprotective hormone adiponectin also inhibits mTORC1 and promotes differentiation in VSMC, analogous to rapamycin. In contrast to rapamycin, adiponectin has many beneficial effects on vascular endothelium. We are also investigating the effects of secondhand smoke exposure on adiponectin, mTORC1 signaling, and vascular disease.

3. Significance of Key Findings Relevant for the Mission of VBT

- Identification of novel mechanisms in intimal hyperplasia
- Identification of additional vascular protective roles of the hormone adiponectin
- Identification of potential novel therapeutic targets

4. Publications:

Gleim S., Kasza Z., Martin K.A., Hwa J. Prostacyclin receptor/thromboxane receptor interactions and cellular responses in human atherothrombotic disease. Current Atherosclerosis Reports 11(3), 227-235 2009.

Kasza Z., Fetalvero K.M., Ding M., Wagner R.J., Acs K., Douville K.L. Rzucidlo E.M., Powell R.J., Hwa J. and Martin K.A. A novel PKA, Akt1 and ERK dependent prostacyclin-induced prostacyclin release propagates differentiation signaling in vascular smooth muscle cells. J Mol Cell Card 46(5),682-694 2009.

Martin K.A., Gleim S, Elderon L, Fetalvero K, Hwa J. The human prostacyclin receptor; from structure function to disease. *Prog Mol Biol Transl Sci* 89C:133-166 2009

K A Martin, E M Rzucidlo, M Ding, B L Merenick, Z Kasza, R J Wagner, and R J Powell. (2010) In Vitro Vascular Cell Culture Systems – V Vascular Smooth Muscle. In: Charlene A. McQueen, Comprehensive Toxicology, volume 6, pp. 69–96 Oxford: Academic Press.

Ding M., Martin K.A. Review of miRNA and oligonucleotide therapeutics sessions, Experimental Biology 2009, New Orleans, LA, USA IDDB Meeting Report 2009, April 18-22. Reference # RF1004634, Thomson Reuters Pharma.

Fei D.L., Li H., Kozul C.D., Black K.E., Singh S., Gosse J.A., DiRenzo J., Martin K.A., Wang B., Hamilton J.W., Karagas M.R., Robbins D.J. Activation of Hedgehog signaling by the environmental toxicant arsenic may contribute to the etiology of arsenic-induced tumors. *Cancer Res.* 2010 Mar 1;70(5):1981-8.

Wagner RJ*, Martin KA*, Powell RJ, Rzucidlo EM. Lovastatin induces VSMC differentiation through inhibition of Rheb and mTOR. *Am J Physiol Cell Physiol.* 2010 Jul;299(1):C119-27.**Denotes equal contribution.*

Ding M, Guzman A, and Martin KA. Pharmacogenetics of Obesity. Chapter in Applications of Pharmacogenomics in Clinical Practice: Present and Future, edited by Hwa, J. and Lewis, L.D. *In press.*

Stitham J., Arehart E., Elderon L., Gleim S., Douville K., Martin K., Hwa J. Comprehensive biochemical analysis of rare prostacyclin receptor variants is useful as a disease biomarker. (2010 *in revision*).

Ding M., Wagner R.J., Carrao, A.C., Liu L., Guzman A.K., Powell R.J., Hwa J., Rzucidlo E.M., Martin K.A. Adiponectin Induces Vascular Smooth Muscle Cell Differentiation via mTORC1 Inhibition. *Submitted to ATVB, in revision.*

Wang Min, Ph.D.
Associate Professor with Tenure, Pathology

1. Overall Goal(s) of the Research Program of the Laboratory: Understanding of the fundamental molecular mechanisms for vasculogenesis, arteriogenesis and angiogenesis may lead to improved therapeutic strategies for treatment of vascular diseases. The goal in my lab is to dissect the signaling pathways in vasculature involved in vascular development, remodeling and repair related to human diseases such as vascular malformation, atherosclerosis, stroke, graft transplant rejection and tumor metastasis. In the past ten years, my lab has extensively employed biochemical, cell biological and mouse genetic approaches to define the critical molecules mediating vascular development, remodeling and repair.

2. Specific accomplishments in the last year:

a. Cerebral cavernous malformations (CCM):

Cerebral cavernous malformations (CCM) are a common vascular malformation that affect the central nervous system vasculature with a prevalence of 0.1-0.5% in the human population. Based on human genetic studies, CCM are caused by mutations in three genes CCM1, CCM2 and CCM3. However, the molecular mechanisms for development of CCM are unknown. In 2005, my group decided to address these issues by dissecting the CCM3 signaling pathways in vitro and by generating CCM3-knockout mice. In the past 5 years, we have extensively characterized mouse phenotype with a deletion of CCM3 gene either globally or endothelial specifically. My lab has uncovered an essential role of CCM3 in vascular development and morphogenesis, providing insights into the mechanism for human CCM pathogenesis (He, Y. et al 2010). I have also collaborated with Dr. Boggon on the structure and function of CCM3. We have made several very important discoveries on the novel aspects of CCM3 from these collaborations (Li, X. *et al*., 2010).

b. AIP1 as a novel inhibitor of tumor metastasis: AIP1 (also called DAB2IP), a novel member of the Ras GTPase-activating protein family, was identified as apoptosis signal-regulating kinase 1(ASK1)-interacting protein. Over the past 6 years, we have extensively investigated the role of AIP1 in vascular system (Zhang, H et al *J. Clin Invest* 2008). More recently, we have explored the role of AIP1 in tumor growth and metastasis. We have identified AIP1/DAB2IP as a tumor metastasis suppressor gene (Xie, D. et al., 2010).

c. Define the in vivo function of TNFR2 in ischemia-mediated arteriogenesis/ angiogenesis, and TNFR2-specific angiogenic pathways. We have previously shown that TNFR2-specific signaling is critical for TNFR2-mediated angiogenesis in vivo tissue and in vitro cultured EC (Luo, D. et al., 2007. *Am. J. Pathol*). TNFR2 also plays a beneficial role in the human heart. Recently we have generated EC-specific transgenic mice expressing TNFR2, and demonstrated that EC-expressed TNFR2 is sufficient to promote ischemia-mediated arteriogenesis and angiogenesis (Luo, Y. et al., 2010).

3.Publications:

Chen, H., Ko, G., Zatti, A., di Giacomo, G., Liu, L., Raiteri, E., Perucco, E., Collesi, C., Min, W., Zeiss, C., De Camilli, P. and Cremona, O (2009). Embryonic arrest at midgestation and disruption of Notch signaling produced by the absence of both epsin 1 and epsin 2 in mice. *Proc. Natl. Acad. Sci*. USA 106(33), 13838-43. PMCID: PMC2728981.

He, Y, Zhang, H., Yu, L., Gunel, M., Boggon, T., Chen, H. and Min, W.* (2010) Stabilization of VEGFR2 signaling by cerebral cavernous malformation 3 is critical for vascular development. *Science Signaling*. 3 (116):ra26.

Li X, Zhang R, Zhang H, He Y, Ji W, Min W*, Boggon TJ*. (2010) Crystal structure of CCM3, a cerebral cavernous malformation protein critical for vascular integrity. *J Biol Chem*. 2010 May 19. [Epub ahead of print]. PMID: 20489202. (*co-corresponding author).

Yu, L., Ji, W., Zhang, H., Renda, MJ, He, Y., Lin, S., Cheng, E., Chen, H., Krause, DS, and Min, W.* (2010). SENP1-mediated GATA1 deSUMOylation is critical for definitive erythropoiesis. *J. Exp Med*. 207(6):1183-95. MCID: PMC2882842.

Wan, T., Liu, T., Zhang, H, Tang, S, and Min, W.* (2010). AIP1 functions as Arf6-GAP to negatively regulate TLR4 signaling. *J. Biol. Chem.* 285(6):3750-7. PMCID: PMC2823516.

Xie, D., Gore, C., Zhou, J., Pong, RC, Zhang, H., Yu, L., Vessella, RL, Min, W., and Hsieh, JT (2009). DAB2IP coordinates both PI3K-Akt and ASK1 pathways for cell survival and apoptosis. *Proc. Natl. Acad. Sci*. USA 106(33), 13838-43. PMCID: PMC2785260.

Xie, D., Gore, C., Liu, J., Pong, RC, Mason, R., Hao, G., Long, M., Kabbani, W., Yu, L., Zhang, H., Chen, H., Sun, X., Boothman, Min, W.*, and Hsieh, JT* (2010). DAB2IP/AIP1 modulates epithelial-to-mesenchymal transition (EMT) and metastasis in prostate cancer *Proc. Natl. Acad. Sci*. USA 107 (6):2485-90. (*co-corresponding authors). PMCID: PMC2823864.

Al-Lamki, R., Brookes, AP, Wang, J., Reid, MJ, Parameshwar, J, Goddard, MJ, Tellides, G, Wan, T, Min, W, Pober, JS, Bradley, JR (2009) TNF receptor differentially signal and are differentially expressed and regulated in the human heart. *Am J. Transplant*. 9(12):2679-96. PMID: 19788501.

Luo, Y, Xu, Z., Wan, T, He, Y, Jones, D., Zhang, H, and Min, W*. (2010) Endothelial-specific transgenesis of TNFR2 promotes adaptive arteriogenesis and angiogenesis *Arterioscler Thromb Vasc Biol*. 30 (7): 1307-14. PMCID: PMC2889154.

Laura Elizabeth Niklason, M.D., Ph.D.
Professor & Vice-Chair of Anesthesia; Professor of Biomedical Engineering

1. Overall Goal (s) of the Research Program of the Laboratory:
Dr. Niklason's research program focuses on cardiovascular and lung tissue engineering, utilization of stem cells for tissue regeneration, and on mechanical characteristics of native and engineered vascular structures.

2. Specific research accomplishments in the last 12 months:
Work on the engineering of implantable blood vessels has progressed through the assessment of decellularized, engineered arteries as bypass conduits in a porcine model. In comparison with autologous, contralateral vein grafts, decellularized grafts lined with autologous endothelium displayed a decreased propensity for pseudointimal hyperplasia. Speculating that resistance to intimal hyperplasia was related to the mTOR signaling pathway, we assessed expression of p-AKT and p70S6K by immmunoblotting. Results of this analysis showed increased activation of mTOR in vein grafts as compared to engineered grafts, along with an increased rate of cellular proliferation in walls of vein grafts. These results together imply that a relative resistance to mTOR activation and consequent cellular replication underlie the observed resistance to neointimal formation. This resulted in improved patency of decellularized, engineered grafts as compared to vein grafts over just a 30-day period. Future studies will look further at the mechanisms of remodeling of implanted, decellularized grafts. In addition, future work will focus on surface functionalization of engineered grafts to reduce thrombosis. Such surface treatments will include both covalent attachment of heparin to the collagenous graft lumen, as well as coating of the lumen with thrombospondin2-null matrix, which has been shown to inhibit platelet adhesion and activation.

Based upon our previous work using adult-derived mesenchymal stem cells to produce vascular smooth muscle for vascular engineering, we are also studying the roles of specific cytokines and matrix substrates in the differentiation of smooth muscle from both human embryonic stem cells and from human induced pluripotent stem cells. The appeal of iPS cells rests with their genetic identity with the intended recipient, though safety issues and tumorigenicity of these cells remain significant issues. Work with engineered vessels is funded by the National Institutes of Health, while the human ES and iPS work is funded by the Connectictut Stem Cell Program.

Lastly, we have developed a system for culturing functional, implantable lung tissues using decellularized native lung scaffolds that are repopulated with lung cell types within a biomimetic bioreactor. We recently showed that mixed populations of neonatal rat pulmonary epithelial cells, when seeded into an acellular lung matrix, generally adhere appropriate anatomic locations. This implies either that cellular adhesion events are driven by specific local matrix cues within the lung tissue, or that the differentiation of relatively plastic neonatal rat epithelium is driven by local cues – or both. In this work we have also shown that breathing movements, analogous to those observed during fetal life, are important for lung epithelial survival and differentiation within the acellular lung matrix. Engineered lungs are implantable in allogeneic rat hosts and participate in efficient gas exchange for periods of several hours.

3. Publications:
Norotte, C., Marga, F., Niklason, L. and Forgacs, G. Scaffold-free vascular tissue engineering using bioprinting. *Biomaterials* 30(30): 5910-5917, 2009.

Petersen, T.H., Hitchcock, T., Muto, A., Calle, E.A., Zhao, L., Gong, Z., Gui, L., Dardik, A., Bowles, D.E., Counter, C.M., and Niklason, L.E. Utility of telomerase-pot1 fusion protein in vascular tissue engineering. *Cell Transplant*, 19:79-87, 2010.

Lee, E.J., Niklason, L. A novel flow bioreactor for in vitro microvascularization. *Tissue Engineering Part C Methods* 2010 Apr 6 [Epub ahead of print].

Pang, Z., Niklason, L.E., Truskey, G.A. Porcine endothelial cells cocultured with smooth muscle cells become procoagulant in vitro. *Tissue Engineering Part A* 16: 1835-1844, 2010.

Niklason, L.E., Yeh, A.T., Calle, E.A., Bai, Y., Valentin, A., Humphrey, J.D. Regenerative Medicine Special Feature: Enabling tools for engineering collagenous tissues integrating bioreactors, intravital imaging, and biomechanical modeling. *PNAS* 107: 3335-3339, 2010.

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Xu, X., Mughal, M.R., Scott Hall F., Perona, M.T., Pistell, P.J., Lathia, J.D., Chigurupati, S., Becker, K.G., Ladenheim, B., Niklason, L.E., Uhl, G.R., Cadet, J.L., Mattson, M.P., Dietary restriction mitigates cocaine-induced alterations of olfactory bulb cellular plasticity and gene expression, and behavior. *J. Neurochemistry* 114: 323-334, 2010.

Miller, C., George, S., Niklason, L., Developing a tissue-engineered model of the human bronchiole. *J. Tissue Eng. Regen. Med.* 2010 Jul 5 [Epub ahead of print].

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

1. Overall Goal (s) of the Research Program of the Laboratory: The Pober laboratory studies interactions of the immune and vascular systems. Emphases include recruitment and activation of T cells by endothelial cells (EC), interactions of T cells with other vessel wall cell types (smooth muscle cells, pericytes); activation of ECs by T cell-derived cytokines; immune mediated injury and resistance to injury of vascular cells, and application of vascular cells to engineered tissues.

2. Specific Research Accomplishments in the last 12 months: In the last year we have continued our analysis of human T cell recruitment into inflammatory sites, delineating further differences between chemokine-based and antigen recognition-based recruitment pathways. We have also extended our studies of TNF effects on human EC, demonstrating that specific TNF-induced miRNAs provide negative feedback for inflammatory changes and further elucidating the regulation of TNF responses through regulation of TNF receptors. We have also advanced our development of microvascular grafts for tissue engineering by developing an approach to isolate human pericytes that may be used for this purpose.

3. Significance of Key Findings Relevant for the Mission of VBT: The vascular system is a key component of the immune response and the findings of the Pober lab regarding T cell recruitment has identified new therapeutic targets for regulating immunity. At the same time, evidence is increasing that the immune system plays a key role in normal vascular homeostasis as well as in pathological vascular changes and the Pober lab's analysis of TNF responses are important for understanding how this works. Finally, the Pober lab's application of vascular cell biology to tissue engineering, in collaboration with Mark Saltzman, is addressing a key issue in this field, namely how to achieve adequate perfusion of engineered tissues.

4. Publications:

Li J, D'Alessio A, Pober J. Lipopolysaccharide triggers both caspase and cathepsin B death responses in human endothelial cells. Am J Pathol. 2009; 175:1124-1135.PMCID: PMC2731131

Ding B, Kirkiles-Smith NC, Pober JS. FOXO3A regulates oxygen-responsive expression of tumor necrosis factor receptor (TNFR)2 in human dermal microvascular endothelial cells. J Biol Chem. 2009; 284:19331-19339. PMCID: PMC2740558

Lepus CM, Gibson TF, Gerber SA, Kawikova I, Ablamunits V, Kirkiles-Smith N, Szczepanik M, Houssain J, Donis RO, Herold KC, Bothwell AL, Pober JS, Harding MJ. Comparison of human fetal liver, umbilical cord blood, and adult blood hematopoietic stem cell engraftment in NOD-*scid*/$\gamma c^{-/-}$, Balb/c-*Rag1*$^{-/-}$,$\gamma c^{-/-}$, and C.B-17-*scid*/bg immunodeficient mice. Human Immunol. 2009; 70:790-802.

Gerber SA, Yatsula B, Maier CL, Sadler TJ, Whittaker LW, Pober JS. Interferon-gamma induces prolyl hydroxylase (PHD)3 through a STAT1-dependent mechanism in human endothelial cells. Arterio, Thrombo and Vasc Biol. 2009; 29:1363-1369.

PMCID: PMC2757736

Choy JC, Pober JS. Paracrine generation of NO by bystander human CD8 T cellaugments allogeneic responses by inhibiting cytokine deprivation-induced cell death. Am J Transplant. 2009; 9:2281-2291.

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Jay SM, Shepherd BR, Andrejecsk JW, Kyriakides TR, Pober JS, Saltzman WM. Delivery of VEGF and MCP-1 to support endothelial cell transplantation for therapeutic vascularization. Biomaterials. 2010; 301:3054-3062. PMCID PMC2827647

Roh JD, Sawah-Martinez R, Brennan MP, Jay SM, Devine L, Rao DA, Yi T, Mirensky TL, Nalbandian A, Udelsman B. Hibino N, Shinoka T, Saltzman WM, Snyder E, Kyriakides TR, Pober JS, Breuer CK. Tissue-engineered vascular grafts transform into mature blood vessels via an inflammation mediated process of vascular remodeling. Proc Natl Acad Sci (USA). 2010; 107:4669-4674. PMCID PMC2842056

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Maier CL, Sheperd BR, Pober JS. Explant outgrowth, propagation and characterization of human pericytes. Microcirculation. 2010 (in press).

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Ahmad U, Ali R, Lebastchi A, Qin L, Lo SL, Yakimov AO, Khan SF, Choy JC, Geirsson A, Pober JS, Tellides G. Interferon-γ primes intact human coronary arteries and cultured coronary smooth muscle cells to dsRNA and self-RNA induced inflammatory responses by upregulating TLR3 and MDA5. J Immunol. 2010; (in press).

Zhang P, Manes TD, Pober JS, Tellides G. Human vascular smooth muscle cells lack essential costimulatory molecules to activate allogeneic memory T cells. ATVB 2010; (in press).

Zhang J, Silva T, Yarovinsky T, Manes TD, Tavakoli S, Nie L, Tellides G, Pober JS, Bender JR, Sadeghi MM. VEGF blockade inhibits lymphocyte recruitment and ameliorates immune-mediated vascular remodeling. Circ Res. 2010; (in press).

D'Alessio A, Kluger MS, Li JH, Al-Lamki R, Bradley JR, Pober JS. Targeting of tumor necrosis factor eceptor 1 to low density plasma membrane domains in human endothelial cells. J Biol Chem. 2010; (in press).

Zhou J, Tang PCY, Qin L, Gayed PM, Li W, Skokos EA, Kyriakides TR, Pober JS, Tellides G. CXCR3-dependent accumulation and activation of perivascular macrophages is necessary for homeostatic arterial remodeling tohemodynamic stresses. J Exp Med. 2010 (in press).

Nancy H. Ruddle, Ph.D.
John Rodman Paul Professor Emerita and Senior Research Scientist, Departments of Epidemiology and Public Health and Immunobiology

1. Overall Goal (s) of the Research Program of the Laboratory:
The overall goals of the laboratory are to understand cell trafficking in inflammation and lymphoid organ development and to relate these two seemingly disparate phenomena.

2. Specific Research Accomplishments relevant to VBT:
a. Development of transgenic mice for analysis of human insulin-specific T cell expression and antigen recognition. Mice transgenic for a construct consisting of the human insulin gene and a GFP tag have been produced and analyzed, with appropriate regulation in pancreas and thymus. These mice will be crossed to mice transgenic for human HLA-A2 and for reconstitution cells from human patients and evaluated for human cell trafficking to the pancreas.
b. Development of mice with fluorescent high endothelial venules and lymphatic vessels. Mice transgenic for a construct that includes an high endothelial venule (HEV) restricted gene driving a green fluorescent protein reporter (HEC6ST-GFP) have been produced and analzed. Other mice transgenic for a construct that includes the lymphatic vessel gene, PROX-1, driving expression of td tomato have also been produced and analyzed. These mice are being used to evaluate the immune and autoimmune response in living mice by multiphoton microscopy.
c. The role of lymphotoxin in lymphangiogenesis. LTα contributes to the function of lymphatic vessels and to lymphangiogenesis during inflammation by evaluating lymph flow velocities and interstitial fluid pressure and several inflammatory models.

3. Significance of Key Findings Relevant for the Mission of VBT
Mice with fluorescent lymphatic vessels and high endothelial venules will be invaluable for in vivo analysis of lymphangiogenesis and in inflammation and ontogeny. Our studies provide insight into mechanisms of lymphatic vessel development and HEV regulation.

4. Publications:
Akirav, E., Bergman, C.M., Hill, M., Ruddle, N.H. Depletion of CD4+CD25+ T cells exacerbates experimental autoimmune encephalomyelitis induced by mouse, but not rat, antigens. *J. Neurosci.Res.* 87: 3511-3519, 2009. PMID: 19125411

Ruddle, N.H. and Akirav, E.M. Secondary lymphoid organs: Responding to genetic and environmental cues in ontogeny and the immune response. J. Immunol. 183:2205-2212, 2009, PMID: 19661265

Penaranda,C., Tang, Q., Ruddle, N.H., Bluestone, J.A. Prevention of diabetes by FTY720-mediated stabilization of peri-islet tertiary lymphoid organs. *Diabetes*. 59:1461-1468, 2010 PMID: 20299465

Sasaki, M., Lankford, K.L., Brown, R.J., Ruddle, N.H., Kocsis, J.D. Focal experimental autoimmune encephalomyelitis in the Lewis rat induced by immunization with MOG and intraspinal injection of VEGF. Glia 58:1523-31,2010 PMID:20655414

Mounzer R.H., Svendsen O.S., Baluk P., Bergman C.M., Padera T.P., Wiig H., Jain R.K., McDonald D.M., Ruddle N.H. Lymphotoxin alpha contributes to lymphangiogenesis. *Blood.* 2010 Jun 21. [Epub ahead of print]PMID:20566898

Bentley, K.L., Shashikant, C.S., Wang, W., Ruddle, N.H., Ruddle, F.H. A yeast-based recombinogenic targeting toolset for transgenic analysis of human disease genes of interest. Annals of the N.Y. Acad. of Sci. in press, 2010.

Bentley, K.L., Stranford, S.,Liao, S., Mounzer, R.H., Ruddle, F.H., Ruddle, N.H. High endothelial reporter mice to probe regulation of lymph node vasculature. In Advances in TNF Family Research, (D. Wallach, A. Kovalenko, and M. Feldmann, eds.) Springer Press, NY, in press.

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

1. Overall Goal (s) of the Research Program of the Laboratory:

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.

2. Specific Research Accomplishments in the last 12 months:

Over the past year, we have successfully developed and implemented 2 inducible models of endothelial selective neuregulin knockout. Using these models, we have shown that neuregulin expression in endothelium is critical for vascular maintenance, growth of new vessels in response to injury and protection of 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 also have a core for cardiovascular imaging of murine models of cardiovascular disease using the Vevo770 system. This facility has been used by multiple diverse faculty at the University and Medical School.

3. 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. In addition, we have found that some of these factors can both protect myocytes and contribute to vascular preservation and angiogenesis.

4. Publications:

Wendler, CC, Busovsky-McNeal, M, Ghatpande, S, Kalinowski, A., Russell, KS, Rivkees, SA. Embryonic caffeine exposure induces adverse effects in adulthood. *FASEB J*. 2009;23:1272-1278.

Ozcan C, Miller EJ, Russell KS, Dewar ML, Rosenfeld LE. An unusual case of nonbacterial thrombotic (marantic) endocarditis. *J Thorac Cardiovasc Surg.* 2009;137(1):239-241.

Parks, J, Fong , PM, Lu, J, Russell, KS, Booth, CJ, Saltzman, MW, Fahmy TM. PEGylated PLGA nanoparticles for the improved delivery of doxorubicin. *Nanomedicine.* 2009; 5:410-418.

Kalinowski, A, Huang, Q, Plowes, NJR, Berdejo-Izquierdo, C, Russell, RR, Russell, KS.

Metaloproteinase-dependent cleavage of neuregulin and autocrine stimulation of vascular endothelial cells, FASEB J, in press 2010.

Velazquez, H, Wu, Y, Wang, P, Liu, D, Russell KS, Russell, RR, Xu, J, Flavell, R, Giordano, F, Desir, GV. Renalase modulates redox metabolism and is cardioprotective in mice. 2009: In submission, *J Clin Invest*

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

1. Overall Goal (s) of the Research Program of the Laboratory:
The main goal of our research is to develop novel molecular imaging approaches for cardiovascular diseases, with an emphasis on the vascular system. Vascular remodeling, changes in the vessel geometry and/or composition, is a common feature of a broad spectrum of vasculopathies, from atherosclerosis to graft arteriosclerosis and aneurysm. We have focused on four examples of vascular remodeling, namely injury-induced vascular remodeling, graft arteriosclerosis, aneurysm, and atherosclerosis. 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 relevant animal models, and use a dedicated microSPECT/CT small animal imaging system to detect the process in vivo. Studies of the pathophysiology of vascular remodeling, to identify novel targets for imaging and to understand the biology of the targets identified, is an integral part of our research.

2. Specific Research Accomplishments in the last 12 months:
Over the past several years, we have established the feasibility of MMP-targeted imaging of injury-induced vascular remodeling and aneurysm in the mouse, and have demonstrated that MMP-targeted imaging may be used to track the remodeling process in vivo. We have recently demonstrated that using this approach we can predict a murine aneurysm's propensity to expansion in vivo, and track the effect of dietary intervention on vascular remodeling. Other work has focused on imaging VEGF receptors in vascular remodeling. In parallel studies, we have continued our work on characterization of a potentially novel target for imaging vascular remodeling, namely, endothelial and smooth muscle derived neuropilin-like protein (ESDN) identified in previous years through genomic approaches. We have established the pattern of ESDN expression in graft arteriosclerosis, demonstrating little ESDN expression in normal arteries and high levels of expression during the course of vascular remodeling in parallel with cell proliferation. Furthermore, we have demonstrated that ESDN plays a key role in vascular smooth muscle cell differentiation, and modulates receptor tyrosine kinase signaling. Other work has focused on the interaction between growth factors, integrins and ESDN in graft arteriosclerosis, demonstrating that inhibition of VEGF drastically reduces vascular remodeling in graft arteriosclerosis through effects on leukocyte trafficking. This led to identification of a new subset of T lymphocytes that express VEGFR-1. These findings may potentially lead to the development of novel diagnostic and therapeutic approaches for vascular remodeling.

3. Publications:
Guo X, Nie L, Esmailzadeh L, Zhang J, Bender JR, Sadeghi MM. "Endothelial and Smooth Muscle-Derived Neuropilin-Like Protein (ESDN) Modulates Platelet-Derived Growth Factor (PDGF) Signaling in Human Vascular Smooth Muscle Cells", *Journal of Biological Chemistry*, 2009, 284, 29376-29382.

Dobrucki LW, Meoli DF, Hu J, Sadeghi MM, Sinusas AJ, "Regional Hypoxia Correlates with the Uptake of a Radiolabeled Targeted Marker of Angiogenesis in Rat Model of Myocardial Hypertrophy and Ischemic Injury", *Journal of Physiology and Pharmacology*, 2009, 60 Suppl 4:117-23.

Sadeghi MM, Glover DK, Lanza GM, Fayad ZA, Johnson LL. " Imaging Atherosclerosis and the Vulnerable Plaque", *Journal of Nuclear Medicine*, 2010, 51:1S-15S.

Razavian M, Zhang J, Nie L, Tavakoli S, Razavian N, Dobrucki LW, Sinusas AJ, Edwards DS, Azure M, Sadeghi MM. "Molecular Imaging of Matrix Metalloproteinase Activation to Predict Aneurysm Expansion in vivo", *Journal of Nuclear Medicine*, 2010, 51:1107-15.

Zhang J, Silva T, Yarovinsky T, Manes TD, Tavakoli S, Nie L, Tellides G, Pober JS, Bender JR, Sadeghi MM. "VEGF Blockade Inhibits Lymphocyte Recruitment and Ameliorates Immune-Mediated Vascular Remodeling", *Circulation Research*, 2010, 107:408-17.

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

1. Overall Goal(s) of the Research Program of the Laboratory:

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.

2. Publications:

Jay SM and Saltzman WM. Controlled Delivery of VEGF Via Modulation of Alginate Microparticle Ionic Crosslinking. *Journal of Controlled Release* 134:26-34 (2009).

Cartiera MS, Johnson KM, Rajendran V, Caplan MJ, and Saltzman WM. The uptake and intracellular fate of PLGA nanoparticles in epithelial cells, *Biomaterials* 30:2790-2798 (2009).

Woodrow KA, Cu Y, Booth CJ, Saucier-Sawyer J, Wood MJ, and Saltzman WM. Intravaginal gene silencing using biodegradable nanoparticles densely loaded with small-interfering RNA, *Nature Materials* 8:526-533 (2009).

Bennewitz M and Saltzman WM. Nanotechnology for delivery of drugs to the brain for epilepsy, *Neurotherapeutics* 6(2):323-336 (2009).

Lo CT, Van Tassel PR, and Saltzman WM. Simultaneous Release of Multiple Compounds from Mixed Nanoparticles Adsorbed to the Surface of an Implantable Medical Device, *Biomaterials* 30:4889-4897 (2009).

Liu J, Jiang Z, Zhang S, and Saltzman WM. Poly(*ω*-pentadecalactone-*co*-butylene-*co*-succinate) nanoparticles as biodegradable carriers for camptothecin delivery, *Biomaterials* 30:5707-5719 (2009).

Cu Y, Lemoellic C, Caplan MJ, and Saltzman WM. Ligand-modified gene carriers increase uptake in target cells but reduce DNA release and transfection efficiency, *Nanomedicine* 6:334-343 (2010).

Cartiera MS, Ferreira EC, Caputa C, Egan ME, Caplan MJ, and Saltzman WM. Partial correction of cystic fibrosis defects with PLGA nanoparticles encapsulating curcumin, *Molecular Pharmaceutics* 7: 86-93 (2010).

Sirianni R, Jang EH, Miller KM, and Saltzman WM. Parameter estimation methodology in a model of hydrophobic drug release from a polymer coating, *Journal of Controlled Release* 142:474-482 (2010). PMID: 19958804

Sirianni RW, Olausson P, Chiu AS, Taylor JR, and Saltzman WM. The behavioral and biochemical effects of BDNF containing polymers implanted in the hippocampus of rats. *Brain Research* 1321:40-50 (2010).

Jay SM, Shepherd BR, Andrejecsk JW, Kyriakides TR, Pober JS, and Saltzman WM. Dual delivery of VEGF and MCP-1 to support endothelial cell transplantation for therapeutic vascularization, *Biomaterials* 31:3054-3062 (2010). PMID: 20110124 [PubMed - in process].

Lo CT, Van Tassel PR, and Saltzman WM. Poly(lactide-co-glycolide) nanoparticle assembly for highly efficient delivery of potent therapeutic agents from medical devices, *Biomaterials* 31: 3631-3642 (2010).

Rubin J, Paltiel AD, and Saltzman WM. Are we studying what matters? Health priorities and NIH-funded biomedical engineering research. *Annals of Biomedical Engineering* 38:2237-2251 (2010).

Roh JD, Sawh-Martinez R, Brennan MP, Jay SM, Devine L, Rao DA, Yi T, Mirensky TL, Nalbandian A, Udelsman B, Hibino N, Shinoka T, Saltzman WM, Snyder E, Kyriakides TR, Pober JS, Breuer CK. Tissue-engineered vascular grafts transform into mature blood vessel via an inflammation-mediated process of vascular remodeling. *Proceedings of the National Academy of Sciences*, 107:4669-74 (2010). PMID: 20207947 [PubMed - in process].

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

1. Overall Goal (s) of the Research Program of the Laboratory:
Our laboratory is very interested in endothelial cell biology, signaling and regulation of post-natal angiogenesis/ arteriogenesis and atherosclerosis.

2. Specific accomplishments in the last year:
In the past year, we have made successful inroads into two areas: 1. miRNAs in smooth muscle cells; 2. Proteomics of caveolae/lipid rafts. In the first area, significant progress was made by the smooth muscle specific generation of mice lacking the rate limiting enzyme in miRNA synthesis, Dicer1. The mice exhibit impaired vascular development, smooth muscle differentiation and contractility. A VSM specific miR, miR 145, strongly regulates the actin cytoskeleton. In the second area, we have performed quantitative proteomic analysis of proteins found in caveolae in vivo. In this analysis, we have discovered that the genetic loss of Caveolin-1, reduces the levels of Caveolin-2 , Cavin -1 and Cavin-2 in lipid rafts. siRNA knockdown of Cavin-1 reduces Caveolin-1 and 2 levels and blocks endothelial cell growth and migration.

3. Publications:
Albinsson S, Sessa WC. Can microRNAs control vascular smooth muscle phenotypic modulation and the response to injury? Physiol Genomics. 2010 Sep 14. [Epub ahead of print] PubMed PMID: 20841497.

Davalos A, Fernandez-Hernando C, Sowa G, Derakhshan B, Lin MI, Lee JY, Zhao H, Luo R, Colangelo C, Sessa WC. Quantitative proteomics of caveolin-1 regulated proteins: Characterization of PTRF/Cavin-1 in endothelial cells. Mol Cell Proteomics. 2010 Jun 28. [Epub ahead of print] PubMed PMID: 20585024.

Fernández-Hernando C, Yu J, Dávalos A, Prendergast J, Sessa WC. Endothelial-specific overexpression of caveolin-1 accelerates atherosclerosis in apolipoprotein E-deficient mice. Am J Pathol. 2010 Aug;177(2):998-1003. Epub 2010 Jun 25. PubMed PMID: 20581061; PubMed Central PMCID: PMC2913373.

Albinsson S, Suarez Y, Skoura A, Offermanns S, Miano JM, Sessa WC. MicroRNAs are necessary for vascular smooth muscle growth, differentiation, and function. Arterioscler Thromb Vasc Biol. 2010 Jun;30(6):1118-26. Epub 2010 Apr 8. PubMed

PMID: 20378849; PubMed Central PMCID: PMC2880481.

Chidlow JH Jr, Sessa WC. Caveolae, caveolins, and cavins: complex control of cellular signalling and inflammation. Cardiovasc Res. 2010 May 1;86(2):219-25. Epub 2010 Mar 3. Review. PubMed PMID: 20202978;

Michael Simons, M.D.
RW Berliner Professor of Medicine & Cell Biology
Chief, Section of Cardiovascular Medicine

1. Overall Goals of the Research Program of the Laboratory

Our laboratory is interested in regulation of arterial morphogenesis and angiogenic growth factor signaling. These process are investigated at all levels, including in vitro signaling studies, in vivo mouse transgenic and knock-out models and translational studies in larger animal models and early phase clinical trials.

2. Specific Research Accomplishments in the last 12 months

We have made significant advances in unraveling AKT-ERK cross-talk in regulation of arterial morphogenesis and in the role of receptor trafficking in control of VEGF-R2 signaling.

In studying arterial morphogenesis we have established that synectin controls arterial maturation by controlling endothelial PDGF-BB production.

3. Significance of Key Findings Relevant for the Mission of VBT

These findings advance our knowledge of molecular details of regulation of vascular development and signaling and should eventually enable the development of new therapeutic paradigms.

4. Publications

Muders MH, Vohra PK, Dutta SK, Wang E, Ikeda Y, Wang L, Udugamsooriya DG, Memic A, Rupashinghe CN, Baretton GB, Aust DE, Langer S, Datta K, Simons M, Spaller MR, Mukhopadhyay D. Targeting GIPC/Synectin in pancreatic cancer inhibits tumor growth. *Clin Cancer Res* 2009; 15:4095-4103

Chen PY, Simons M, Friesel R. FRS2 via FGFR1 is required for PDGFRβ-mediated regulation of vascular smooth muscle marker gene expression. *J Biol Chem* 2009; 284:15980-15992

Elfenbein A, Rhodes JM, Meller J, Schwartz MA, Matsuda M, Simons M. Suppression of RhoG activity is mediated by a Syndecan 4/Synectin/RhoGDI1 complex, and is reversed by PKCα in a Rac1 activation pathway. *J Cell Biol* 2009; 186:75-83

Ren B, Deng Y, Mukhopadhyay A, Lanahan AL, Zhuang ZW, Moodie KL, Mulligan-Kehoe MJ, Byzova TV, Peterson RT, Simons M. Erk1/2-Akt1 cross-talk-dependent regulation of arteriogenesis. *J Clin Invest*, 2010; 120:1271-1228

Lanahan A, Hermans K, Claes F, Kerley-Hamilton JS, Zhuang ZW, Giordano FJ, Carmeliet P, Simons M. VEGF receptor 2 endocytic trafficking regulates arterial morphogenesis. *Developmental Cell* 2010; 18:713-724.

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

1. Overall Goal (s) of the Research Program of the Laboratory

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 of the cardiovascular system. The laboratory is currently focused on targeted molecular imaging, developing noninvasive 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. The laboratory also evaluates the role of angiogenesis on post-MI remodeling.

2. Publications:

Suh JW, Scheinost D, Dione DP, Dobrucki LW, Sinusas AJ, Papademetris X. A non-rigid registration method for serial microCT mouse hindlimb images. Med Image Comput Comput Assist Interv. 2009;12 (Pt 1):688-95. PubMed PMID: 20426048.

Dobrucki LW, Dione DP, Kalinowski L, Dione D, Mendizabal M, Yu J, Papademetris X, Sessa WC, Sinusas AJ. Serial noninvasive targeted imaging of peripheral angiogenesis: validation and application of a semiautomated quantitative approach. J Nucl Med. 2009 Aug;50(8):1356-63. Epub 2009 Jul 17. PubMed PMID: 19617325.

Dobrucki LW, Meoli DF, Hu J, Sadeghi MM, Sinusas AJ. Regional hypoxia correlates with the uptake of a radiolabeled targeted marker of angiogenesis in rat model of myocardial hypertrophy and ischemic injury. J Physiol Pharmacol. 2009 Oct; 60 Suppl 4:117-23. PubMed PMID: 20083860.

Dobrucki LW, Sinusas AJ. PET and SPECT in cardiovascular molecular imaging. Nat Rev Cardiol. 2010 Jan;7(1):38-47. Epub 2009 Nov 24. Review. PubMed PMID: 19935740.

Morrison AR, Sinusas AJ. Advances in radionuclide molecular imaging in myocardial biology. J Nucl Cardiol. 2010 Jan-Feb;17(1):116-34. PubMed PMID: 20012514; PubMed Central PMCID: PMC2823801.

Sinusas AJ. Molecular imaging in nuclear cardiology: translating research concepts into clinical applications. Q J Nucl Med Mol Imaging. 2010 Apr;54(2):230-40. PubMed PMID: 20588216.

Dobrucki LW, Tsutsumi Y, Kalinowski L, Dean J, Gavin M, Sen S, Mendizabal M, Sinusas AJ, Aikawa R. Analysis of angiogenesis induced by local IGF-1 expression after myocardial infarction using microSPECT-CT imaging. J Mol Cell Cardiol. 2010 Jun;48(6):1071-9. Epub 2009 Oct 20. PubMed PMID: 19850049; PubMed Central PMCID: PMC2866767.

Suh JW, Scheinost D, Dione DP, Dobrucki LW, Sinusas AJ, Papademetris X. A non-rigid registration method for serial lower extremity hybrid SPECT/CT imaging. Medical Image Analysis 2010 (in press)

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

1. Overall Goal (s) of the Research Program of the Laboratory:

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 VBT Core D Cell Collection and Processing Laboratory can provide, as requested, cell purification services. 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 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/ 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, 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.

2. Specific Accomplishments in the last 12 months:

In 2009-2010, Core D performed 22 MNC apheresis collections for Program Leaders' research

3. Publications:

Kirkiles-Smith NC, Snyder, EL, et al. Development of a humanized mouse model to study the role of macrophages in allograft injury.Transplantation 2009;87:189-97.

Tormey CA, Sweeney JD, Champion M, Pisciotto PT, Snyder EL, Wu YY. Analysis of transfusion reactions associated with pre-storage pooled platelet components. Transfusion 2009;49:1242-1247

McClaskey,J, Xu M, Snyder EL, Tormey C. Clinical trials for pathogen reduction in transfusion medicine: a review. Transfusion and Apheresis Science 2009;41:217-25.

Tormey CA, Peddinghaus ME, Snyder EL. Improved plasma removal efficiency for therapeutic plasma exchange using a new apheresis platform, Transfusion 2010;50:471-7

Roback JD, Caldwell S, Carson J, Snyder, E, Djulbegovic B. Evidence-based practice guidelines for plasma transfusion. Transfusion 2010;50:1227-1239.

Tadokoro, H., Reesink H W, .Snyder, E L, Champion M H. Problems with irradiators. Vox Sang 2010;98:78-84

Tormey C, Whitley P, Miripol J, Manlove T, Snyder EL. In vitro and in vivo evaluation of a whole blood platelet-sparing leukoreduction filtration system. Transfusion 2010 (in press)

Roh JD, Sawh-Martinez R, Brennan MP, Jay SM, Devine L, Rao DA, Yi T, Mirensky TL, Nalbandian A, Udelsman B, Hibino N, Shinoka T, Saltzman WM, Snyder E, Kyriakides TR, Pober JS, Breuer CK. Tissue-engineered vascular grafts transform into mature blood vessels via an inflammation-mediated process of vascular remodeling. Proc Natl Acad Sci 2010 107:4669-74.

Vassallo R, Adamson J, Gottschall J, Snyder EL, Lee W, Houghton J. In vitro and in vivo evaluation of apheresis platelets stored for 5 days in 65% platelet additive solution/35% plasma. Transfusion 2010, (in press).

Snyder EL, Choate, J. The emergence of cellular therapy: impact on transfusion medicine. Transfusion 2010 (in press).

Wu YY, Mantha S, Snyder EL. Transfusion Reactions. In: Benz EJ, Cohen HJ, Furie B, Hoffman R, Shattil S (eds): HEMATOLOGY Basic Principles and Practice 5th ed. Churchill Livingstone, NY, 2009.

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

Overall Goals of the Research Program of the Laboratory:
The overall goal of the research program of this 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 two MAPK upstream activators MEKK2 and MEKK3, and Sin1, a key component of mTOR complex (TORC) 2. With combined biochemistry and molecular biology approaches, we investigate the biology and regulation of the MEK2/3-MAPK pathway, and the mTORC2 pathway in the immune system and in the vascular system. In the past 12 months, we have studied the roles of MEKK2 and MEKK3 in T cell development, survival and effector differentiation. We also study the role of MEKK3 in endothelial cells and angiogenesis during development. We utilize MEKK3 T cell and endothelial cell conditional knockout mice to understand the specific role of MEKK3 in T cells and endothelia cells. We have revealed critical functions of MEKK3 in T cell homeostasis, cytokine production, and development. We also used inducible gene knockout approaches and revealed an essential function of MEKK3 in brain vascular integrity. Furthermore, we found that both MEKK2 and MEKK3 regulate Treg and Th17 cell differentiation.

The other area of research focus is to investigate the role of Sin1-mTORC2 in embryonic angiogenesis and development. At the molecular level, we revealed a novel function of Sin1-mTORC2 in regulating the protein stability of Akt and PKC through phosphorylation of a conserved threonine residue. We also identified a critical role of Sin1-mTORC2 in B cell development and survival. Our findings from studying both the MEKK3 and 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

Yamazaki K, Gohda J, Kanayama A, Miyamoto Y, Sakurai H, Yamamoto M, Akira S, Hayashi H, **Su B,** Inoue J. Two mechanistically and temporally distinct NF-kappaB activation pathways in IL-1 signaling. Sci. Signal. 2009, 2(93): ra66.

Adam S Lazorchak, Dou Liu, Valeria Facchinetti, Annarita Di Lorenzo, William C Sessa, David G Schatz, **Su B.** Sin1-mTORC2 suppresses *rag* and *il7r* gene expression through Akt2 in B cells

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

1. Overall Goals of the Research Program of the Laboratory:
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. We are also investigating how developmental and environmental signals may modulate vascular smooth muscle cell phenotype by epigenetic mechanisms.

2. Significance of Key Findings Relevant for the Mission of VBT:
Participation in NIH program project and interdisciplinary grant from the Williams Syndrome Association. The support of junior cardiac surgeon-scientist faculty in their research careers.

3. Publications:

Zhou J, Tang PC, Qin L, Gayed PM, Li W, Skokos EA, Kyriakides TR, Pober JS, Tellides G. CXCR3-dependent accumulation and activation of perivascular macrophages is necessary for homeostatic arterial remodeling to hemodynamic stresses. J Exp Med. 2010 Aug 30;207(9):1951-66. Epub 2010 Aug 23. PubMed PMID:20733031; PubMed Central PMCID: PMC2931170.

Ahmad U, Ali R, Lebastchi AH, Qin L, Lo SF, Yakimov AO, Khan SF, Choy JC, Geirsson A, Pober JS, Tellides G. IFN-gamma primes intact human coronary arteries and cultured coronary smooth muscle cells to double-stranded RNA- and self-RNA-induced inflammatory responses by upregulating TLR3 and melanoma differentiation-associated gene 5. J Immunol. 2010 Jul 15;185(2):1283-94. Epub 2010 Jun 18. PubMed PMID: 20562257; PubMed Central PMCID: PMC2902797.

Zhang P, Manes TD, Pober JS, Tellides G. Human vascular smooth muscle cells lack essential costimulatory molecules to activate allogeneic memory T cells. Arterioscler Thromb Vasc Biol. 2010 Sep;30(9):1795-801. Epub 2010 Jun 10. PubMed PMID: 20539019; PubMed Central PMCID: PMC2924449.

Zhang J, Silva T, Yarovinsky T, Manes TD, Tavakoli S, Nie L, Tellides G, Pober JS, Bender JR, Sadeghi MM. VEGF blockade inhibits lymphocyte recruitment and ameliorates immune-mediated vascular remodeling. Circ Res. 2010 Aug 6;107(3):408-17. Epub 2010 Jun 10. PubMed PMID: 20538685; PubMed Central PMCID:PMC2929975.

Al-Lamki RS, Brookes AP, Wang J, Reid MJ, Parameshwar J, Goddard MJ, Tellides G, Wan T, Min W, Pober JS, Bradley JR. TNF receptors differentially signal and are differentially expressed and regulated in the human heart. Am J Transplant. 2009 Dec;9(12):2679-96. Epub 2009 Sep 25. PubMed PMID: 19788501.

Eid RE, Rao DA, Zhou J, Lo SF, Ranjbaran H, Gallo A, Sokol SI, Pfau S, Pober JS, Tellides G. Interleukin-17 and interferon-gamma are produced concomitantly by human coronary artery-infiltrating T cells and act synergistically on vascular smooth muscle cells. Circulation. 2009 Mar 17;119(10):1424-32. Epub 2009 Mar 2. PubMed PMID: 19255340; PubMed Central PMCID: PMC2898514.

Kirkiles-Smith NC, Harding MJ, Shepherd BR, Fader SA, Yi T, Wang Y, McNiff JM,Snyder EL, Lorber MI, Tellides G, Pober JS. Development of a humanized mouse model to study the role of macrophages in allograft injury. Transplantation. 2009 Jan 27;87(2):189-97. PubMed PMID: 19155972; PubMed Central PMCID: PMC2776641.

Agnès Vignery, DDS, PhD
Associate Professor of Orthopaedics and Rehabilitation

1. Overall Goals of our Research Program

Research in our laboratory focuses on two lines of investigation, the commonality of which is osteoporosis, a disease that is at the cross road of the immune, vascular and nervous system. The first 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 are mononucleat cells that 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.
The second research focus is the targeted induction of new bone to specific sites of the skeleton. One of our patent applications was granted last year while two more have been submitted this year.

2. Specific Research Accomplishments in the last 12 months:

Conferences

July 1-6, 2009, chair of the session on macrophage fusion, second Gordon Research Conference (GRC) on Cell-Cell Fusion, a new GRC that I initiated and chaired in 2007: http://www.grc.org/programs.aspx?year=2009&program=cellcell
October 08-11, 2009, Keynote speaker, conference on “Progenitor cells, Microenvironment and Cell Fusion in Cancer Progression” in Stockholm, Sweden

3. Significance of Key Findings Relevant for the Mission of VBT

To initiate the formation of intramembranous bone, in contrast with endochondral bone, requires extensive vascularization. Our finding that new bone forms in targeted sites of 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 vascularisation, which we are actively exploring. In addition, our long-term collaboration with Dan Wu has led to a manuscript in press in PNAS.

4. Publications

Carlson J, Zhang Q, Bennett AM, Vignery A. Deletion of mitogen activated protein kinase phosphatase-1 (MKP-1) modifies the response to mechanical bone marrow ablation in a mouse model. Comparative Medicine 59: 221-226, 2009

Carlson J, Cui W, Zhang Q, Mercant F, Bennett AM, Vignery A. Role of MKP-1 in osteoclasts and bone homeostasis. Am J Pathology, 175:1564-1573; 2009. Epub 2009 Sep 17.

Zhang Q, Carlson J, Ke H-Z, Li J, Kim M, Murphy K, Gilligan J, Mehta N, Vignery A. Dramatic increase in cortical thickness induced by femoral marrow ablation followed by a three-month treatment with PTH in rats. J Bone and Min Res, 25:1350-1359, 2010

Kang H, Chang W, Hurley M, Vignery A, Wu D. Important Roles of PI3Kγ in Osteoclastogenesis and Bone Homeostasis. Proc Natl Acad Sci USA*, In press*

Zhang Q, Carlson J, Gilligan J, Mehta N, Vignery A. Aging potentiates the site-specific formation of new bone after marrow ablation and treatment with PTH in rats. *In preparation*

Vignery, A. “Macrophage Fusion: the making of a new cell”: invited chapter in book entitled ”Cell Fusions: Regulation and Control", Lars-Inge Larsson Ed., Springer, *in press*

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. VBT continues to explore additional opportunities for university-corporate partnerships, especially in translational applications of VBT scientific discoveries. VBT members continue the relationship with Boehringer-Ingelheim with two new research projects.

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 support to our annual retreat

WILLIAMS SYNDROME/CHLOE'S QUEST FOUNDATION
VBT members have started a 2 year pilot project and continue to meet with Chloe's Quest Foundation.

APPENDIX 1

The Ninth Annual VBT Retreat

The Ninth Annual Retreat of Vascular Biology and Therapeutics Program held in conjunction with Cardiovascular Medicine

Saturday, November 7, 2009, 8:00 A.M. – 5:00 P.M. (Lunch Included)

8:00 -8:30	Registration - Continental Breakfast
SESSION I	
8:30-10:00	**Tissue Engineering - Session Chair – Themis Kyriakides, PhD**
8:30-8:50	Joe Madri, MD, PhD – "Modeling the neurovascular niche: Implications for recovery from CNS injury"
8:50-9:00	Questions
9:00-9:20	Mark Saltzman, PhD – "Controlled Delivery of Proteins, Genes, and Oligonucleotides in Tissue Engineering"
9:20-9:30	Questions
9:30-9:50	Zhenwu Zhuang, MS, MD - "microCT & tissue engineering"
9:50-10:00	Questions
10:00 -10:20	**Coffee Break**
SESSION II	
10:20-11:50	**Vascular Cell Signaling - Session Chair – Michael Simons, MD**
10:20-10:40	Hyung Chun – "Apelin Singaling in Animal Models of Vascular Pathology"
10:40-10:50	Questions
10:50-11:10	Kathleen Martin, PhD – "mTOR signaling in VSMC differentiation"
11:10-11:20	Questions
11:20-11:40	Wang Min, PhD – "Bone marrow tyrosine kinase Bmx in inflammatory arteriogenesis, angiogenesis and lymphangiogenesis"
11:40-11:50	Questions
11:50-1:30	**Lunch and Poster Session**

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

SESSION III

1:30 – 3:20	**Mechanisms of Inflammation – Session Chair – Jack Elias, MD**
1:30 -1:50	Patty Lee, MD - "TLR4-mediated Anti-Oxidant Pathways"
1:50 – 2:00	Questions
2:00 – 2:20	Je-Min Choi, Ph.D., "Regulation of inflammation by delivery of cell permeable Foxp3 protein"
2:20 – 2:30	Questions
2:30 – 2:50	Bing Su, PhD - "MEKK2 and MEKK3 suppresses TGF-beta signals in helper T cell differentiation"
2:50 – 3:00	Questions
3:00 – 3:20	**Coffee Break**
3:20 -3:30	**Introduction Keynote Speaker – William Sessa**
3:30 – 4:20	**Keynote Address** **William Aird, MD, Beth Israel Deaconess Medical Center, Harvard University** **Title: "The brain arachnoid displays a unique pluripotent vascular phenotype"**
4:20 – 4:30	Questions
4:45	**Announcement of Poster Contest Winners**

APPENDIX 2

The 8th Annual Meeting of the Joint Cambridge-Yale Program in Cardiovascular Research

Cambridge Yale Cardiovascular Research Program

11- 13 September 2009

Amistad Research Building

Friday, September 11, 2009

7:00 The Graduate Club
155 Elm St, **New Haven**, **CT** 06511
(203) 624-3197

Saturday, September 12, 2009

8:30 – 9:00 Continental Breakfast, Amistad

SESSION I: VASCULAR BIOLOGY 9-10:30; 11-12:00 (Chair- Sir Keith Peters)

9:00 - 9:15 Jordan Pober & John Bradley
Welcome & Introduction

9:15 – 9:30 Martin Bennett
"Mesenchymal stem cell-derived vascular smooth muscle cells in atherosclerosis"

9:30 – 9:45 Mike Simons
"Location, duration, causation"

9:45 – 10:00 Willem Ouwehand
"Quantitave trait loci for mean platelet volume and platelet count"

10:00 – 10:15 Bill Sessa
"Akt-eNOS signaling in angiogenesis"

10:15 - 10:30 Ziad Mallatt
"Immuno-regulatory pathways in atherosclerosis"

10:30 – 11:00 Break

11:00 – 11:15 Jack Elias
"Regulation of VEGF Responses in the Lung"

11:15 – 11:30 Steve Charnock-Jones
"sFlt-1, endothelial cell activation and pre-eclampsia"

11:30 – 11:45 Sylvia Nurnberg
"Transcriptional regulation of the megakaryocyte – From common genetic variation to platelet phenotype

11:45 – 12:00 Andrew Bradley
"Mechanisms of allorecognition"

SESSION II. TRANSPLANTATION 11:45-12:30: 1:30-2:30 (Chair- John Wallwork)

12:00 – 12:15 George Tellides
"Medial Immunoprivilege"

12:15 – 12:30 Reza Motallebzadeh
"The role of LTbetaR signaling in the formation of tertiary lymphoid organs in cardiac allografts"

12:30 - 12:45 Jordan Pober
"The Immunology of Human Pericytes"

12:45 -1:45 Lunch

1:45 – 2:00 Craig Taylor
"Physiochemical modelling of HLA class I amino acid polymorphism enables prediction of the magnitude of humoral immune responses"

2:00 – 2:15 Daniel Goldstein
"A new pathway of inflammation and alloreactivity with aging"

2:15 – 2:30 John Bradley
"In situ cardiac responses to TNF"

SESSION III: IMMUNOLOGY I 3-4 (Chair- Nancy Ruddle)

2:30 – 2:45 Doug Fearon
"An Immunosuppressive Stromal Cell Associated with Chronic Inflammation and Cancer"

3:45 – 3:15 Break

3:15 – 3:30 Sue Kaech
"Memory T cell differentiation"

3:30 – 3:45 Menna Clatworthy
"Immune inhibition in infection and autoimmunity"

3:45 – 4:00 Eitan Akirav
"Control of beta cell function by islet vasculature in Type 1 diabetes"

4:00 – 4:15 Su Metcalfe
"Targeted nanotherapy to modulate T lymphocyte function"

4:15 - 4:30 Paul Lehner
"Ubiquitin E3 ligases link MHC I molecules to renal cancer""

4:30 – 4:45 Pete Cresswell
"Antigen cross-presentation to MHC class I-how do external proteins get in"

6:00 Central Steak House
99 Orange Street - New Haven - Ct - 06510
(203) 787 – 7885 http://centralsteakhouse.com/

Sunday, September 13, 2009

8:30 – 9:00 Continental Breakfast, Amistad

SESSION IV: IMMUNOLOGY II 9-10:45; 11-11:30 (Chair-Patrick Sissons)

9:00 – 9:15 Andres Floto
"How mycobacterial heat shock proteins shape host immunity"

9:15 – 9:30 John Todd
"From gene to phenotype in autoimmune disease using a genotype-selectable bioresource"

9:30 – 9:45 Judy Cho
"Genetics and genomics of inflammatory bowel disease"

9:45 – 10:00 Richard Flavell
"Human immune system mice"

10:00 – 10:30 Break

SESSION V; STEM CELLS 10:45 - 12:30; 1:30-2:30 (Chair- Laura Niklason)

10:30– 10:45 Roger Pedersen
"Mechanisms of pluripotency and differentiation in mammalian embryonic stem cells"

10:45 – 11:00 Yingquin Huang
Molecular function of Lin28 in human embroyonic stem cells

11:00 – 11:15 Ludovic Vallier
"Similarities and divergences between human Embryonic Stem Cells and Induced Pluripotent Stem cells"

11:15 – 12:00 Sherman Weissman
"Genomic approaches to study of in vitro myelopoiesis and erytrhropoiesis"

12:00 -1:00 Lunch

1:00 – 1:15 Diane Krause
"MKL1 in hematopoiesis and leukemogenesis"

1:15 – 1:30	Harold Ayetey “Induction of Pluripotency for Disease Modelling in Cariovascular Medicine”
1:30 – 1:45	Haifan Lin “ A novel Small RNA-Mediated Epigenetic Mechanism Related to Stem Cells”
1:45 – 2.00	Sanjay Sinha “Regulation of vascular smooth muscle cell disease phenotype – novel roles for myocardin”
2:00 – 2.15	Chris Breuer “Tissue engineered vascular grafts”
2:15 –2:30	Sheikh Tamir Rashid “Development of in vitro models to study metabolic diseases using human Induced Pluripotent Stem Cells”