



SURGERY RESEARCH

DEPARTMENT OF SURGERY 2007 REPORT



UW Medicine
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Research in the Department of Surgery University of Washington School of Medicine

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UNIVERSITY OF WASHINGTON

DEPARTMENT OF SURGERY

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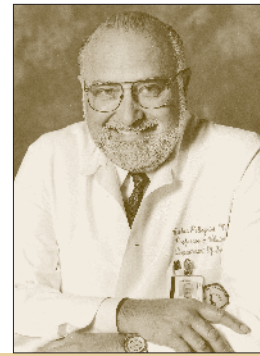
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Quality of Life Depends on Basic Research



Research is a most important component of the life of our Department and our School. It serves us directly by answering questions that eventually result in the development of newer therapeutic modalities, but its effect extends well beyond our Department. Indeed, research offers a unique way to develop the mind, the character and the judgment of many of our young trainees; it provides a unique niche to members of our faculty who through research become “differentiated” and eventually gain national recognition; and it is an important stimulus to our senior faculty as well as a unique opportunity to mentor people.

For many years our Department concentrated all its research efforts in the basic science arena. This form of research—the more “pure” science—led to the development of programs in inflammation, vascular biology, wound healing and ischemia reperfusion, to name just a few that became well-funded and nationally recognized. This enterprise serves as a springboard to many residents, fellows and junior faculty who eventually gain substantial reputation and the respect of their peers because of it. This type of research continues to be at the core of the enterprise in the Department, as can be seen throughout this report. Its application to practical problems (applied and/or translational research) has, in turn, contributed to the enhancement of the quality of life for your family and loved ones.

At the same time, over the last few years our Department has developed major research interests in the health services. At Harborview, for example, we have the Injury Prevention & Research Center where **Dr. Charles (Charlie) Mock** served as director. As a result of the excellence of his work, the World Health Organization recently offered Charlie a leadership position in the area of trauma. Although he remains on our faculty, this honor does require him to relocate to Geneva for the next few years.

Likewise under the leadership of **Dr. David Flum**, we have created a powerhouse at the UW campus. The Surgical Outcomes Research Center (SORCE) emphasizes an evidence-based approach to surgical care and concentrates the interests of researchers who evaluate the processes of care that relate to optimal outcomes. Using the tools of epidemiology, health economics, and the behavioral sciences, SORCE integrates aspects of vascular, thoracic, plastic, neurosurgical, urologic and gastrointestinal surgery. With faculty from the Schools of Medicine and Public Health, it crosses surgical specialties and institutions to train fellows, develop research ideas, secure funding for studies, implement protocols, and complete high impact research. It also works with state and

national agencies to develop consortium-based research and quality improvement activities that incorporate evidence-based practice to improve surgical care. SORCE also serves as the research home for the Surgical Care and Outcomes Assessment Program (SCOAP)—a statewide quality improvement initiative linking state hospitals in a prospective surveillance and response system for surgical safety and quality.

It has become clear through the years that the older model of research—namely, the single isolated investigator working in his/her laboratory with a team—is no longer capable of delivering the high-end output and to sustain the effort in most fields. Instead, researchers today join forces through the disciplines and create large groups of individuals with a single general theme. This “working together in a focused area” yields the best chances of success in a modern world.

While no single criterion exists to establish the quality of research conducted at an academic institution, the level of National Institutes of Health (NIH) funding often is accepted as a benchmark in terms of both quantity and quality. During this past fiscal year (FY06), our faculty held a total of 91 active grants and received a total of \$7.8 million in NIH funding. While this still represents a healthy program, it does reflect a 16% decrease in funding over FY05. This decrease primarily reflects the overall NIH funding cutbacks and is being felt at other academic institutions across the nation. Other funding sources include the Centers for Disease Control, U.S. Army & Navy, and other federal & private peer-reviewed sources. During FY06, we received \$3.2 million in non-NIH federal monies and \$2.8 million in non-federal funding including sponsored funds and research gift funds.

Thus, our Department continues to focus on our three-fold mission. First, we offer the best surgical care to you, your family, and your friends. Second, we conduct research to improve the manner in which healthcare is delivered. Finally, we share this knowledge with our medical colleagues for use in their practice. If you would like more information about how you can help further any of the research projects in this report, please contact Lynn Hogan, Executive Director of Medical Affairs Development, at (206) 543-5686 (lhogan@u.washington.edu).

A handwritten signature in dark ink, appearing to read 'Pellegrini'.

Carlos A. Pellegrini, M.D.

The Henry N. Harkins Professor and Chairman

SELECTED RESEARCH HONORS & AWARDS FROM 2006

JOSEPH CUSCHIERI, M.D., ASSISTANT PROFESSOR, received the *Joseph Susman Memorial Award* at the Surgical Infection Society for his paper titled “Acid Sphingomyelinase Is Required for LPS-Mediated Macrophage Activation.”

LOREN ENGRAV, M.D., PROFESSOR, was awarded an NIH R21 (High Risk/High Gain) grant for his study, “Hypertrophic Scarring after Partial-Thickness Wounds.” At age 63, Dr. Engrav ranks in the oldest 1% of NIH R21 New Investigators. **Drs. Kathy Zhu, Nicole Gibran, Frank Isik,** the Washington State Council of Firefighters Burn Foundation, and the National Institute on Disability and Rehabilitation Research made this grant possible.

JEFFREY FRIEDRICH, M.D., ASSISTANT PROFESSOR (then R6), won the award for Best Hand Paper at the Plastic Surgery Senior Residents Conference. The title of his paper is “Upper Extremity Reconstruction with Radial Forearm Fascia Flaps.”

AARON R. JENSEN, M.D., (then) R2, was awarded a one-year, home-site Surgical Education Research Fellowship (SERF). Supported jointly by the Association for Surgical Education and Ortho Biotech, SERF is designed to equip investigators with the skills and knowledge needed to plan, implement and report research studies in the field of surgical education. Dr. Jensen is one of only 12 fellows accepted into the program.

GRANT O’KEEFE, M.D., PH.D., PROFESSOR, received a Royalty Research Grant from the American Surgical Association. The title of Dr. O’Keefe’s work is “B-Adrenergic Blockade and Inflammatory Responses in Post-Traumatic Sepsis.”

STEPHEN SULLIVAN, M.D., (then) R5, won the award for best resident paper at the annual meeting of the Northwest Society of Plastic Surgeons. The title of his paper is “Malignant Melanoma of the Head and Neck and Safety of Immediate Reconstruction.”

SPECIAL MENTION: *The New England Journal of Medicine* published a national study authored by **Drs. Ellen MacKenzie, Fred Rivara, & Jerry Jurkovich** on the effectiveness of Level I trauma centers. Jointly funded by the Centers for Disease Control and Prevention and the National Institutes of Health, this analysis of data from the National Study on the Costs and Outcomes of Trauma is among the first to provide strong evidence that level I trauma centers are effective in preventing deaths from injuries.

This study, “A National Evaluation of the Effect of Trauma Center Care on Mortality,” analyzed the outcomes of 5,190 adult trauma patients who received treatment at 18 hospitals with Level I trauma centers and 51 hospitals without a trauma center. It found that care at Level I trauma centers can lower the risk of death for injured patients by 25% compared to treatment received at non-trauma centers.

CARDIOTHORACIC SURGERY

GABRIEL S. ALDEA, M.D.

MICHAEL S. MULLIGAN, M.D.

EDWARD D. VERRIER, M.D.

Gabriel S. Aldea, M.D.

• Minimizing Morbidity of Cardiopulmonary Bypass



FUNDING

National Institutes of Health
SCCOR grant
Edwards Cardiovascular
The Medicines Company
Medtronic, Inc.

National Research Service
Award in Heart and
Vascular Diseases
Proctor and Gambel (Alexion)

Despite advances in traditional techniques, coronary artery bypass graft cardiac surgery is associated with a mortality rate of 1-4%, as well as a 1-4% incidence of perioperative myocardial infarction (MI) and stroke, or changes in neurological and neuropsychological function. Alternatives to traditional cardiac surgical methods, including “minimally invasive” techniques, are being developed to limit morbidity associated with conventional cardiac surgery. Although much effort has been focused on smaller alternative incisions to median sternotomy, much of the morbidity of cardiac surgery is related to manipulation of an atherosclerotic aorta (embolization) and artificial perfusion and to the

and leave no residual deficits, they can lead to long-lasting cardiac, pulmonary, renal and neurological dysfunction in a subset of patients with limited reserve.

Using recent advances in perfusion technology and research in biomaterial sciences we have developed specific surgical techniques that have resulted in the routine application of more biocompatible circuits, such as heparin-bonded cardiopulmonary bypass circuits with alternatives to full anticoagulation protocol. In the laboratory, these techniques have been demonstrated to blunt the inflammatory response to CPB and promote hemostasis.

Our results suggest that cardiotomy suction should be eliminated whenever possible.

biological response of the body to artificial perfusion and gas exchange through the non-endothelialized cardiopulmonary bypass (CPB) circuit. These effects may be compounded by the effects of autologous transfusion.

Within seconds of CPB, formed and unformed blood elements come into contact with the large surface area of the CPB circuit. Despite anticoagulation with heparin, this interaction results in extensive activation of platelets, neutrophils, complement, cytokines and the fibrinolytic system, producing a complex and intense “inflammatory” response. Furthermore, response to CPB is very heterogeneous and varies tremendously between patients, with some patients manifesting marked inflammatory changes and other little or none. Although these responses are usually short lived

Clinically, the use of these circuits and techniques reduced the need for homologous transfusion and decreased neutrophil and complement activation, resulting in a reduction in thromboembolic complications, myocardial and pulmonary dysfunction, postoperative morbidity, and cost. The use of heparin-bonded circuits also has resulted in a dramatic decrease in the incidence of perioperative MI to less than 1%, neurological deficits to less than 1%, and pulmonary complications to 1.5%. Compared to previous reports, the incidence of neurological and persistent neuropsychological deficits following CABG were markedly reduced to near baseline.

Figure 1 shows a representative scanning EM at 200-fold magnification of the arterial filter (the last barrier to debris before the blood from the CPB circuit reaches the

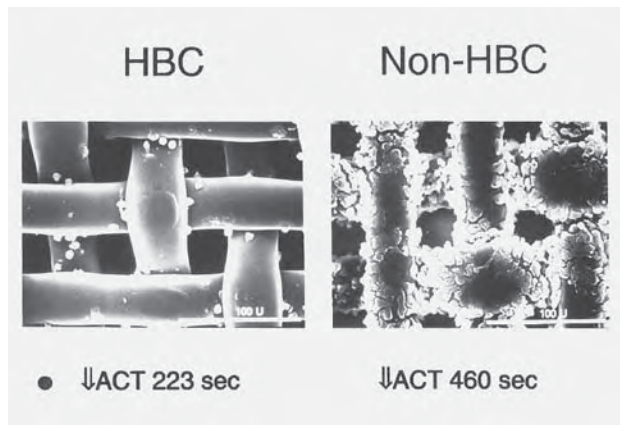


FIGURE 1: Scanning electron micrographs at 200 fold magnification of arterial filter. Lowest activating times (ACT) in seconds are noted. HBC= heparin-bonded circuits. Non-HBC- control non-heparin-bonded circuits.

systemic circulation). This comparison demonstrates dramatic reduction (quantified in 60 patients to be >80% reduction) in debris and inflammation resulting from the use of biocompatible heparin-bonded circuits with reduced anticoagulation protocol (HBC) compared with conventional non-biocompatible circuits with full anti-coagulation.

We are involved in several ongoing clinical investigations to study ways to disassociate the contribution of biocompatible circuits from the specific surgical techniques (the effects of cardiomy suction vs. use of cell saver technology) on markers of hemostasis, inflammation, neurological and neuropsychological deficits. Although both result in blood conservation, one (cardiomy suction) re-infuses blood directly from the surgical field into the arterial side of the CPB machine. Cell saver technology, though not perfect, washes the cells prior to intravenous re-infusion. These different approaches result in markedly different effects on inflammation and thrombin generation during artificial perfusion. This research may lead to changes in both the design and application of this technology.

Heparin bonded circuits (HBC) have been proven to be effective in several research groups, including our own, in preserving platelet function and decreasing inflammation during CPB. However, markers of thrombin generation (PF1.2), inflammation (IL-6, IL-8, elastase, complement), platelet function (β -thromboglobulin) and neurological injury (neuron specific enolase, S-100b) are *all* nearly completely blunted when HBC are used and cardiomy suction is eliminated during CPB. Our results suggest that cardiomy suction should be eliminated whenever possible. Our results challenge long held precepts that adverse

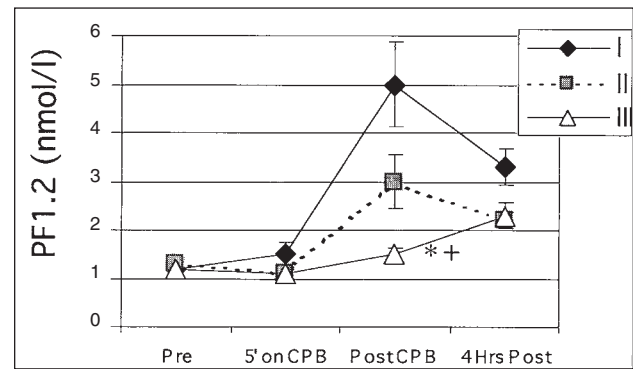


FIGURE 2: PF1.2 for thrombin generation.

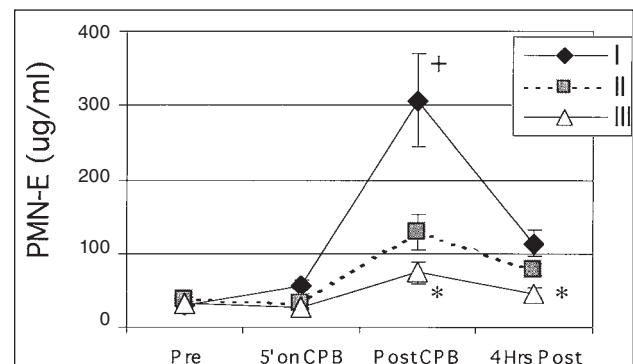


FIGURE 3: PMN-E for elastase.

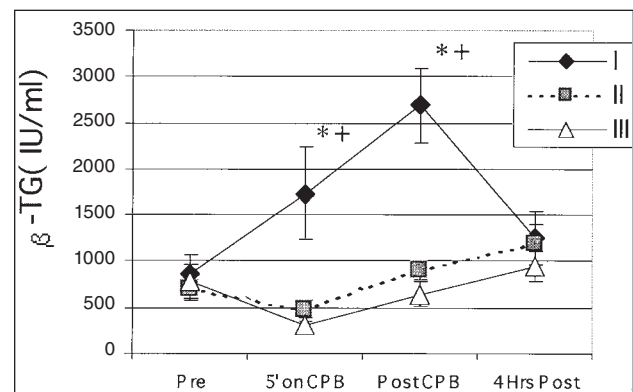


FIGURE 4: β -Thromboglobulin for platelet activation.

outcomes possibly associated with thrombin generation, inflammation and platelet activation are inevitable whenever CPB is used (Figures 2-4).

We continue to investigate novel targeted pharmacological interventions as well as further biomaterial modifications of the perfusion surface to further attenuate platelet, neutrophil, and complement activation, and cytokine release.

With the increasing incidence and awareness of HIT(T) we have evaluated alternatives to heparin anticoagulation using the short acting direct thrombin inhibitor Bivalirudin and have demonstrated safety and efficacy. The significance of post CPB HIT antibody conversion on long-term outcomes and the importance of limiting ubiquitous uncontrolled use of UFH is the focus of our future studies.

We are becoming more aware of differences and individual variability between individual patients in expressing such responses to CPB with some patients having a minimal response and others having very accentuated responses to CPB. We are trying to determine ways to identify individual biological susceptibility *prior* to surgery so we

can alter surgical technique (either avoid CPB altogether or use a combination of altered equipment, techniques and pharmacological therapy) and hope to develop reliable specific biological assays to predict an individual patient's response to artificial perfusion and direct clinical therapy. Finally, we also recognize that both CPB and transfusion may change patients' immunity and immunization and perhaps affect long term outcomes. We will study these interactions in collaborations with Drs. Nelson and Slichter in a three-year NIH SCCOR-sponsored study.

RELATED PUBLICATIONS

1. Vander Salm TJ, Kip KE, Jones RH, Schaff HV, Shemin RJ, Aldea GS, Detre KM. What constitutes optimal surgical revascularization? Answers from the Bypass Angioplasty Revascularization Investigation (BARI). *J Am Coll Cardiol* 2002; 39:565-72.
2. Aldea GS, Soltow LO, Chandler WL, Triggs CM, Vocolka CR, Crockett GI, Shin YT, Curtis WE, Verrier ED. Limitation of thrombin generation platelet activation and inflammation by elimination of cardiomy suction in patients undergoing coronary artery bypass grafting treated with heparin-bonded circuits. *J Thorac Cardiovascular Surg* 2002; Apr 123(4):742-55.
3. Aldea GS, Goss R, Boyle EM et al. Use of off-pump and on-pump CABG strategies in current clinical practice: the Clinical Outcomes Assessment Program of the State of Washington. *J Card Surg*; 2003;18:206-215.
4. Dabal RJ, Goss R, Maynard C and Aldea GS. The effect of left internal mammary artery utilization on short-term outcomes after coronary revascularization. *Ann Thorac Surg*; 2003; 76:464-70.
5. Eisses MJ, Aldea GS, Chandler WL. Reducing Hemostatic Activation during CPB: a Combined Approach. *Anesth & Analg*; 2004; 98(5):1208-16.
6. Aldea GS. Total leukocyte control for elective coronary bypass surgery does not improve short-term outcome (Invited Commentary). *Ann Thorac Surg*, 2005;79(6):2038-9
7. Eisses MJ, Velan, T, Aldea GS, Chandler WL. Strategies to reduce hemostatic activation during cardiopulmonary bypass. *Thrombosis Research* 2005; Jul 1.
8. J Richard Goss, MD, MPH, Charles Maynard, PhD, Gabriel S Aldea, MD, et al. Effects of a statewide physician-led quality improvement program on the quality of cardiac care. *American Heart Journal* 2006; 151: 1040-9.
9. Koster A, Dyke CM, Aldea GS, Smedira NG, McCarthy HL, Aronson S, Hetzer R, Avery E, Speiss B, and Lincoff M. Bivalirudin during cardiopulmonary bypass in patients with previous or acute heparin-induced thrombocytopenia and heparin antibodies: results of the CHOOSE-On Trial. *Ann Thorac Surg* 2007; 83: 572-79.
10. Aldea GS, Takayama H, Soltow L et al. Differential expressions of markers for thrombin generation, platelet activation and inflammation in cell saver versus systemic blood in patients undergoing on pump CABG. *J Card Anesth*, 2007: publication pending

DEPARTMENT CO-INVESTIGATORS

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Michael S. Mulligan, M.D.

• Cytokines and Chemokines in Direct Ischemia Reperfusion Injury of Lung and Cardiothoracic Transplant Rejection



AWARDS

Schilling Lecture, University of Washington
Seattle Surgical Society, Best Presentation
Resident Teaching Award 2000

FUNDING

Bayer Corporation
Novartis
PrimeSource Surgical
Thoracic Society Directors Association

Lung transplantation, which was introduced into clinical practice nearly twenty years ago, has become an option for selected patients with end stage lung disease. Refinements in patient selection, perioperative care and immunosuppression have resulted in improved three-year survivals of 70%. Despite these improved outcomes, ischemia-reperfusion, an unavoidable consequence of transplantation, compromises the early and late function of the transplanted lung. Twenty-five percent of transplant recipients experience some degree of reperfusion injury. In addition to acute morbidity, this acute inflammatory injury may compromise the long-term viability of the graft.

Attempts to alleviate immediate reperfusion injury in the grafted lung have focused on improving preservation techniques, minimizing ischemic times and modifying preservation solutions. More recently a number of studies investigated the role of cytokines and inflammatory peptides in the pathophysiology of reperfusion injury. Roles for several cytokines in reperfusion injury in clinical lung transplantation have been postulated for some time and animal studies suggest that these mediators may play a critical role. Chemokines of the IL-8 family have been isolated in various models of inflammation and may be involved in mediating reperfusion injury.

The chemokines are a family of chemotactic cytokines with a high degree of specificity for subpopulations of leukocytes. Four groups of chemokines have been characterized based on the structure of the peptides, CC, CXC, CX3C, and C. The CC chemokines or the β chemokines have two adjacent cysteine residues, and function primarily as monocyte and lymphocyte chemotactic agents. Members of this family include MCP-1, RANTES and MIP-1 α , MIP-1 β , to name just a few. The second group, the CXC chemokines,

are also referred to as the α chemokines. This group is characterized by the presence of an amino acid between the two cysteine residues, and includes powerful neutrophil chemoattractants, such as IL-8, MIP-2, and CINC. Two recently discovered groups of chemokines include the C and CX3C families. These chemokines are chemotactic for T lymphocytes and monocytes and include lymphotactin (C) and fractalkine, also known as neurotactin (CX3C).

Reperfusion injury in rat lungs has been shown to be complement-dependent and oxygen radical mediated. It peaks in severity after four hours of reperfusion as assessed by tissue hemorrhage, vascular permeability and accumulation of neutrophils. This is strikingly similar to other models of acute lung injury such as immune-complex alveolitis, anti-basement membrane associated injury and secondary lung injury after remote tissue ischemia. A number of cytokines have been identified (i.e. TNF α , IL-1 β , PAF) as important mediators in these models and to a lesser degree, in lung reperfusion injury.

Likewise three C-C chemokines, MCP-1, MIP-1 α , and RANTES, have been shown to play roles in the development of several of these models, but only IL-8 has been investigated for any potential role in lung ischemia reperfusion injury. MIP-1 α is upregulated *in vitro* following hypoxic stress and increased MIP1 α messenger RNA is found in liver allografts shortly after reperfusion. Secondary lung injury develops following reperfusion of ischemic limbs, and liver that is at least partially regulated by C-C and potentially C-X-C chemokines. These findings would suggest that chemokines are likely to play some role in regulating direct ischemia reperfusion injury of the lung.

A model of hilar isolation for the study of ischemia reperfusion injury of rat lung has been reproducibly established and standardized in our laboratory. A pattern of mRNA expres-

sion for MIP-1 α in reperfusion injury has been suggested by preliminary findings. Unmanipulated control lungs and those from animals undergoing ischemia plus 0.5, 1, 2, 3 and 4 hours of reperfusion were extracted for MIP-1 α mRNA. Message was not detectable in the unmanipulated lung but appeared at 30 minutes of reperfusion and was present throughout the reperfusion period. Using ELISA technology developed in our laboratory, we have also demonstrated increased protein expression MCP-1 (C-C), and CINC (C-X-C) content in BAL fluid from reperfused lungs (data not shown).

Lung injury as assessed by vascular leakage of ^{125}I labeled BSA has been determined as a measure of injury severity. The permeability index among negative (unmanipulated) controls is consistently 0.09 ± 0.05 . Permeability doubled in

In addition to the direct lung ischemia reperfusion projects, we have investigated two *in vivo* models of thoracic transplantation. The first of these models investigates the major impediment to long term survival in lung and heart lung transplantation-chronic rejection which is histologically defined as obliterative bronchiolitis (OB). OB affects 33 -60% of long term lung and heart lung transplant recipients patients in recent series and more than 60% of patients in prior reports. Clinically, OB is characterized by progressive dyspnea, non-productive cough, reductions in the FEV-1 and mid-expiratory flow volumes. Treatment typically consists of intensification of immunosuppressive therapy or substitution of medications in a standard post-transplant triple medication regimen. Such therapy is at best capable of slowing the rate of progression but this disease is characteristically progressive and ultimately fatal.

The alveolar macrophage appears to be the key effector cell early in the reaction and we are looking at its response to hypoxia and reoxygenation in vitro as well.

animals undergoing only thoracotomy and mechanical ventilation. Ninety minutes of ischemia did not significantly increase mean permeability values; however, four hours of reperfusion resulted in an eight-fold rise in lung permeability to a mean index of 0.75 ± 0.01 ($p < .001$ compared to controls). In contrast, animals treated with blocking antibody to MIP 1 α , experienced a mean 35% reduction in permeability compared to injured controls ($p < .001$). The lungs were also analyzed for myeloperoxidase (MPO) content as a measure of tissue neutrophil accumulation.

Increased tissue neutrophil content is detectable after two hours of reperfusion, is significant by three hours and is marked by four hours. In contrast, lungs from animals treated with anti-MIP-1 α demonstrated a 42% reduction in MPO content compared to four hours reperfused controls ($p = .02$). Ongoing studies are also investigating the mechanisms of chemokine regulation of reperfusion injury. The alveolar macrophage appears to be the key effector cell early in the reaction and we are looking at its response to hypoxia and reoxygenation *in vitro* as well. We have also developed strategies for blocking multiple chemokine receptors and interfere with common second messenger pathways. These studies should reveal the maximal effectiveness of chemokine blockade at numerous points in the inflammatory cascade.

Recent investigations have attempted to define the mediators involved in the development of OB but these experiments have been limited by the inability to develop a practical and reproducible model. Whole organ transplants are desirable but such studies are confounded by technical complications, and the costs can be prohibitive. A technically simple model for airway transplantation with histopathologic features of OB has gained acceptance. This technique, originally described in mice and now adapted to rats, produces an experimental OB that is histologically indistinguishable from human OB. We have used this model to investigate the potential role of beta chemokines in the development of experimental OB.

In addition to a variety of other mediators, two of the β - chemokines, MCP-1 and RANTES, were studied for their potential role in the development of obliterative bronchiolitis. Rat tracheas and main stem bronchi were heterotopically transplanted into the subcutaneous tissue of allogeneically mismatched (BN- LEW) or syngeneically matched (LEW-LEW) recipients. Control animals received daily injections of PBS or non- immune rabbit serum; additional animals were treated with polyclonal blocking antibodies against MCP-1 or RANTES. Tissue was explanted at two weeks and examined histologically

to quantify change in airway cross sectional diameter and loss of epithelium. Northern and Western blot analysis were performed to measure upregulation of MCP-1 and RANTES mRNA and protein.

Syngeneic control animals demonstrated mild to moderate peri-tracheal inflammation, but near complete preservation of respiratory epithelium and airway cross sectional area. In contrast, allograft controls demonstrated a dense pan-mural inflammatory response, near complete loss of respiratory epithelium and a 60% reduction in airway cross-sectional area. Animals treated with anti-MCP-1 or anti-RANTES antibodies had more limited histologic changes including only a 12% and 26% reduction in cross-sectional area respectively ($p < .001$). Levels of MCP-1 and RANTES mRNA were also increased in allograft tracheas but not in isografts. These data suggest that MCP-1 and RANTES play important regulatory roles in the development of experimental OB.

A heterotopic rat heart transplant model is also being used to determine the role of CC chemokines in heart allograft function and rejection. This model, which is technically

challenging, involves a precise dissection of the donor heart using a 10x operating microscope followed by a hand sewn anastomosis using 8-0 suture. The hearts are explanted at various time points and the laboratory is currently gathering data on the role of chemokine blockade on cytokine expression and abrogation of rejection.

In addition to the *in vivo* work done in the Mulligan lab, there is significant complementary *in vitro* work. All of the chemokines and cytokines discussed previously will be investigated in tissue sample using ELISA and Western Blot for protein analysis and Northern and RPA blots for mRNA analysis. The *in vivo* work is therefore complemented by sophisticated molecular techniques. With this in mind, the lab has embarked on a project to reconstitute reperfusion injury using cell culture. Specifically culture of type II pneumocytes, alveolar macrophages, pulmonary artery endothelial cells and neutrophils will be undertaken separately and in combination to elucidate the specific response of these cells to hypoxia and reoxygenation.

RELATED PUBLICATIONS

1. Mulligan MS, Lentsch AB, Huber-Lang M, Ren-Feng G, Sarma V, Wright CD, Ulich TR, Ward PA. Anti-inflammatory Effects of Mutant Forms of Secretory Leukocyte Protease Inhibitor. *Am J Pathol* 2000 156 (3) : 1033-1039.
2. Mulligan MS, Warner RL, Rittershaus C, Thomas LJ, Ryan US, Foreman KE, Crouch LD, Till GO, Ward PA. Endothelial Targeting and Enhanced Anti-inflammatory Effects of Complement Inhibitors Possessing Sialyl Lewis X Moieties. *J Immunol* 1999. April 15; 162 (8): 4952-9.
3. Mulligan MS, Lentsch AB, Shanley TP, Miyasaka M, Johnson KJ, Ward PA. Cytokine and Adhesion Molecule Requirements for Lung Injury Induced by Anti-Glomerular Basement Membrane Antibody. *Inflammation*, 22(4): 403-417, 1998.
4. Mulligan MS, Warner RL, Lowe LB, Smith PL, Suzuki Y, Miyasaka M, Yamaguchi S, Ohta Y, Tsukada Y, Kiso M, Hasegawa A, Ward PA. In Vitro and in Vivo Selectin-Blocking Activities of Sulfated Lipids and Sulfated Sialyl Compounds. *Intl. Immunol.* 10 (5): 569-575. 1998
5. Mulligan MS, Miyasaka M, Suzuki Y, Kawashima H, Iizuka M, Suzuki T, Hasegawa A, Kiso M, Warner RL, Ward PA. Anti-inflammatory Effects of Sulfatides in Selectin-Dependent Acute Lung Injury. *Int. Immunology.* 7:10, pp1107-1113. 1998
6. Mulligan MS, Schmid E, Till GO, Friedl HP, Hugli TE, Roth RA, Ward PA. C5a-Dependent Upregulation of P-Selectin in Vivo. *J Immunol.* 1997 Feb. 15; 158(4):1857-61
7. Mulligan MS, Shanley T, Foreback J, Warner R and Ward PA. Protective Effects of IL-4, IL-10, IL-12 and IL-13 in IgG Immune Complex-Induced Lung Injury. *J Immunol:* 1997; 159: 3483-3489.
8. Mulligan MS, Schmid E, Beck-Schimmer B, Till GO, Friedl HP, Rauer RB, Hugli TE, Miyasaka M, Warner RL, Johnson KJ, Ward PA. Requirement for and Role of C5a in Acute Inflammatory Lung Injury in Rats. *J Clin Invest.* 1996; 98: 503-12.
9. Mulligan MS, Warner RL, McDuffie JE, Bolling SF, Sarma V, Ward PA. Regulatory Roles of the Th-2 Cytokines IL-4 and IL-10 in Cardiac Allograft Rejection. *J Exp. Mol. Pathol.* 2000.
10. Mulligan MS, McDuffie JE, Shanley TP, Guo RF, Sarma JV, Warner RL, Ward PA. Role of Rantes in Experimental Cardiac Allograft Rejection. *J Experimental Pathology* Volume 69, Number 3, December 2000, pp. 167-174(8).

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Edward D. Verrier, M.D.

• Cellular and Molecular Mechanisms of Myocardial Ischemia-Reperfusion Injury



FUNDING

Bayer Corporation

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Thoracic Surgery Foundation

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Cardiovascular disease is the leading cause of death in the United States. Although there are a variety of therapeutic options for patients with cardiac disease, heart surgery is a mainstay of treatment for patients with advanced acquired or complex congenital heart disorders. Despite advances in the techniques of heart surgery, ischemic cardiac injury results in considerable morbidity and mortality. To date, the therapy for acute ischemia of the heart has been largely directed towards re-establishing perfusion of ischemic myocardial, or towards the coagulation system to prevent thrombosis. These therapies have arguably reached an efficacious limit.

Our research focuses on understanding how the myocardium responds to ischemia at the molecular, cellular and physiological levels. The goal of our research is to translate an understanding of the molecular mechanisms of ischemic cell signaling into applications for clinical practice.

Ischemia-reperfusion Injury: Paradoxically, restoration of blood flow to oxygen-deprived tissue, the mainstay of therapy for ischemia, often causes further myocardial damage (termed “ischemia-reperfusion [I/R] injury”). I/R injury contributes significantly to morbidity and mortality in surgical patients, and is the principal pathogenetic event in stroke, complications of peripheral vascular disease, hemorrhagic shock, and early transplant graft dysfunction. The reperfusion of oxygen-deprived tissue can cause further myocardial injury by inciting a deleterious inflammatory reaction in and around the reperfused tissue. Because restoration of oxygen delivery to ischemic tissue is critical to survival, a substantial amount of research in the last decade has focused on treating or preventing this detrimental consequence of reperfusion. In our laboratory, we examine the molecular mechanisms of regional I/R injury that often complicate cardiothoracic surgical procedures.

Toll-like receptors: Increased expression of Toll-like receptors (TLRs) has been noted in biopsy samples of patients with severe congestive heart failure, suggesting that TLRs may serve a function apart from their classic role in recognizing microbial antigens. TLRs have been identified on cardiac myocytes, but the function of these receptors of innate immunity in the heart is unknown. We believe that TLRs expressed on cardiac myocytes are activated by reperfusion of ischemic myocardium. We postulate that TLR4 activation during ischemia and reperfusion leads to the activation of mitogen-activated protein kinase (MAPK) signaling pathways and specific transcription factors. These DNA-binding proteins can promote the transcription of genes encoding proteins that cause cardiac apoptosis, or that initiate an acute inflammatory process in the myocardium surrounding an infarction.

Research in our laboratory has identified the involvement of innate immunity receptors in the mechanism of ischemic injury. We have examined mice that are genetically engineered to lack Toll-like receptor 4 (TLR4). Compared to wild-type mice, TLR4-null mice develop a significantly smaller infarct after myocardial I/R injury — illustrating that this innate immune signaling pathway plays a role in the pathogenesis of I/R injury.

TLRs can signal through an adaptor protein called MyD88. MyD88-null mice also develop smaller myocardial infarct after I/R injury, indicating that I/R activates a TLR4- and MyD88-dependent signaling event that results in myocardial damage.

In addition, TLR4 is known to signal through MAP kinases. We have pharmacologically inhibited the activity of the MAP kinase p38, resulting in reduced infarct size after ischemia and reperfusion, compared to mice treated

with vehicle alone. Thus, we are able to apply what we are discovering about the basic science of myocardial I/R injury to potential clinical development.

Ischemic preconditioning: Ischemic preconditioning (IPC) of the myocardium is a phenomenon whereby *brief* repetitive periods of transient ischemia and reperfusion substantially protects the heart against subsequent *prolonged* ischemia. Adaptation of the heart to ischemia following IPC is a biphasic phenomenon. There is an early phase of protection that develops within minutes from the initial ischemic insult and lasts 2–3 hours, and a late (or delayed) phase that is acquired 24 hours later and lasts 3–4 days. The enhanced resistance to infarction and myocardial stunning afforded by IPC and the lasting nature of the response has generated considerable interest in this phenomenon as a potential therapeutic adjunct in the treatment of ischemic heart disease in humans.

Heat shock proteins (HSPs) are highly conserved molecules that participate in protein folding and assembly, as well as the translocation of proteins between cellular compartments following cellular stress. Interestingly, HSP60 and HSP70 have been identified as potential ligands for TLR4. In the heart, HSP70 is the primary stress protein responsive to oxidative stress. Increased expression of HSPs in the myocardium increases resistance to ischemia. Our laboratory has shown evidence that IPC is mediated, in part, by the expression of two inducible members of this family, HSP 70.1 and HSP 70.3. Thus, heat shock proteins are potential mediators of the late phase of IPC, and may work through Toll-like receptors.

The balance: Our studies indicate that TLR4 has a detrimental role in prolonged ischemia, but is necessary for the protective effect observed in brief episodes of ischemia. We hypothesize that IPC causes a shift in

Increased expression of Toll-like receptors (TLRs) has been noted in biopsy samples of patients with severe congestive heart failure, suggesting that TLRs may serve a function apart from their classic role in recognizing microbial antigens.

The mechanism by which IPC exerts this cardioprotection remains unclear. The classic ligand for TLR4 is LPS (lipopolysaccharide; endotoxin), an integral component of the outer membrane of gram-negative bacteria. Transient activation of TLR4 by LPS in the heart confers functional protection from subsequent I/R injury, indicating that LPS treatment can substitute for ischemia in myocardial preconditioning. We have observed that when TLR4-null mice are treated with ischemic preconditioning, the myocardial infarction size remains large compared to the protection seen in wild-type mice, indicating that TLR4 is necessary for early ischemic preconditioning of the heart. However, MyD88-null mice are responsive to IPC, suggesting that the TLR4 signaling involved in myocardial protections does not require MyD88. Research is ongoing in our laboratory to further elucidate the role of Toll-like receptors in preconditioning.

There is also increasing evidence that endogenous ligands can stimulate TLRs, triggering an immune or inflammatory response. Signals from damaged or stressed cells may initiate an inflammatory response even in the absence of

TLR4-mediated signaling, away from a MyD88-dependant pathway (leading to cellular death), and toward a MyD88-independent pathway, leading to the modulation of NFκB activation, ultimately resulting in cellular survival (Figure 1). The regulation of this proposed shift from TLR4-mediated cell death to TLR4-mediated cell survival raises intriguing possibilities for therapeutic intervention, and is an active area of research in our laboratory.

Ischemia reperfusion injury and ischemic preconditioning are critically important in cardiac surgery. Both cyto-destructive (infarction) and cyto-protective (IPC) molecular pathways can be activated following an ischemic event. Our goal is to understand these cellular events so that therapy can be developed to protect against myocardial damage.

Experimental techniques: We utilize cultured cells (cell lines and primary cell isolates) to examine molecular mechanisms that are involved in the response to I/R injury. These studies allow us to examine specific questions about the effects of hypoxia and reoxygenation on molecular pathways in precisely controlled conditions. In addition, cell

culture gives us the capability to move DNA sequences into cells in a controlled fashion to deduce cellular mechanisms of activation based on the over-expression of specific proteins. Finally, by employing differential array and DNA microchip technology, we can identify and characterize novel protein kinases or transcription factors that, in concert with NF- κ B, regulate the cellular response to hypoxia and reoxygenation.

We pair these *in vitro* studies with *in vivo* mouse models of myocardial I/R injury and IPC, in which ischemia is induced in mouse hearts by transient occlusion of the left anterior descending coronary artery. Following reperfusion we determine the size of the infarction to quantify the magnitude of cardiac I/R damage. Although these mouse models are technically challenging, they allow for the use of transgenic and gene knockout strains to examine the effects of specific genotypic changes on myocardial I/R injury.

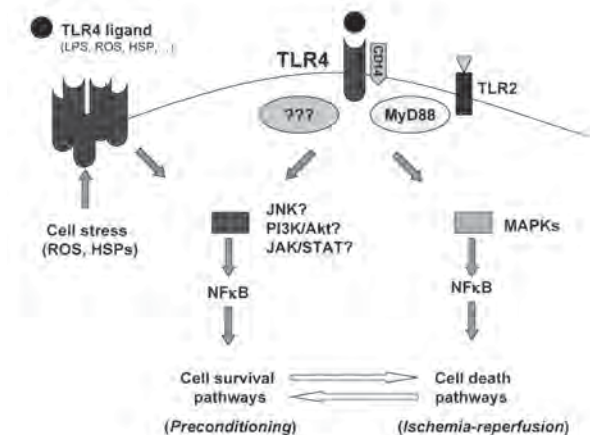


FIGURE 1: TLR4, and possibly other TLRs, are activated by oxidative stress during myocardial I/R injury, either by binding a putative endogenous ligand (e.g., HSPs) that circulates in response to myocardial I/R injury or because of physical alterations by oxygen radical species that cause TLR4 activation in the absence of ligand. Receptor dimerization leads to signal transduction via a MyD88-dependent or -independent pathway, resulting in transcription factor activation (e.g., NF- κ B). NF- κ B translocates to the nucleus to promote the transcription of genes encoding either cell survival proteins (following IPC) or cell death proteins (following I/R). Thus, ischemia-reperfusion can initiate selective myocardial signaling pathways that result in either myocardial damage or myocardial protection, depending on the nature of the stimulus.

RELATED PUBLICATIONS

- Boyle E.M., Jr., Kovacich J.C., Hèbert C, Canty T.G., Jr., Morgan E.N., Pohlman T.H., Verrier E.D. Inhibition of Interleukin-8 Blocks Myocardial Ischemia-reperfusion Injury. *J. Thoracic Cardiovascular Surgery*. 1998; 116: 114-121.
- Kovacich J.C., Boyle E.M., Jr., Morgan E.N., Canty T.G., Jr., Farr A.E., Caps M., Frank N., Pohlman T.H., Verrier E.D. Inhibition of the transcriptional activator protein nuclear factor κ B prevents hemodynamic instability associated with the whole-body inflammatory response syndrome. *J. Thoracic Cardiovascular Surgery*. 1999; 118(1): 154-62.
- Canty T.G., Jr., Boyle E.M., Jr., Farr A.L., Morgan E.N., Verrier E.D., Pohlman T.H. Oxidative Stress-Induced NF- κ B Nuclear Localization without I κ B α Degradation: An Alternative Pathway for Human Endothelial Cell Activation. *Circulation*. 1999; 100(19 Suppl): II361-4.
- Morgan E.N., Boyle E.M., Jr., Yun W., Griscavage-Ennis J.M., Farr A.L., Canty T.G., Jr., Pohlman T.H., Verrier E.D. An Essential Role for NF- κ B in the Cardioadaptive Response to Ischemia. *Ann. Thorac. Surg.* 1999; 68: 377-382.
- Boyle EM Jr, Canty TG Jr, Morgan EN, Yun W, Pohlman TH, Verrier ED. Treating myocardial ischemia-reperfusion injury by targeting endothelial cell transcription. *Ann Thorac Surg*. 1999; 68(5): 1949-53.
- Erlach J.H., Boyle, E.M., Labriola, J., Kovacich, J.C., Santucci, R.A., Fearn, C., Morgan, E.N., Yun, W., Luther, T., Kojikawa, O., Martin, T.R., Pohlman, T.H., Verrier, E.D., and Mackman, N. Inhibition of the tissue-factor-thrombin pathway limits infarct size after myocardial ischemia-reperfusion injury by reducing inflammation. *Am. J. Pathol.* 2000; 157(6): 1849-62.
- Chong AJ, Pohlman TH, Hampton CR, Shimamoto A, Mackman N, Verrier ED. Tissue factor and thrombin mediate myocardial ischemia-reperfusion injury. *Ann Thorac Surg*. 2003; 75(2): S649-55.
- Hampton CR, Shimamoto A, Rothnie CL, Griscavage-Ennis J, Chong A, Dix DJ, Verrier ED, Pohlman TH. HSP70.1 and -70.3 are required for late-phase protection induced by ischemic preconditioning of mouse hearts. *Am J Physiol Heart Circ Physiol*. 2003; 285(2): H866-74. Epub 2003 Apr 24.
- Chong AJ, Shimamoto A, Hampton CR, Takayama H, Spring DJ, Rothnie CL, Yada M, Pohlman TH, Verrier ED. Toll-like receptor 4 mediates ischemia/reperfusion injury of the heart. *J Thorac Cardiovasc Surg*. 2004; 128(2): 170-9.
- Yada M, Shimamoto A, Hampton CR, Chong AJ, Takayama H, Rothnie CL, Spring DJ, Shimpo H, Yada I, Pohlman TH, Verrier ED. FR167653 diminishes infarct size in a murine model of myocardial ischemia-reperfusion injury. *J Thorac Cardiovasc Surg*. 2004; 128(4): 588-94.

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• Inflammatory Signaling Response to Thermal Injury Beta-Blocker Therapy in the Injured Patient



AWARDS

American Association for the Surgery of Trauma
• Peter C. Canizaro Award
• Junior Faculty Research Award
Surgical Infection Society Foundation
• Faculty Research Award

FUNDING

National Institutes of Health

Inflammatory Signaling Response to Thermal Injury

Severe thermal insult induces a major disturbance in the homeostatic mechanisms with significant disturbances in hemodynamic, respiratory, and metabolic pathways. Potential post-injury complications include severe sepsis, multisystem organ failure, and death. Since an aberrant systemic inflammatory response appears to be the underlying mechanism for ultimate organ failure, most studies have focused on systemic therapy to control this over-exuberant immune response. However, systemic administration of several anti-inflammatory or immunomodulatory agents, such as platelet activating

attenuate the subsequent complications such as acute lung injury. In this approach, we use topical agents to inhibit post-injury burn wound inflammatory signaling. The agent that we use is a potent inhibitor of p38MAPK, which is a pro-inflammatory signaling pathway that plays a prominent role in the regulation of inflammatory cell responses. The p38MAPK inhibitors are applied to the burn wound using a simple acetone-olive oil vehicle.

Topical p38MAPK inhibition attenuates the burn wound inflammatory response. There is a significantly less pulmonary inflammatory response via reduction of

Controlling local inflammatory signaling may attenuate the subsequent complications such as acute lung injury.

factor receptor antagonists, anti-TNF antibodies, and IL-1 receptor antagonists, have failed to demonstrate improvement in survival or organ failure. In addition, the systemic administration of immuno-modulators is associated with multiple disadvantages. These agents are not tissue specific and act on multiple organs. In a complex interacting system of cell-specific pathways, systemic inhibition of one pathway may have unpredictable deleterious results.

We therefore propose a new approach which calls for “inflammatory source control.” The hypothesis is that burn injury induces dermal inflammation and production of pro-inflammatory mediators, which act as a lasting trigger stimulating the systemic inflammatory response syndrome. Therefore, controlling local inflammatory signaling may

pulmonary neutrophil sequestration, pulmonary cytokine expression, microvascular injury and edema formation. Topical inhibition of p38 MAPK decreased pulmonary collagen deposition and improved pulmonary function with significantly reduced inspiratory and expiratory time. In a burn-pneumonia model, application of p38 MAPK inhibitor to the wound reduced the mortality rate back to sham level (Figure 1). While dermal gene upregulator ATF-2, a downstream p38 MAPK target, was significantly reduced, there was no reduction in pulmonary ATF-2 expression, arguing against significant systemic absorption of the topical inhibitor. These experiments also confirm the strong interaction and dependence on dermal inflammation to drive the systemic inflammatory response.

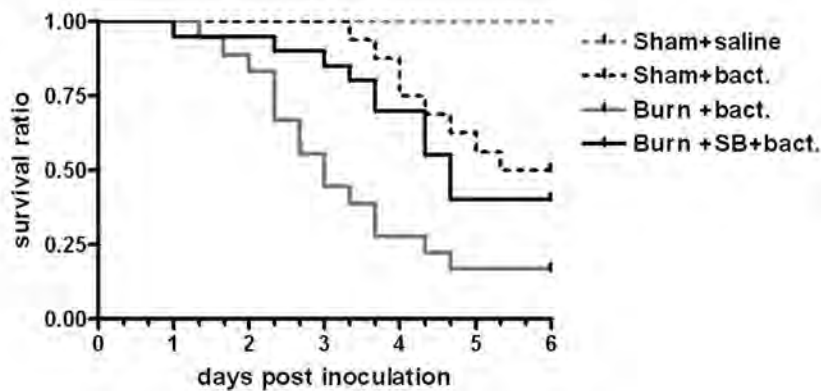


FIGURE 1: Dermal inflammatory source control improves survival in a burn-pneumonia two hit model.

In summary, topical p38 MAPK inhibition in burn wounds to prevent inflammatory cell activation appears to be an effective strategy to reduce the systemic inflammatory response and end-organ failure. This novel therapy is practical and fits the current clinical practice of daily application of topical antimicrobial agents to the burn wound. Moreover, it is tissue restricted and avoids potential side-effects from systemic administration. I have worked on intracellular inflammatory pathways for the last 10 years, elucidating the mechanism of action of p38MAPK in response to injury. My goal is to continue this investigation and develop an effective practical therapy in severe burns.

Beta-Blocker Therapy in the Injured Patient

Major injury induces a significant sustained release of catecholamines for several days or weeks after trauma. This catecholamine surge is especially increased when there is a significant head injury. The highest concentration of beta-adrenoreceptors is in the cerebral cortex. Activation of these receptors by catecholamines increases cerebral metabolism, glucose and oxygen consumption, which may be beneficial by increasing alertness at times of stress. However, increased cerebral oxygen consumption in the presence of elevated intracranial pressure post-trauma may worsen cerebral ischemia and secondary brain injury (Figure 2). Beta-blockers can break this Trauma-Catecholamine-Head Injury cycle by decreasing the cerebral oxygen requirement, which may attenuate cerebral ischemia and secondary brain injury. Overall, beta-blockers can be beneficial by decreasing hypermetabolism, alleviating cardiac workload and ischemia, and by decreasing cerebral oxygen requirement in head injury.

Following our original study in burn patients, we reviewed outcomes for 4,711 trauma patients from 2001 to 2004 and found that 7% received beta-blockers. In the beta-cohort, 45% of patients were on beta-blockers pre-injury. The most common reason to initiate beta-blocker therapy was blood pressure (60%) and heart rate (20%) control. The overall mortality rate was 5.6%, and head injury was considered to be the major cause of death. After adjusting for age, ISS, blood pressure, GCS, respiratory status, and mechanism of injury, the odds ratio for fatal outcome was 0.3 ($p < 0.001$) for beta-blocker cohort as compared to control. Decreased risk of fatal outcome was more pronounced in patients with a significant head injury. We concluded that beta-blocker therapy is safe and may be beneficial in selected trauma patients with or without head injury. We are planning further studies looking at beta-blocker therapy in trauma patients and their effect on cerebral metabolism.

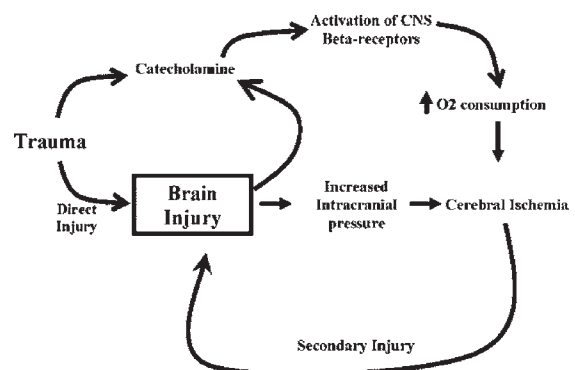


FIGURE 2: Trauma-Catecholamine-Head Injury Cycle

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Eileen Bulger, M.D.

- Hypertonic Resuscitation for Blunt Trauma
- Prehospital Airway Management & Treatment for Traumatic Brain Injury
- National Variability in Prehospital Care following Injury
- Immunomodulation of the Alveolar Macrophage
- Management of Necrotizing Soft Tissue Infections
- Rib Fracture Management
- Crash Injury Research and Engineering Network (CIREN)



AWARDS

American Association for the Surgery of Trauma

- Wyeth-Ayert Research Scholarship Award
- John H. Davis Research Scholarship Award
- Peter C. Cannizaro Award 2004

FUNDING

Brain Trauma Foundation

- Medic One Foundation
- National Institutes of Health
- National Highway Transportation and Safety Administration

Based on a strong interest in trauma and critical care, my research has focused on injury prevention, important clinical questions regarding patient management, and elucidating the cellular biology of the systemic inflammatory response. My clinical research has focused on the prehospital care of patients following traumatic injury, including airway management and fluid resuscitation strategies. My laboratory efforts, in collaboration with Dr. Ronald V. Maier & Dr. Joseph Cuschieri, have focused on the immunomodulation of the alveolar macrophage, which plays a key role in the development of the acute respiratory distress syndrome (ARDS). In addition, a collaborative study with Dr. Avery Nathens seeks to explore the predictors of poor outcome following necrotizing soft tissue infection. Additional clinical trials address the pain management options for patients with rib fractures and the development of clinical care guidelines for these patients. To address the injury prevention side of the equation, I have recently become the local PI for the Crash Injury Research and Engineering Network (CIREN), which collects detailed data regarding the biomechanics of injury associated with motor vehicle crashes. These data will allow us to make recommendations regarding automobile design and crash test parameters that will translate into a reduction in occupant injury.

Hypertonic Resuscitation for Blunt Trauma

An evolving body of evidence suggests that resuscitation with hypertonic fluids following injury may improve outcome. The potential benefits of hypertonic resuscitation include more rapid restoration of tissue perfusion, preservation of cerebral perfusion while lowering intracranial pressure for brain-injured patients, and modulation of the inflammatory response at the time of reperfusion, thus lessening the subsequent development of inflammatory organ injury such as ARDS. With the support of the National

Heart, Lung, and Blood Institute of the NIH, we have embarked on clinical trials to answer these questions. We recently closed a local trial in which randomized patients received either hypertonic saline/dextran (HSD) or lactated ringers as their first resuscitation fluid, administered by the paramedics at the scene of the injury.

The primary outcome variable was ARDS-free survival within 28 days. Secondary outcomes include mortality, infectious complications, multiple organ dysfunction, and long term neurological function for patients with traumatic brain injury. We have subsequently used the lessons learned from this trial to design a multicenter trial to be conducted by the Resuscitation Outcomes Consortium (ROC). The ROC involves 10 clinical centers in the US and Canada and a data coordinating center based at the University of Washington (PI: Scott Emerson; Co-PIs: Graham Nichol, Eileen Bulger). The Seattle and King County Medic One programs are two of the regional clinical centers (PI: Peter Kudenchuk; Co-PIs: Tom Rea and Eileen Bulger).

The ROC, which is supported by the NIH, Department of Defense and Canadian Institute for Health Research, is charged to conduct prehospital clinical trials of promising therapies for both cardiac arrest and life threatening trauma. The trial of hypertonic resuscitation will enroll nearly 6,000 patients in a three arm trial of HSD, hypertonic saline without dextran and normal saline as the initial resuscitation fluid for a hypovolemic shock cohort and a traumatic brain injury cohort. These trials are designed as definitive Phase III trials to determine the efficacy of this resuscitation strategy. These trials are currently enrolling patients. Investigators from three of the clinical centers including Seattle, San Diego, and Toronto have also submitted an R01 application to conduct detailed studies of the immunoinflammatory response of patients enrolled in the clinical trial (PI: Bulger).

Prehospital Airway Management & Treatment for Traumatic Brain Injury

Currently supported by two grants from the Medic One Foundation, we have been investigating the airway management strategies employed in Seattle, with a particular focus on the management of patients with anatomy or injuries that make endotracheal intubation particularly challenging. We have reported that with the aid of paralytic agents to facilitate intubation, the Seattle Medic One program has the highest success rate for intubation in the literature at 98.4% and the lowest surgical airway rate at 1.1%. (*J Emerg Med*, 2002). We have subsequently established a prospective data collection process to allow us to track the impact of different airway management strategies on patient outcome.

Among injured patients, the group that may benefit the most from early airway control and resuscitation is that of patients with traumatic brain injury (TBI). It has been well established that hypoxia and hypotension contribute to the development of secondary brain injury and worsen outcome following TBI. A single episode of prehospital hypotension has been associated with a two-fold increase in the incidence of adverse outcome (severely disabled, vegetative, or dead) following severe brain injury. With the support of the Brain Trauma Foundation we recently completed a study investigating the relationship between prehospital interventions and outcome following TBI. We identified that patients undergoing prehospital intubation facilitated by neuromuscular blocking agents actually had a better outcome than those intubated without these medications (*J Trauma* 2005).

We next turned our attention to the impact of prehospital ventilation on outcome following TBI. Hyperventilation may lead to cerebral vasoconstriction and thus impair cerebral blood flow. Hypoventilation may lead to cerebral vasodilation and thus raise intracranial pressure. Hyperventilation has been reported to be a common problem following prehospital intubation. We have undertaken a series of studies aimed at defining the optimal ventilation strategy for injured patients. For trauma patients intubated in the prehospital setting, those with an arrival arterial pCO₂ between 30-35mmHg demonstrated improved outcome, which was most marked for those with severe TBI (*J Trauma*, in press). Further studies have examined the impact of correcting patients into a target range in the Emergency Department and our current studies are examining the utility of end tidal CO₂ monitoring for this patient population both in the field and in the ED. Taken together, these studies will allow us to design an optimal ventilation strategy for these patients early after injury.

National Variability in Prehospital Care following Injury

In collaboration with Drs. Jerry Jurkovich and Fred Rivara, co-PIs on the National Study of Costs and Outcome for Trauma (NSCOT), we have utilized data collected from 14 geographic regions in the US to assess the variability in prehospital care provided to victims of traumatic injury. We have identified substantial variability in prehospital care among the regions including: prehospital intubation (5-48%), use of neuromuscular blocking agents or sedatives to facilitate intubation (0-100%), surgical airway access (0.1-3.5%), peripheral and central intravenous access (22-95%), and needle thoracentesis (0-5%). Intubation success rates averaged 94% in patients receiving neuromuscular blocking agents vs. 67% for those who did not ($p < 0.001$). This variability persisted even when patients were stratified based on their injury severity and physiology. Understanding this national variability in care and EMS system design is critical to interpreting the various studies in the literature and to designing future multi-center trials.

Immunomodulation of the Alveolar Macrophage

ARDS is a process of acute inflammatory lung injury, which affects a diverse array of surgical and medical patients. The etiology of this process is thought to involve an excessive overexpression of the inflammatory response, leading to the destruction of host tissue. The alveolar macrophage is a key cell in the coordination of this response. Our laboratory has focused on all aspects of this response using endotoxin as a prototypic inflammatory stimulant. In previous studies we have demonstrated that treatment of alveolar macrophages with certain antioxidants, in vitro, results in significant inhibition of the macrophage cytokine response. This work was extended to an in vivo model of enteral Vitamin E supplementation in rats with similar results and a recently completed prospective, randomized trial of high dose enteral Vitamin E and C vs. placebo in the surgical ICU.

Recently we have also investigated the use of platelet activating factor acetylhydrolase (PAF AH) in vitro. PAF is a pro-inflammatory lipid mediator which has been implicated in several animal models of lung injury. PAF AH is the endogenous enzyme for PAF metabolism. These studies have demonstrated profound inhibition of cytokine production by macrophages treated with PAF AH prior to and following LPS stimulation. With the support of the American Association for the Surgery of Trauma Research Scholarship, we have developed an animal model of ARDS and have begun to test promising modulators of macrophage activation in this model. We have demonstrated that

Among injured patients, the group that may benefit the most from early airway control and resuscitation is that of patients with traumatic brain injury.

both PAF-AH and hypertonic saline, when given intravenously, dramatically down-regulate alveolar macrophage activation in response to inflammatory stimuli.

In collaboration with Dr. Pat Stayton in the Department of Bioengineering, we have secured NIH funding to test a novel intracellular drug delivery system as a means to modulate alveolar macrophage activation, in vivo. We will utilize our established model of ARDS to test the delivery of antisense IRAK and iNOS to alveolar macrophages and the impact of this therapy on subsequent cytokine production.

Management of Necrotizing Soft Tissue Infection

Harborview Medical Center serves as a regional referral center for patients with severe necrotizing soft tissue infection and as a result has seen dramatic increase in the number of these cases over the past several years. In an effort to define the morbidity and mortality of this population, we undertook a retrospective review of our experience over a five year period (Anaya et al, *Arch Surg* 2005). In this review we identified clinical predictors of mortality and limb loss based on data available at the time of patient admission. In a subsequent study we incorporated data from patients treated at the University of Texas in Houston and developed a clinical prediction rule which was internally validated. We are also working with the Surgical Infection Society to generate evidence-based guidelines for the management of these patients.

Rib Fracture Management

Rib fractures are a common injury in the blunt trauma population and are often under-appreciated in the setting of multiple injuries. The elderly are particularly susceptible to complications resulting from rib fractures and underlying pulmonary injury. We recently reviewed all patients > age 65 admitted to HMC with rib fractures over the past ten years and compared these to a cohort of younger patients.

Of note, there was a nearly linear increase in mortality and complication rates associated with increasing rib fracture number in the elderly group. An elderly patient with only 3-4 rib fractures had a 19% mortality and a 31% rate of pneumonia. For an elderly patient with > 6 rib fractures, mortality was 33% with a pneumonia rate of 51%.

The key strategy in the management of these patients involves the ability to obtain adequate pain control to optimize pulmonary status. To determine the best pain management strategy for these patients, we undertook a prospective, randomized trial of thoracic epidural vs. intravenous narcotics. We demonstrated that epidural analgesia decreased the rate of nosocomial pneumonia and shortened the duration of mechanical ventilation (*Ann Surg* 2005). In recognition of the ongoing controversy regarding the indications and contraindications for epidural placement in multiply injured patients, we next conducted a survey of pain service directors at all Level 1 trauma centers in the United States. We plan to use the results of this survey to stimulate the generation of guidelines for the use of thoracic epidural analgesia after injury.

Crash Injury Research and Engineering Network (CIREN)

The Harborview Injury Prevention and Research Center houses one of eight national CIREN centers supported by the National Highway Transportation and Safety Administration. These centers collect detailed injury and crash investigation data following motor vehicle crashes to identify the forces responsible for injury. Some of our current research projects include: examining mechanisms of injury associated with renal injuries, patterns of injury associated with misuse of child restraints, the impact of seat back position on outcome following frontal crashes, the relationship between obesity and lower extremity fractures, and the development of prehospital triage guidelines.

RELATED PUBLICATIONS

1. Bulger EM, Gourlay D, Cuschieri J, Jelacic S, Staudenmeyer K, Garcia I, Maier RV. Platelet Activating Factor Acetylhydrolase inhibits alveolar macrophage activation, in vivo. *Shock* 20:17-22, 2003.
2. Bulger EM, Garcia I, Maier RV. Induction of heme-oxygenase 1 inhibits the proinflammatory response of endothelial cells. *Surgery* 134:146-52, 2003.
3. Bulger EM, Edwards T, Klotz P, Jurkovich GJ. Epidural analgesia improves outcome following multiple rib fractures. *Surgery* 136(2): 426-30, 2004
4. Bulger EM, Jurkovich GJ, Farver CL, Klotz P, Maier RV. Oxandrolone dose not improve outcome for chronically ventilated surgical patients. *Annals of Surgery* 240(3):472-8, 2004.
5. Anaya D, McMahon K, Nathens AB, Sullivan S, Foy H, Bulger EM. Predictors of Mortality and Limb Loss in Necrotizing Soft Tissue Infections. *Archives of Surgery* 140:151-157, 2005.
6. Bulger EM, Copass MK, Sabath D, Maier RV, Jurkovich GJ. The use of Neuromuscular Blocking Agents to Facilitate Prehospital Intubation does not Impair Outcome following Traumatic Brain Injury, *J. Trauma*, 58 (4):718-724, 2005.
7. Pickens J, Copass MK, Bulger EM. Trauma Patients Receiving CPR: Predictors of Survival. *J Trauma*, 58(5):951-958, 2005.
8. Staudenmeyer K, Maier RV, Jelacic S, Bulger EM. Hypertonic saline modulates innate immunity in a model of systemic inflammation. *Shock* 23 (5): 459-463, 2005.
9. Bulger EM, Nathens AB, Rivara FP, MacKensie E, Copass MK, Sabbath D, Jurkovich GJ. National variability in out-of-hospital treatment after traumatic injury. *Ann Emerg Med*, in press, 2006.
10. Bulger EM, Jurkovich GJ, Nathens AB, Copass MK, Hanson H, Cooper C, Liu PY, Warner K, Maier RV. Hypertonic resuscitation of hypovolemic shock after blunt trauma: a randomized controlled trial. *Arch Surg*. In press, 2006.
11. Bulger EM, Cuschieri J, Warner K, Maier RV Hypertonic Resuscitation modulates the inflammatory response in patients with traumatic hemorrhagic shock. *Ann Surg*, in press 2006.

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Joseph Cuschieri, M.D.

- Toll-Mediated Signaling
- Trauma Induced Mononuclear Cell Reprogramming
- Proposed Mechanism of Lipid Raft Clustering and Reprogramming
- Trauma Induced Phenotypic Alterations
- Class Prediction Based on Cytokine Profiles



AWARDS

American College of Surgeons

- Committee on Trauma Region X Competition, Best Basic Science Paper

Seattle Surgical Society Award

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Severe injury results in the activation of the innate immune system characterized by the systemic inflammatory response syndrome (SIRS).

Although this state may persist, resulting in early development of multiple organ dysfunction syndrome (MODS), the majority of injured patients develop a compensatory response that is characterized by a state of dysregulated immune responsiveness. During this state of dysregulated responsiveness, patients are at increased risk for the development of opportunistic or nosocomial infections. If invasive infection occurs following this state, an exaggerated inflammatory response ensues, leading to the MODS development (Figure 1).

The mechanism responsible for this dysregulated immune activation remains poorly understood. This state has been modeled and characterized by the “two-hit” hypothesis. According to this hypothesis, severe injury results in the reprogramming of innate immune cells so that during subsequent infection an exaggerated host response occurs, resulting in tissue injury. Both the peripheral blood monocyte and tissue-fixed macrophage appear to play critical roles during this state. The primary mechanism in which these cells interact with invading organisms is through the

Toll-like receptors (TLRs), a family of pattern recognition proteins. Activation of these receptors by inflammatory factors, such as lipopolysaccharide (LPS), leads to the liberation of various cytokines and chemokines that are in part responsible for eradication of invading organisms. However, when exaggerated, as is the case following severe injury, liberation of the factors leads to subsequent tissue injury and the development of MODS.

The mechanism in which the TLRs are activated and affected by severe injury remains an area of intense investigation. Recently, we have demonstrated that activation of the TLRs, in particular TLR4, requires the formation of a receptor complex with CD14 and other constituents on specialized membrane components termed lipid rafts. In particular, attenuation and augmentation of this receptor complex formation on these membrane platforms results in dysregulated inflammatory mediator liberation. My laboratory efforts, therefore, are to elucidate the cellular mechanisms involved in mononuclear cell reprogramming in patients suffering from MODS and acute respiratory distress syndrome (ARDS) following trauma. If this is accomplished, it would provide the foundation for the development of novel early therapeutic interventions that could be used during the resuscitative period.

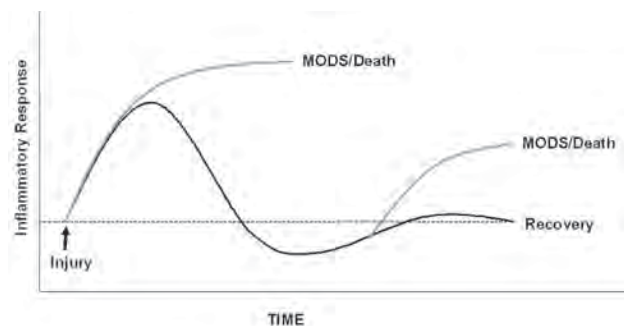


FIGURE 1

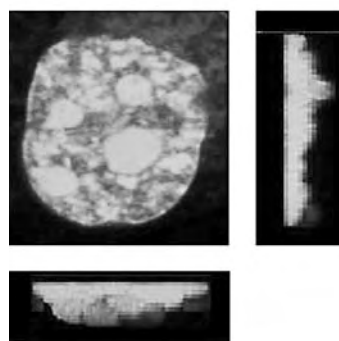


FIGURE 2

Toll-Mediated Signaling

The peripheral blood monocyte and tissue fixed macrophage are activated by pathogen-associated molecular patterns. These are structures that are characteristic of large groups of microorganisms, such as bacterial cell wall components and nucleic acid motifs. Unlike the adaptive immune response which requires antigen-specific antibodies, innate immune cells are able to respond rapidly to invading organisms without the need for prior exposure.

In mammalian cells, the key component to this response is the family of TLRs. These receptors are responsible for the recognition of the pathogen-associated molecular patterns and lead to the subsequent activation of the monocyte and macrophage. The founding member of the TLR family is the *Drosophila* protein, Toll, which was initially identified through its ability to control dorsoventral patterning in fruit fly embryos. Recognition of the importance of Toll in the *Drosophila* innate response prompted exploration for a possible mammalian counterpart.

Currently, a total of 10 human TLRs have been identified that share structural homology and signaling components. Each of the described TLRs, except for TLR9, are all transmembrane molecules. The extracellular amino termini have variable leucine-rich repeat domains, which are involved in the recognition of pathogen-associated molecular patterns. The intracellular domains contain a conserved Toll/interleukin-1 (IL-1) receptor (TIR) domain. The TIR domain, a defining characteristic of the Toll/IL-1 receptor superfamily, is involved in the association with downstream signaling molecules that mediate the response to TLR stimulation.

Toll-like receptor 4 is part of a complex that recognizes LPS. Lipopolysaccharide is an abundant glycolipid present on the outer membrane of gram-negative bacteria. During Gram-negative infections, the highly conserved lipid A component of LPS activates the immune system, leading to generalized inflammation, manifested clinically as sepsis and septic shock. Lipopolysaccharide released from Gram-negative bacteria is present as an aggregate due to the amphiphilic structure of the molecule. Spontaneous diffusion of LPS monomers from these aggregates to CD14 occurs at a very low rate. However, LPS is transformed into monomers through the action of plasmatic LBP. LBP is a lipid transfer molecule catalyzing movement of phospholipids, in particular LPS monomers from LPS aggregates to CD14. This process results in either cell activation through CD14 or neutralization of LPS. Thus, the rate of either process will determine the response of

the host to LPS. Kinetic studies have shown that LPS/LBP complexes bind to CD14 before LPS is transferred to HDL. This suggests that normally LPS first activates immune cells before it is neutralized to prevent overstimulation of the immune system.

Membrane bound CD14 is a 53-kDa glycoprotein present within the plasma membrane via a glycerophosphate inositol (GPI) anchor. CD14 is essential as both a functional receptor and scavenger for LPS. The functional role of CD14 leading to LPS-induced cell activation was initially established using neutralizing antibodies to CD14. Transfection of CD14-negative cells with CD14 greatly enhances sensitivity to LPS. Similarly, mice with a disrupted CD14 gene do not respond to low doses of LPS. Under physiological conditions, LPS-induced cell activation involves the formation of a ternary complex with LBP and CD14 within lipid rafts on the monocytic cell surface leading to cellular activation.

The classical fluid mosaic model proposed by Singer and Nicolson in 1972 has been modified in recent years to accommodate a role for distinct microdomains in the cell membrane, which appear to serve as signaling platforms (Figure 2). The cell membrane is mainly composed of glycerophospholipids, sphingolipids and cholesterol. The headgroups of sphingolipids trigger a lateral association of lipids of this class with one another, which is further enhanced by hydrophobic interactions between the saturated side chains. Cholesterol seems to fill voids between the large glycerosphingolipids, and tightly interacts with sphingolipids, in particular sphingomyelin, by hydrogen bonding. The tight interaction of sphingolipids with one another and with cholesterol results in the segregation of these lipids into discrete membrane structures characterized by a gel-like phase, while glycerophospholipids in the bulk of the cell membrane reside in a more fluid liquid-disordered phase.

These distinct sphingolipid- and cholesterol-enriched membrane microdomains are considered to be floating in an "ocean" of phospholipids, and hence have been termed lipid rafts. In addition to the selective lipid composition, selected proteins are preferentially targeted or constitutively found within the lipid raft. Within mononuclear cells, these modified proteins are composed of saturated acyl-chain proteins, including GPI-anchored proteins, such as CD14, and double acylated proteins. Other receptor proteins, such as the TLRs, are not constitutively found on rafts, but during activation these proteins are recruited into rafts

Severe injury is associated with increased susceptibility to life-threatening infections and sepsis, leading to the development of MODS.

through a mechanism that remains unclear, AZ resulting in the formation of receptor complexes and the presentation of the inciting stimulus.

Rafts appear more prominent and more central to the function during activation of the monocyte and macrophage. In resting cells, rafts appear small and unstable, and consensus now suggests that they are smaller than the optical diffraction limit (250 nm). Upon stimulation, the raft-preferring receptors are clustered through a poorly defined mechanism leading to the generation of lipid raft macrodomains, allowing LPS to be briefly released into the lipid bilayer where it finally interacts with the complex of receptors, including TLR4. Due to the abundance of sphingolipids within the raft membrane, it is our hypothesis that sphingomyelinase activation resulting in degradation of lipid raft sphingolipids into the secondary messenger ceramide is the likely candidate involved in lipid raft reorganization within mononuclear cells.

The sphingomyelin pathway is initiated by the rapid hydrolysis of plasma membrane sphingomyelin to the second messenger ceramide via the action of sphingomyelinase. This is believed to result in the reorganization of lipid rafts. Ceramide, which has the unique property of fusing membranes, appears to drive the coalescence of raft microdomains to form large, ceramide-enriched membrane platforms, which exclude cholesterol. Recently, we have been able to demonstrate the formation of these lipid raft ceramide fused macrodomains following LPS stimulation.

The formation of these ceramide-enriched membrane platforms serves to trap and cluster receptor molecules, and potentially exclude other receptor complexes. We have been able to demonstrate that initial binding of LPS to CD14 results in the activation of acid sphingomyelinase resulting in the liberation of ceramide, and the formation of TLR4 raft associated complexes. The mechanism responsible for sphingomyelinase activity, however, remains unresolved but may occur through the activation of phosphatidylcholine (PC)-specific phospholipase C (PC-PLC).

Once this membrane platform is formed, the signaling pathways leading from LPS/CD14 binding to TLR4 complex assembly are not well understood and are

important because of the potential for early and selective pharmacological intervention. Although PC-PLC and sphingomyelinase may play a role through the induction of ceramide, the subsequent events leading to TLR4 complex assembly remain for the most part uncertain. Recently, we have been able to shed some light on this mechanism by demonstrating that activation of the PKC isoform, PKC- ζ , is involved. Although the full effects of PKC- ζ remain to be elucidated, it appears that the mechanism is ceramide dependent and results in the engagement of integrins and the recruitment of various raft associated proteins.

The high degree of organization observed within lipid raft structures, coupled with their dynamic nature, appears to be important in modulating and integrating signals by providing a signaling microenvironment that is tailored to produce specific biological responses. Changes in protein or lipid composition, size, structure, number, or membrane localization of lipid rafts could potentially affect the functional capabilities of these domains in signaling with important physiological consequences.

Thus, the clustering of lipid rafts and receptor proteins appears to be an efficient means in regulating cell signaling during activation. Additionally, pre-assembly of these factors could be induced following injury and may result in amplification or modulation of signals in a spatially regulated manner. This alteration, induced in part by ceramide content and PKC- ζ activation, may be involved in not only augmenting signaling but could also negatively regulate signaling by sequestering or excluding signaling components in an inactive state.

Among the proteins that are targeted to form clusters within rafts are those that are anchored in part on the outer leaflet of the membrane and can covalently attach to the GPI-protein, CD14. Examples of such proteins include TLR4, HSP70, HSP90, CXCR4 and CD55. Other proteins that are linked to saturated acyl chains, such as the SRC family of kinases, in particular Lyn, and various integrins, such as Cdc42, CD11b and CD18, are also targeted to rafts and may additionally affect raft morphology and function. The formation of these complexes is induced by factors such as LPS, but the effects of severe injury remain unknown.

Trauma Induced Mononuclear Cell Reprogramming

Severe injury is associated with increased susceptibility to life-threatening infections and sepsis, leading to the development of MODS. Severely injured patients appear to have a dysregulated innate immune response following injury, which appears to be central to the development of these clinical syndromes. The effect of trauma on mononuclear cell phagocytosis, killing of microorganisms, antigen presentation, cytokine production, and induction of cytotoxic effector cells has been characterized. However, the mechanisms responsible remain unknown due to both exaggerated pro- and anti-inflammatory responses. Insight into the mechanisms involved, however, can be determined through in vitro modeling of factors induced by severe injury, including PAF, oxidant stress and C5a, and through the induction of tolerance.

Treatment of mononuclear cells with various agents, including PAF, oxidant stress and C5a, results in a heightened responsiveness to subsequently encountered stimuli such as LPS. Critical to this reprogramming is cellular adherence. This is fortunate, since it is difficult to envision an in vivo situation where local tissue injury might occur from stimulation of suspension phase cells.

Common to these various agents is the mobilization of calcium and subsequent activation of CaMK II that we have demonstrated to occur following exposure to each of the reprogramming conditions. Although the cellular source of calcium varies, each factor results in the autophosphorylation and sustained activation of CaMK II. Sustained activation had been previously demonstrated in a number of cell types during sepsis, including cardiac myocytes and smooth muscle cells. Recently, we have demonstrated a similar sustained activation of CaMK II in bronchoalveolar macrophages obtained from injured patients that have gone on to develop ARDS. This is the first example of increased activation of CaMK II following injury, and provides support that cellular alteration of calcium may be an important event in immune cell reprogramming.

In addition to the activation of the regulatory kinase, CaMK II, recent evidence has suggested that sphingomyelinase activation and ceramide production may play additional regulatory roles. In fact, intracellular ceramide levels along with serum TNF- α have been demonstrated to be elevated in patients suffering from severe sepsis. This strong correlation between cell-associated ceramide and serum TNF- α supports the hypothesis that ceramide, along with sphingomyelinase, plays a role in sepsis and subsequent organ dysfunction. Although sphingomyelinase activation

and ceramide production may prove to be important following acute injury, this exploration has only just begun.

Desensitization or tolerance is characterized by diminished responsiveness due to repeated stimulation. Lipopolysaccharide has been consistently shown to induce desensitization in mononuclear cells. Cells in the LPS tolerant state respond to a much lesser extent than the initial stimulation resulting in attenuated liberation of chemokines and cytokines. Tolerance has been shown to attenuate several endotoxin mediated components, including IRAK-1, NF- κ B and the MAPK. Recently, we have demonstrated that endotoxin tolerance does in fact effect recruitment and formation of the TLR4 complex on lipid rafts. In fact, this attenuation in recruitment of TLR4 and HSP70 during tolerance is reversed by non-specific PKC activation with PMA. This finding is consistent with previous observation that demonstrated reversal of tolerance with PMA administration. Thus, limited recruitment of receptor complexes to the lipid raft receptor platform may underlie the increased risk associated with a subgroup of injured patients at risk for devastating infections.

Putting these data together, we have just begun to demonstrate that cellular reprogramming following trauma is associated with marked alterations in raft protein and lipid composition. These changes in composition place various regulatory proteins in association leading to either enhanced or attenuated activation. Due to these changes, immune cells following injury may predispose these patients to either nosocomial infections or the development of MODS. It is therefore our current goal to evaluate these changes, using various high throughput proteomic and HPLC techniques to categorize them.

Proposed Mechanism of Lipid Raft Clustering and Reprogramming

Based upon our findings, we have developed the following model for lipid raft receptor clustering and severe injury induced reprogramming (Figure 3). Activation is initiated by LPS/LBP binding to CD14 on lipid rafts. This ligand specific binding results in the activation of PC-PLC and the generation of DAG. Liberation of DAG results in the membrane recruitment and activation of sphingomyelinase, leading to lipid raft sphingolipid conversion to ceramide within the lipid raft. Ceramide then results in the clustering of lipid raft proteins through the fusion within lipid rafts leading to increased gel phase fluidity and the activation of various kinases, in particular PKC- ζ . Activation of PKC- ζ then potentially leads to the engagement of β_2 integrins on lipid rafts, leading to the formation of macrodomains, as

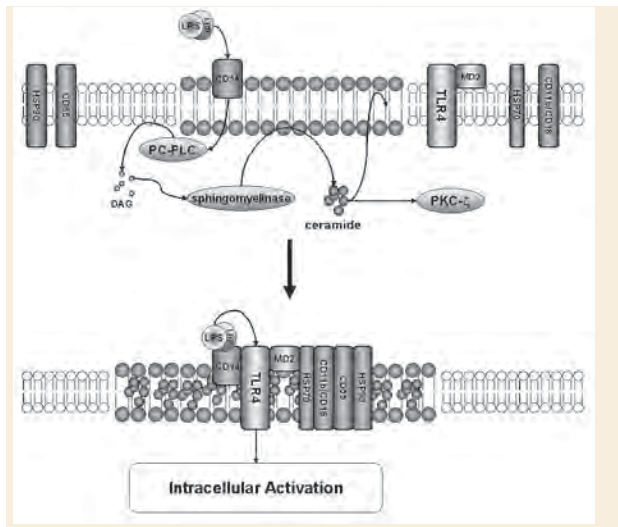


FIGURE 3A

well as cytoskeletal changes resulting in lipid raft recruitment of TLR4 components and scaffolding proteins. These cytoskeletal changes are perhaps induced through engagement of $\beta 2$ integrin intracytoplasmic tails of paxillin, Pyk2 and other adapter and scaffolding molecules and kinases. As a result, these adapter proteins are phosphorylated and activated, leading to cytoskeletal reorganization and protein reorganization and recruitment of TLR components (Figure 3A).

Reprogramming following injury is associated with changes in both protein and lipid content within rafts. These changes are due to local generation of ceramide through the activation of sphingomyelinases by reprogramming factors, such as PAF, oxidant stress and C5a. Generation of ceramide leads to calcium mobilization, followed by the sustained activation of CaMK II. Activation of CaMK II, along with lipid raft ceramide fusion, leads to the early mobilization of TLR components, such as HSP70. This clustering and pre-assembly of kinases and scaffolding proteins results in altered signaling induced by subsequent stimuli (Figure 3B).

Trauma Induced Phenotypic Alterations

Peripheral blood CD14 positive monocytes have been recently divided into two subpopulations, namely one with CD16 surface expression but with diminished CD14 expression (CD14⁺CD16⁺) and one without any CD16 expression (CD14⁺CD16⁻). The population of CD14⁺CD16⁺ monocytes normally represents about 10% of monocytes in healthy adults. These CD14⁺CD16⁺ cells demonstrate features of differentiated monocytes or tissue macrophages such as increased migration into tissues. They have also been described as “pro-inflammatory” in nature,

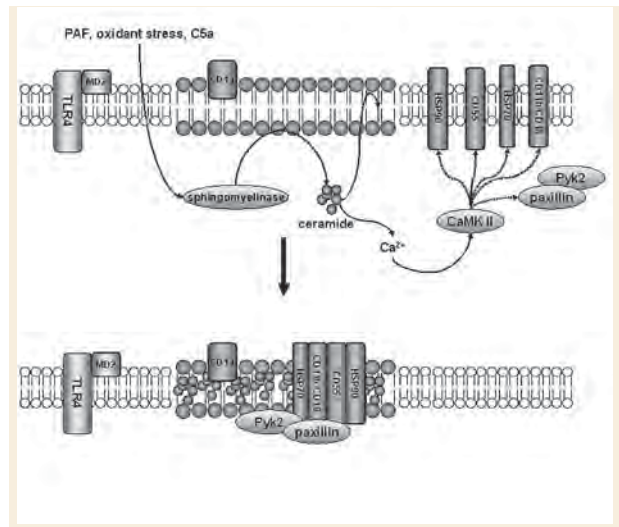


FIGURE 3B

producing high levels of pro-inflammatory cytokines, increased HLA-DR expression and little to no anti-inflammatory cytokines. Although not previously investigated following severe injury, the percentages and absolute number of CD14⁺CD16⁺ monocytes have been shown to be significantly increased in patients with monocytosis associated with cancer, septicemia, acquired immunodeficiency syndrome, and chronic renal failure undergoing dialysis. These findings suggest that CD14⁺CD16⁺ cells may play a key regulatory role following severe injury and may therefore be prognostic.

As a result, we have begun to explore changes in the phenotypic makeup of monocytes following injury. We have been able to consistently demonstrate an increase in the number of CD14⁺CD16⁺ monocytes. Sustained elevation in the expression of this phenotype following injury is associated with the subsequent development of ARDS and MODS. Although causality has not been examined, these cells do liberate increased levels of pro-inflammatory chemokines and cytokines that may in part be responsible for the development of ARDS and MODS.

The mechanism responsible for the development of this phenotype has, however, remained poorly elucidated. Recently, we have demonstrated that circulating monocytes subjected to reprogramming factors, such as oxidant stress, results in the surface expression of CD16. This increased expression of CD16 appears to be cytoskeletally regulated. Therefore, minimizing changes in cellular architecture following injury by therapeutic interventions, such as hypertonic saline, may become a means leading to improved outcome following injury.

Class Prediction Based on Cytokine Profiles

In addition to the alterations in immune cells following injury, we have recently begun to explore the relative changes in cytokine expression profiles following injury. As a result of our multi-center collaboration with the Host Response to Injury and Inflammation consortium, we have examined the early and sustained changes in cytokine expressions following severe injury. To date, we have demonstrated that early elevation in IL-6 to 350 pg/ml within the first 24 hours is predictive of the development of MODS. Although mortality was not predicted by this cytokine profile, patients with elevation in IL-6 were demonstrated to have prolonged ventilator requirements, ICU LOS, hospital LOS, and risk for infection (Table 1).

Similar effects appear to occur with other mediators in a time dependent fashion. These alterations following initial injury may serve to be predictive of poor outcome, and potentially more importantly serve to distinguish future therapies based on innate immunity. Specific therapies targeted at different immune responses would lead to directed individual therapy, rather than non-specific disease based therapy.

	Group 1 (IL-6<350pg/ml) N=47	Group 2 (IL-6>350pg/ml) N=32	p value
IL-6 (pg/ml)	160.3 ± 13.45	1450 ± 390.7	0.0028
Age (yr)	34.64 ± 1.58	32.00 ± 1.62	ns
Gender (% Male)	20(42.5)	25 (78.1)	0.0014
Initial Base Deficit	-8.43 ± 0.529	-8.59 ± 0.67	ns
ER Lowest SBP	87.89 ± 3.33	86.06 ± 4.15	ns
APACHE II score	25.13 ± 0.96	29.75 ± 1.11	0.0026
ISS	28.49 ± 1.90	33.69 ± 2.32	ns
RBCs first 24hrs	1987 ± 181.8	4124 ± 605.4	0.0018
ICU LOS	8.25 ± 0.88	16.28 ± 2.11	0.0011
ICU VENT days	5.72 ± 0.83	13.41 ± 1.86	0.0005
Hospital LOS	16.79 ± 1.99	28.66 ± 4.06	0.0120
Mortality	2 (4.2)	2 (6.2)	ns

TABLE 1

RELATED PUBLICATIONS

1. Cuschieri J, Gourlay D, Garcia I, Jelacic S, Maier RV. Hypertonic Preconditioning Inhibits Macrophage Responsiveness to Endotoxin. *J Immuno* 2002;168(3):1389-96.
2. Cuschieri J, Gourlay D, Bulger E, Garcia I, Jelacic S, Maier RV. Platelet Activating Factor (PAF) Priming of Inflammatory Cell Activity Requires Cellular Adherence. *Surgery* 2002;132:157-166.
3. Cuschieri J, Gourlay D, Garcia I, Jelacic S, Maier RV. Implications of Proteasome Inhibition: Enhanced Anti-inflammatory Macrophage Function. *Cell Immuno*. 2004;227(2):140-7.
4. Cuschieri J, Umanskiy K, Solomkin J, Protein Kinase-Zeta is Essential Toward Endotoxin-Induced Macrophage Activation. *J Surg Res*. 2004;121(1):76-83.
5. Cuschieri J, Implications of Lipid Raft Disintegration: Enhanced Anti-Inflammatory Macrophage Phenotype. *Surgery* 2004;136(2):169-75.
6. Cuschieri J, Gourlay D, Garcia I, Jelacic S, Maier RV. Calcium/Calmodulin-Dependent Kinase II is Required for Platelet Activating Factor (PAF) Priming of Inflammatory Cells. *Shock* 2005;23(2):99-106.
7. Cuschieri J, Bulger E, Garcia I, Maier RV. Oxidative induced calcium mobilization is dependent on annexin VI release from lipid rafts. *Surgery* 2005;138(2):158-64.
8. Cuschieri J, Billgren J, Maier RV, Phosphatidylcholine-specific phospholipase C (PC-PLC) is required for LPS-mediated macrophage activation through CD14. *J Leukoc Biol* 2006;80(2):407-14.
9. Cuschieri J, Billgren J, Maier RV, Endotoxin tolerance attenuates LPS-induced TLR4 mobilization to lipid rafts: A condition reversed by PKC activation. *J Leukoc Biol* 2006;80(6):1289-97.
10. Cuschieri J, Bulger E, Billgren J, Garcia I, Maier RV, Vitamin E inhibits endotoxin-mediated transport of phosphatases to lipid rafts. *Shock* 2007;27(1):19-24.

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- Burn Wound Repair
- Cytokine Response to Thermal Injury
- Neuroinflammatory Response to Wound Repair



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- WA State Association of Fire Fighters Burn Foundation

Wound repair constitutes an essential component of every surgical subspecialty. The health care system spends millions of dollars annually to apply the latest “*goo du jour*” onto wounds. But in spite of all we know about response to injury, we still do not offer good solutions to patients with chronic non-healing wounds or with hypertrophic scars and keloids. Our collective efforts have been focused on understanding the response to cutaneous injury for wounds with either insufficient or exuberant responses.

Burn Wound Repair

With increased patient survival following burn injuries, rehabilitation and problems associated with scarring such as hypertrophy and itching become important. Since early civilization, we have been adapting topical treatments for wounds. While the growth factors that we apply to wounds today are more sophisticated than the honey, wine, oil or resins that were used in ancient medical practices, we still do not know what the growth factors do or when they should be applied.

Valuable studies over the past 30 years have augmented our understanding of the progression of repair from an acute injury through coagulation, inflammation, blood vessel formation, fibrogenesis and epithelialization, and finally to remodeling. Nevertheless, we still do not fully understand normal wound repair and thus, how to therapeutically modulate repair in compromised wounds.

We designed our basic science efforts to define cellular and extracellular inflammatory processes in normal burns. Our aim has been to better understand what deviations result in non-healing wounds or in abnormal scars in order to know when to perturb the healing process with a repair accelerant.

We have studied the temporal and spatial localization of dermal inflammatory cells, basic fibroblast growth factor, macrophage chemoattractant protein-1, and collagenase during repair. Collectively, our data support the theory that the skin itself is a component of the immune system and that non-inflammatory cells may contribute to the initiation and maintenance of the inflammation at the wound site. Furthermore, these studies have accented the notion that inflammatory mediators at the wound site are present at specific phases in the repair process, and that interventions with exogenous mediators must be timely.

Inflammatory Responses to Thermal Injury

With introduction of early excision and grafting and improved critical care, mortality following burn injury has dramatically decreased over the past 30 years; future improvements in survival will require innovative pharmaceutical and wound coverage interventions. For the past five years we have collaborated in a multicenter, multispecialty effort to understand host responses to injury. After five years and a renewal in 2006, we are positioned to correlate clinical data with corresponding genomic and proteomic analyses from patients with severe burn injuries. Ability to predict patients who are likely to develop multi-organ failure or die after a severe injury is the first step in understanding potential targets for therapeutic intervention.

Neuroinflammatory Responses to Wound Repair

Our lab has been dedicated to defining neuroinflammatory responses to wound repair. The sensory nerves in skin regulate pain transmission, but also a local inflammatory response within the wound bed. We have identified normal temporal and spatial distribution of pain fibers in human burn wounds.

We have demonstrated that patients with sensory deficits due to both spinal cord injury and diabetes mellitus have a dramatic reduction in cutaneous sensory nerves, especially

Ability to predict patients who are likely to develop multi-organ failure or die after a severe injury is the first step in understanding potential targets for therapeutic intervention.

in the wound beds. We have also recently determined that activity levels of neutral endopeptidase, a membrane bound enzyme that degrades substance P, is elevated in the wounds and skin of patients and mice with diabetes. Therefore, it was not a surprise to us that exogenous substance P shortens time to healing in a model of delayed wound repair in diabetic mice. We have also observed increased levels of the enzyme neutral endopeptidase in skin and wounds from diabetic mice. We have shown that increased glucose and fatty acids increases neutral endopeptidase levels in cultured endothelial cells. Most interestingly, this increase can be inhibited with antioxidant treatment.

Following injury, sensory nerves are absent within the injury site. With time there appears to be a transient abnormal increase in neuroinflammatory mediator within the wound that eventually approaches normal. These findings are important because itching, which is mediated by neuropeptides, is a major complaint of patients with thermal injuries. Hypertrophic scars have elevated levels of substance P and decreased neutral endopeptidase activity compared to uninjured skin and normal scars.

Our lab is focused on determining endothelial cell derived signals that govern nerve cell differentiation. Sensory nerve-derived neuropeptides stimulate endothelial cells following injury to round up, proliferate and synthesize adhesion molecules and cytokines. These studies are currently focused on intracellular signaling pathways that mediate substance P mediated changes to the endothelial cell. Activated endothelial cells stimulate reinnervation of the injury site. We have defined this process to be a neuro-endothelial axis and believe that it may contribute to the pathophysiology of hypertrophic scar formation. Our latest effort has been to determine the mechanism by which substance P upregulates an inflammatory response. We have evidence that change in substance P-induced cell shape with the accompanying reorganization of the cytoskeleton may be an intermediary step. Most recently we have focused on the role of nitric oxide synthase as means of mediating substance P activity. In wound repair it appears that some cellular responses to substance P involve nitric oxide but others are independent of the reactive oxidative species.

RELATED PUBLICATIONS

1. Pham, T.N. et al. The Clinical Pulmonary Infection Score Poorly Predicts Pneumonia in Patients With Burns. *J Burn Care Res* 28, 76-79 (2007).
2. Imahara, S.D. et al. SCORTEN Overestimates Mortality in the Setting of a Standardized Treatment Protocol. *J Burn Care Res* 27, 270-275 (2006).
3. Muangman, P. et al. The optimal time for early excision in major burn injury. *J Med Assoc Thai* 89, 29-36 (2006).
4. Klein, M.B. et al. Inflammation and the host response to injury: an overview of the multicenter study of the genomic and proteomic response to burn injury. *J Burn Care Res* 27, 448-51 (2006).
5. Scott, J.R., Gibran, N.S., Engrav, L.H., Mack, C.D. & Rivara, F.P. Incidence and characteristics of hospitalized patients with pressure ulcers: State of Washington, 1987 to 2000. *Plast Reconstr Surg* 117, 630-4 (2006).
6. Sullivan, S.R. et al. Elevated orbital pressure: another untoward effect of massive resuscitation after burn injury. *J Trauma* 60, 72-6 (2006).
7. Scott, J.R. et al. Substance P levels and neutral endopeptidase activity in acute burn wounds and hypertrophic scar. *Plast Reconstr Surg* 115, 1095-102 (2005).
8. Saffle, J.R., Gibran, N. & Jordan, M. Defining the Ratio of Outcomes to Resources for Triage of Burn Patients in Mass Casualties. *J Burn Care Rehabil* 26, 478-482 (2005).
9. Cobb, J.P. et al. Application of genome-wide expression analysis to human health and disease. *Proc Natl Acad Sci U S A* (2005).
10. Muangman, P., Tamura, R.N. & Gibran, N.S. Antioxidants inhibit fatty acid and glucose-mediated induction of neutral endopeptidase gene expression in human microvascular endothelial cells. *J Am Coll Surg* 200, 208-15 (2005).

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- Washington State Trauma Registry and Central Region CQI
- Post-Traumatic Stress Disorder in Trauma Patients
- Field Triage Document and Implementation Toolkit



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- National Center for Injury Prevention and Control

National Highway Traffic Safety Administration

National Institute of Mental Health

National Study on Costs and Effectiveness of Trauma Care

For the past five years the University of Washington and Johns Hopkins University have been collaborating on the largest extramural grant ever awarded by the National Center for Injury Prevention and Control of the Centers for Disease Control and Prevention (CDC) for the study of injury. This project, titled “The National Study on Costs and Effectiveness of Trauma Center Care,” has as its principle investigator at Johns Hopkins University Dr. Ellen MacKenzie, Professor of Health Policy, Senior Associate Dean for Academic Affairs in the School of Public Health, and Director of the Johns Hopkins Center for Injury Research. The Principle Investigators at the University of Washington are Dr. Gregory J. Jurkovich, Professor of Surgery, Chief of Trauma at Harborview, and Director of the Acute Care Section of the Harborview Injury Prevention and Research Center (HIPRC) and Dr. Fred Rivara, George Atkins Professor of Pediatrics and past Director of Harborview Injury Prevention and Research Center.

The purpose of this \$4.8 million, direct-cost grant is to examine variations in trauma care, and outcomes from trauma care, in designated trauma centers compared to non-trauma centers across the United States. Specific outcomes to be addressed include mortality, morbidity, functional outcome, and quality of life status. Estimates of costs associated with care will also be conducted at Level I Trauma Centers, Level II Trauma Centers, and non-trauma centers.

The specific aims of this research project are to:

- Examine variation in trauma care between trauma centers and non-trauma centers;
- Examine the relationship between treatment received and mortality, complications, & functional outcome;
- Estimate the costs of care at trauma centers vs. non-trauma centers; and
- Describe the relationship between cost and effectiveness of care.

The study has carefully selected 14 regions of the country and 80 hospitals from which we recruited patients. These locations were selected based on data from the Area Resource File, the American Hospital Association, and trauma center designation databases. These hospitals were selected to represent a wide range of volumes and hospital characteristics in these 14 regions. We identified lead physicians for the study at each of these hospitals and collected comprehensive data from each institution on available resources for the care of trauma patients. In addition to IRB approval by Johns Hopkins and the University of Washington, we sought and obtained IRB approval (and annual renewals) from each of these 80 hospitals. From this initial total we ended with 18 Level 1 trauma centers and 51 non-trauma center hospitals in 12 states.

We hired skilled nurses to serve as regional coordinators in each of these 14 regions and undertook rigorous training of them in patient identification procedures and chart abstraction to guarantee high quality data collection. We collected ongoing data on all hospital discharges for trauma in each of the study hospitals for 15 months, and developed new software to identify eligible patients on the basis of

injury severity, age and body region injured. We developed a sophisticated sampling algorithm and instituted this to identify 8,000 trauma patients for the study.

We contracted with Westat, one of the leading survey research firms in the world, to conduct follow-up phone interviews at 3 and 12 months after injury. We spent a great deal of time developing, piloting and revising measures to determine functional outcomes at these follow-up times. We culled the literature, consulted our National Advisory Committee, consulted experts and developers of measures to come up with the most comprehensive, sensitive group of indicators of functional outcome. We have completed all three-month patient interviews and 12-month interviews, for an 80% follow-up rate.

We developed software for chart abstraction, trained our regional coordinators in it, and have abstracted about 2,000 fields of chart data. We have obtained charts from transferring hospitals as well as charts on re-hospitalizations.

To determine costs of care, we have obtained hospital bills on each of the study patients and abstracted them using the UB-92 standard format. To supplement the CDC funds for this project, we wrote a grant and were funded by the National Institute on Aging to obtain Medicare data on the study patients aged 65 and older. We obtained data from MarketScan to determine national data on professional fee costs for trauma.

The products from this study will be remarkable. Just a few of them are:

- Determination of for which types of patients and kinds of injuries trauma center care has better outcomes than care in non-trauma centers.
- The most complete data available on the cost of trauma, payor mix and how these vary by type of hospital.
- Relationship between cost of trauma care and outcome.
- We will be able to recommend the best measures to be used for examining functional outcome of trauma.
- Determination of the types of hospital resources which make the most significant impact on outcome from trauma.
- Determination of the types of pre-hospital resources which make the most significant impact on outcome from trauma.
- Relationship between volume of trauma care and outcome for a wide variety of injury problems.
- Determination of how transfer status affects outcome.
- Understanding of how trauma systems interact with trauma center status of hospitals to influence outcomes.

Data collection for this study is complete, and includes 1,104 patients who died in hospital and 4,087 patients who were discharged alive. Our first major publication is in the *New England Journal of Medicine*, focusing on the mortality advantage seen in trauma centers compared to non-trauma centers. We used propensity-score weighting to adjust for observable differences between patients treated at trauma centers and those treated at hospitals without a trauma center. We have demonstrated a 20% reduction in in-patient deaths at trauma centers vs. non-trauma centers (7.6% vs. 9.5%) and a 25% one-year death rate reduction (10.4% vs. 13.8%). The life-saving beneficial effects of trauma center care is most evident in the younger (age <55), more severely injured patients (AIS 4-5), with a relative risk of death within 30 days of injury between 0.67 and 0.78 (CI <1.0). Vexing questions remain on why this dramatic beneficial effect is not seen in the elderly, and will be the focus of further studies. Ongoing evaluation of this data set has determined that the best functional outcomes for lower extremity fractures are also obtained in Level I trauma centers. Disappointingly, we have been unable to show any variation in the functional outcome following head injury based on center type of acute care provided. Cost effectiveness evaluation is ongoing.

Washington State Trauma Registry and Central Region CQI

Washington State now has a trauma system that has been in place for approximately eight years. Previous studies (See Nathens et al) have suggested that it takes about this length of time for a trauma system to mature, and to show benefits in life-saving effects of trauma center care. Central Region (conforming geographically to King County) is one of eight designated trauma and emergency medical regions in the state, and has been collecting trauma registry data such information for the past eight years.

The Central Region Quality Assurance Committee oversees the collection and analysis of these data, in an effort to analyze and improve trauma care and outcomes in the Central Region. This committee, along with personnel from the Harborview Injury Prevention Center and the State Department of EMS and Trauma Care, is analyzing the data in an effort to address a variety of trauma system issues which remain largely unanswered in today's trauma systems. These include such questions as, "How long is too long in the pre-hospital phase of care?"; "How many patients and of what severity are essential to maintain skills and good outcome?"; and "When should you bypass the closest lowest level trauma center for the highest level trauma center?"

Youth admitted to the hospital for physical injury are at increased risk for recurrent traumatic life events; identifiable risk factors appear to be assault injury and history of injury prior to inpatient admission.

Ongoing or recently completed data analysis includes the outcomes of elderly patients with hip fractures in Central Region trauma and non-trauma hospitals, the distribution of the most severely injured patients (ISS>15) within the regional trauma centers, and Airlift Northwest landing zone delays by site location, the outcome on non-operated splenic injuries, and an assessment of preventable mortality in the region. A comparison of Central Region trauma patient outcomes to a national reference, the Major Trauma Outcome Study, reveals a significantly lower mortality for both adult blunt and penetrating trauma patients treated in the Central Region compared to this national norm.

Post-Traumatic Stress Disorder in Trauma Patients

A valued addition to the Department of Psychiatry at Harborview Medical Center is Dr. Doug Zatzick. He has a special interest in post traumatic stress disorder (PTSD) in trauma patients, and is responsible for initiating cooperative studies between surgery, pediatrics, and psychiatry on the assessment and treatment of PTSD in trauma patients. PTSD occurs in 20-40% of patients over the course of the year after physical injury. Youth admitted to the hospital for physical injury are at increased risk for recurrent traumatic life events; identifiable risk factors appear to be assault injury and history of injury prior to inpatient admission. Further, in a study comparing PTSD at Harborview and UC Sacramento, 58% of 269 randomly selected injury survivors who were screened for PTSD, depressive, and peritraumatic dissociative symptoms demonstrated high levels of immediate posttraumatic distress and/or alcohol abuse/dependence. Regression analyses identified greater prior trauma, non-white ethnicity, and site as significant independent predictors of high levels of posttraumatic distress. Early mental health screening and intervention procedures that target both PTSD and alcohol use should be developed for acute care settings.

Studies conducted at Harborview have also demonstrated that injured adolescents represent a high-risk pediatric population, with almost 40% reporting no source of primary care, 30% showing signs of PTSD, 11% with high depression symptom levels, and 17% with problem alcohol use. The burden of these largely unrecognized and untreated medical psychiatric issues is likely to include significant recidivism.

A growing body of clinical trials research suggests that PTSD may be efficaciously treated with psychotherapeutic and psychopharmacological interventions. Also, there is now evidence that pediatricians can successfully detect and intervene with youth and their families who are suffering from psychosocial disturbances. An additional aim of the investigation is to elucidate the clinical, family and community infrastructures available to support the implementation of psychosocial interventions for injured youth with PTSD. The overarching goal of the proposed investigation is to provide preliminary data that will inform the development of a larger scale R01 funded randomized intervention trial targeting PTSD and posttraumatic functional impairment among injured adolescents.

In a remarkable blend of basic molecular science and clinical care, some researchers are beginning to investigate the gene expression signatures on peripheral blood cells (monocytes), and preliminary work suggests that such genetic expression is distinct and recognizable and predictive of those who go on to develop PTSD and those who do not. (Shefi et al, *Molecular Psychiatry* 2005).

Triage of Trauma Patients from the Field

The CDC and National Highway Traffic Safety Administration (NHTSA) have asked me to chair a diverse working group of individuals representing health care providers from emergency medicine, prehospital care, surgical care, and bioengineering to revise, disseminate, and implement national guidelines for the triage of injured patients from the field. This working group has revised the “Field Triage

Document” as published by the American College of Surgeons Committee on Trauma Resource Document of Care of the Injured Patient. The next step is wide dissemination of these guidelines, and the development of a “tool kit” for implementing these guidelines in trauma system design across the country.

RELATED PUBLICATIONS

1. Bulger EM, Jurkovich GJ, Farver CL, Klotz P, Maier RV: Oxandrolone does not improve outcome of ventilator dependent surgical patients. (American Surgical, April, 2004) *Ann Surg* 240(3): 472-80, 2004.
2. Nathens AB, Jurkovich GJ, MacKenzie EJ, Rivara FP: A resource-based assessment of trauma care in the United States. *J Trauma* 56(1):173-178, 2004.
3. Zatzick D, Jurkovich GJ, Russo J, Roy-Byrne P, Katon W, Wagner A, Dunn C, Wisner D, Grossman D, Rivara F: Posttraumatic distress, substance intoxication, and recurrent trauma across Level 1 trauma center sites. *J Trauma* 57(2): 360-366, 2004.
4. Zatzick, DF, Roy-Byrne P, Russo J, Rivara FP, Driesch R, Wagner A, Dunn C, Jurkovich GJ, Uehara E, and Katon W. A randomized effectiveness trial of stepped collaborative care for acutely injured trauma survivors. *Arch Gen Psychiatry* 61(5): 498-506, 2004.
5. Bulger EM, Copass MK, Sabath D, Maier RV, Jurkovich GJ: The use of neuromuscular blocking agents to facilitate prehospital intubation does not impair outcome following traumatic brain injury. (presented at the AAST, Sept, 2004): *J Trauma* 58:718-24, 2005.
6. McIntyre LK, Schiff M, Jurkovich GJ. Failure of non-operative management of splenic injuries: causes and consequences. *Arch Surg* 140(6): 563-569, 2005.
7. Zatzick DF, Russo J, Pittman R, Rivara FP, Jurkovich GJ, and Roy-Byrne P. Reevaluating the association between emergency department heart rate and the development of PTSD: A public health approach. *Biol Psychiatry* 57(1): 91-95, 2005.
8. Zatzick, DF, Russo J, Rivara FP, Jurkovich GJ, Roy-Byrne P and Katon W. The detection and treatment of patients with posttraumatic distress and substance intoxication in the acute care medical setting. *Gen Hosp Psychiatry* 27(1):57-62, 2005.
9. Zatzick, D, Grossman DC, Russo J, Pynoos R, Berliner L, Jurkovich GJ, Sabin J, McCauley E, Ghesquiere A, Rivara FP: Predicting posttraumatic stress symptoms longitudinally in a representative sample of hospitalized injured adolescents. *J Am Acad Child Adolesc Psychiatry*. 2006 Oct; 45(10):1188-95.
10. Zatzick, D, Russo J, Grossman DC, Jurkovich GJ, Sabin J, Berliner L, Rivara F: Posttraumatic stress and depressive symptoms, alcohol use, and recurrent traumatic life events in a representative sample of injured adolescents and their parents. *J Ped Psychology* 2006.
11. Utter GH, Maier RV, Rivara FP, Mock CN, Jurkovich GJ, Nathens AB: Inclusive trauma systems: do they improve triage or outcomes of the severely injured? (Presented, AAST Sept 2005) *J Trauma*. 2006 Mar;60(3):529-35; discussion 535-37.
12. Esposito TJ, Leon L, Jurkovich GJ: The shape of things to come: results from a national survey of trauma surgeons on issues concerning their future. (presented, AAST September 2005). *J Trauma* 60(1): 8-16, 2006.
13. MacKenzie EJ, Rivara FP, Jurkovich GJ, Nathens AB, Frey KP, Egelston BL, Salkever DS and , Scharfstein DO: A national evaluation of the effect of trauma center care on mortality. *N Eng J Med* 354 (4): 366-378, 2006.
14. Hunt RC, Jurkovich GJ: Field triage: opportunities to save lives. *Prehosp Emerg Care* 2006 Jul-Sept; 10(3): 282-3.
15. Hunt RC, Jurkovich GJ: Deliberations and recommendations. *Prehosp Emerg Care* 2006 Jul-Sept; 10(3): 355.

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Ronald V. Maier, M.D.

- Harborview Injury Prevention and Research Center
- Clinical Trials in the Surgical Intensive Care Unit
- Modulation of the Excessive Inflammatory Response to Biomaterials
- Modulation of the Trauma-Related Macrophage Inflammatory Response to Prevent ARDS, MOFS and Death
- Genomic Controlled Phenotypic Response to Severe Injury



AWARDS

- American Trauma Society
 - William S. Stone Award
- National Institutes of Health
 - NIGMS Institutional NRSA
- Shock Society
 - Scientific Achievement Award

FUNDING

- Centers for Disease Control
 - National Center for Injury Prevention and Control
- Engineering Research Center (ERC)

National Institutes of Health

- Institutional National Research Service Award
- National Heart, Lung and Blood Institute
- National Institute of General Medical Sciences

National Science Foundation

Trauma remains a major cause of death and morbidity in America. It is the number one cause of mortality among 1-45-year-olds and is the overall number one cause of loss of productive years of life in America. Death due to injury occurs in three peaks: 1) at the scene; 2) during the acute resuscitation phase; and 3) late, after one to two weeks of ICU support, secondary to multiple organ failure and sepsis. My research focuses on each of these phases. Prevention provides the best means to minimize deaths at the scene. Trauma system developments and improvements in acute care, including early resuscitation will reduce early deaths and minimize subsequent morbidity. Finally, elucidation of the genomic and molecular responses to severe injury will identify treatment modalities to prevent the autodestructive inflammatory response causing organ dysfunction and death following trauma.

Harborview Injury Prevention and Research Center

Dr. Maier is Senior Advisor of the Harborview Injury Prevention and Research Center (HIPRC). HIPRC is linked closely with the Northwest Regional Trauma Center at Harborview Medical Center. The goal of HIPRC is to diminish the impact of trauma on people's lives and to draw on the effectiveness of the Northwest Regional Trauma Center's injury prevention and trauma treatment programs. Established at HMC in 1985, HIPRC is a component of the University of Washington and the Schools of Medicine and Public Health.

Current projects include identifying the risk factors for injury while developing new techniques for the application of epidemiology in the field of trauma research. Further goals are to develop and utilize systematic, high-quality data systems to document the types, causes, treatment

and consequences of injuries in a wide variety of settings. A particular focus is on assessment of outcomes and the impact of trauma system development. In addition, development and assessment of new, more effective means to resuscitate and treat injured patients along the entire spectrum of care from prehospital to rehabilitation is ongoing. Following are examples of current investigations:

EVALUATION OF THE EFFECT OF STATE FIREARM LEGISLATION ON FIREARM MORTALITY

Firearm-related mortality continues to comprise approximately twenty percent of all injury related deaths in this country, despite the implementation of "preventive" legislation regulating handguns. Numerous handgun laws have been enacted, and the ultimate effect of such legislation on firearm violence is questionable and highly debated. We have investigated whether a "shall issue" law permitting unrestricted carrying of concealed handguns, a minimum age of twenty-one for private purchase, a minimum age of twenty one for possession, a mandatory registration law, restricting purchases to "one gun a month," or a ban on "junk guns" would reduce firearm related mortality.

We have reviewed vital statistics for the entire United States from 1979-1998 looking at total and firearm, homicide and suicide death rates. "Shall issue" and mandatory registration laws were associated with a respective 17% and 21% increase in homicide rates. Mandatory registration and a ban on "junk guns" reduced firearm suicide rates. Individual gun legislation varies in regard to the effect on firearm mortality. Permitting unrestricted carrying of concealed weapons through "shall issue" laws increases firearm and total homicide rates. Implementing laws restricting the purchase or possession of handguns by persons younger than twenty-one years of age reduces firearm homicide and firearm suicide rates in youths.

RELATIONSHIP BETWEEN TRAUMA CENTER VOLUME AND OUTCOME

The premise underlying regionalization of trauma care is that optimal outcomes can be achieved at greatest efficiency if care is restricted to relatively few dedicated trauma centers. Implicit in this premise is that higher patient volumes will lead to greater experience and this experience translates into better outcomes. This relationship appears to hold for other areas of surgical care involving complex procedures but, in contrast, there is no such relationship when less complex procedures are evaluated. Previous studies evaluating the relationship between institutional volume and outcomes in trauma patients are difficult to interpret because of multiple logistic issues.

Two distinct cohorts of trauma patients are being evaluated, including penetrating abdominal injury and multisystem blunt trauma with a minimum head injury and lower extremity, long bone fracture, treated at 31 academic Level I or Level II trauma centers across the United States, participating in the University Health System Consortium. Results indicate a strong association exists between trauma center volume and outcome, with significant improvements in mortality and length of stay, but only when the volume exceeds at least 600 cases per year, and these benefits were only evident in patients at the highest risk for adverse outcomes and not in the vast majority of lesser-injured patients.

Clinical Trials in the Surgical Intensive Care Unit

We are performing multiple ongoing trials based on the pathophysiologic response of the severely injured patient, many in conjunction with the Division of Pulmonary and Critical Care in the Department of Medicine. In particular, clinical studies and associated basic investigations are focused on the acute respiratory distress syndrome (ARDS), which affects critically ill and injured patients.

ARDS is largely responsible for the prolonged intensive care unit and hospital stay, and contributes significantly to mortality in these patients. Management is primarily supportive while the underlying disease process stabilizes and resolves. Attempts to reduce the consequences of ARDS have focused upon 1) pharmacologic manipulation of the inflammatory response, and 2) modifying positive pressure ventilation techniques to reduce the potential iatrogenic ventilator-associated lung injury. Examples of current studies are:

LOW TIDAL VOLUME VENTILATION IN ARDS

The mortality rate from acute lung injury and ARDS is approximately 40-50%. Traditional approaches to mechanical ventilation use tidal volumes of 10-15 ml/kg of body weight. These volumes are much larger than those in normal subjects at rest, but are frequently necessary to achieve normal values for partial pressure of arterial carbon dioxide and pH. Since atelectasis and edema reduce aerated lung volumes, inspiratory airway pressures are often excessively high to achieve these parameters, suggesting the presence of excessive distension, or "stretch," of the remaining aerated lung.

Thus, this traditional approach to mechanical ventilation exacerbates or perpetuates lung injury and, in contrast, the use of lower tidal volumes during ventilation reduces or prevents this deleterious process. Previous uncontrolled studies suggest that lower tidal volumes may improve survival. However, this approach may necessitate acceptance of significant acidosis and decreased arterial oxygenation, or increased levels of PEEP. A clinical trial in conjunction with the ARDS Network tested whether lower tidal volumes during mechanical ventilation in patients with acute lung injury improved ARDS severity and/or survival. The trial has been stopped after enrollment of 861 patients because mortality was lower in the group treated with lower tidal volumes. Mean tidal volumes were 6 cc/kg vs. 12 cc/kg, with a subsequent reduction of mean plateau pressures to 25 cm compared to 34 cm of water. Thus, in patients with acute lung injury and ARDS, mechanical ventilation with a lower tidal volume and, subsequently, a lower mean plateau pressure results in decreased mortality.

MODULATION OF THE INFLAMMATORY RESPONSE

The potentially auto-destructive excessive immuno-inflammatory response is thought to contribute to the initiation and progression of ARDS and to ultimately affect patient outcome. Work at Harborview Medical Center (HMC) has shown a high incidence of Vitamin C and potential Vitamin E deficiency in trauma patients admitted to the HMC intensive care unit. A study of patient admissions to HMC found that 64% of patients had plasma Vitamin C levels below the reference range and 23% of patients had plasma Vitamin C levels less than 0.20mg/dL, indicating Vitamin C deficiency as defined by the World Health Organization. Reports from other institutions document a low plasma Vitamin C concentration in 28-83% of select hospitalized patient populations and 12-21% in a random sample of all new hospital admissions.

Our HMC study demonstrated that supplementing 3 grams/day of Vitamin C and 3000 IU/day of Vitamin E in patients with initially low levels resulted in plasma levels within the normal reference range within seven days. Patients not receiving supplements remained in the low or below the reference range. The significance of Vitamin C deficiency in these patients is illustrated by a study of 78 patients with 105 fractures of the mandible treated at HMC: those patients who had fracture complications (infection, malunion) had significantly lower serum Vitamin C concentration than those with good fracture outcomes. In addition, patients with ARDS have been shown to have high levels of oxidants and suppressed levels of antioxidants, such as Vitamin C and Vitamin E, in bronchoalveolar lavage (BAL) specimens.

We hypothesize that routine supplementation of Vitamin C and E will protect against oxidant-induced injury in severely injured and stressed patients, and avoid the diffuse insult predisposing to ARDS and other organ dysfunction, and also secondary nosocomial infections such as ventilator-associated pneumonia and wound infections. In a prospective observational study, all trauma admissions to the HMC surgical ICU had three grams of Vitamin C or 3,000 IU of Vitamin E, divided over three doses per day, started at the time of admission. Otherwise, care was standard and the populations were followed to determine the incidence of ARDS, duration in the ICU, mortality and infectious complications. In addition, we studied BAL samples for evidence of oxidant injury and cytokine production.

The results show that the treatment with anti-oxidant supplementation on admission to the surgical ICU produced a 50% reduction in evidence of oxidant injury in the BAL solution, along with a 50% reduction in the production of inflammatory mediators, while having no detrimental effect on the production of antibacterial mediators of the immune system. Concomitant with this decrease in intrapulmonary inflammatory response, there was a decrease by 50% in the incidence of ARDS and a significant decrease in length of stay and ventilator days in these critically ill patients. Concomitant with this decrease in development of ARDS and inflammation was a 50% reduction in mortality in the treated population.

Modulation of the Excessive Inflammatory Response to Biomaterials

The production and release of potent inflammatory mediators by tissue-fixed macrophages coordinate and orchestrate a series of biologic events that lead to either normal wound healing or abnormal chronic granulation

and typical “foreign body” reaction. The goal of the experiments performed in conjunction with the University of Washington Engineered Biomaterials (UWEB) program funded by the NSF is to define the cell signaling processes that control the pro-inflammatory phenotype of the macrophage in response to various biomaterials and cause the subsequent chronic inflammatory response that leads to non-healing and extrusion of biomaterials.

Preliminary experiments demonstrated that adherence by the macrophage to various surfaces primes the macrophage for activation. Subsequent steps in the inflammatory response lead to multi-nucleated giant cell formation and subsequent capsule formation, secretion of extracellular matrix, vascular budding, and fibroblast proliferation with thick collagen deposition. Prevention of the pro-inflammatory phenotype may well equate with prevention of foreign body reaction. In current studies, we are investigating coating of biomaterials with various molecules. These include osteopontin and various anti-inflammatory agents, such as anti-oxidants, including Vitamin E and components of the extracellular matrix, such as hyaluronic acid derivatives, to test the subsequent response of adherent macrophages to inflammatory stimuli, such as endotoxin.

In addition, we are studying materials of various selected pore sizes to minimize cell spreading and to test spatial structural impact on macrophage response to inflammatory stimuli. End-product analysis of inflammatory mediators, such as TNF, procoagulant activity and IL-8, along with the normally produced anti-inflammatory mediators, IL-10 and PGE2, are monitored. These mediators exist in a delicate balance and time sequence to produce normal, as opposed to abnormal, wound healing and chronic inflammation.

The ultimate goal is to modulate the surface characteristics of biomaterials so that they may be adapted as “compatible” and elicit a normal host response and normal wound healing with incorporation of the biomaterial — “true healing.”

Modulation of the Trauma-Related Macrophage Inflammatory Response to Prevent ARDS, MOFS and Death

The last major area of investigation is based on the aberrant host immuno-inflammatory response to trauma and sepsis. This auto-destructive response is thought to be responsible for the induction and persistence of the “malignant systemic inflammatory response” underlying ARDS and multiple organ failure syndrome (MOFS). ARDS and MOFS are the major determinants of late death following trauma.

The primary etiology of ARDS and MOFS leading to late mortality following trauma is the clinical “sepsis syndrome,” or systemic inflammatory response syndrome (SIRS). This diffuse inflammatory response causes disseminated tissue injury and subsequent organ dysfunction. The long-lived, highly diverse tissue-fixed macrophage is a crucial central coordinator of both the normal and the aberrant host immuno-inflammatory response. The macrophage is both primed and activated by a multitude of stimuli during the inflammatory response.

Until now, therapeutic approaches have focused on control or inhibition of single components of the overall inflammatory response. However, since the inflammatory response is replete with redundancy and feedback amplification mechanisms, it is appealing to take a broader approach to control the inflammatory response and subsequent injury to multiple diffuse organ beds. To

A major focus is on the ability of anti-oxidants, such as vitamin E, or cytoskeletal spatial disruption with agents, such as cytochalasin D, to modify the cellular response to inflammatory stimuli. Recent investigations have also demonstrated that hypertonic preconditioning similarly disrupts the signaling pathways in the macrophage. Hypertonic saline has been shown to produce an adequate resuscitation for the severely injured while limiting the excessive inflammatory response. Recent investigations have confirmed that hypertonic saline led to a reduction in ERK1/2 phosphorylation with no effect on p38. This was correlated with an inhibition of stress fiber formation in the macrophages and appears to link the necessity for cytoskeletal polymerization for optimal MAP kinase signal transduction and inflammatory mediator production. Thus, hypertonic saline early in the response of the host to reperfusion injury could lead to a reduction in subsequent organ injury and failure. Elucidation and control of these

We have reviewed vital statistics for the entire United States from 1979–1998 looking at total and firearm, homicide and suicide death rates. “Shall issue” and mandatory registration laws were associated with a respective 17% and 21% increase in homicide rates.

achieve this goal in these basic laboratory investigations, we are focusing on the cellular and molecular mechanisms involved in macrophage signaling and activation by inflammatory stimuli and the subsequent production of multiple inflammatory cytokines.

The goal is to develop therapeutic interventions based on controlling these intracellular transduction pathways and to modulate the over-aggressive macrophage response and the subsequent auto-destructive immuno-inflammatory response. Currently, we are studying the manipulation of cellular signal transduction mechanisms that control inflammatory mediator genes by altering the intracellular levels and release of calcium, the regulation of levels of cyclic AMP and the delineation of regulatory protein kinase signal transduction pathways, particularly the MAP kinase family, including ERK1/2, JNK and p38. In addition, we are investigating signaling processes activated through formation of focal adhesion complexes induced by adherence of the monocyte/macrophage as critical to the host inflammatory cell response.

macrophage cellular mechanisms will permit development of future safe therapies to prevent ARDS, MOFS and death in the critically ill surgical patient.

Genomic Controlled Phenotypic Response to Severe Injury

To better understand the pathophysiologic phenotype in the severely injured patient, a collaborative study has been developed, funded by the NIH-NIGMS for a “Glue Grant,” a consortium and large-scale project grant. The intent is to study the entire human genomic response across time to the severe stress of injury, resuscitation and subsequent nosocomial infections. To enable this, the technologic developments necessary for reproducible, high quality isolation of RNA and analysis via microarray chips have been developed through this consortium. The analysis of gene expression data in clinical medicine has been plagued by a lack of critical evaluation of accepted methodologies for the collection, processing, and labeling of RNA.

Using whole blood obtained from healthy subjects, the blood was either untreated or stimulated ex vivo with SEB. Blood samples were also collected from trauma patients, but were not stimulated ex vivo. Total RNA was isolated from the whole blood with the PAXgene proprietary blood collection system or from isolated leukocytes. Biotin cRNA was hybridized to Affymatrix GeneChips. Correlation coefficients for gene expression measurements and replicates from healthy subjects using both techniques is excellent. Unsupervised analyses, including hierarchical cluster analysis, however, revealed that the RNA isolation method resulted in greater differences in gene expression than stimulation

with SEB or among different trauma patients. The intraclass correlation as a measure of signal-to-noise ratio of the difference between SEB stimulated and unstimulated blood from healthy subjects was significantly higher in the leukocyte-derived samples, than in whole blood. Thus, the isolation of RNA from whole blood using the buffycoat is critical to the validity of the microarray analyses. For ongoing studies, the buffycoat and subpopulations are being employed to analyze the serial sequence of genomic responses in the severely injured to identify patterns predictive of trajectory and subsequent outcome.

RELATED PUBLICATIONS

1. "US Trauma Center Crisis: Lost in the Scramble for Terror Resources." Board of Directors, National Foundation for Trauma Care. May 2004.
2. Jurkovich GJ, Maier RV, Rivara F, Johansen J: CDC Injury Research Agenda: Identification of Acute Care Research Topics of Interest to the CDC-NCIPC. *J Trauma*, 56:1166-70, 2004.
3. Feezor RJ, Baker HV, Mindrinos M, Hayden D, Tannahill CL, Brownstein BH, Fay A, MacMillan S, Laramie J, Xiao W, Moldawer LL, Cob JP, Miller-Graziano CL, Maier RV, Schoenfeld D, Davis RW, Tompkins RG. Inflammation and the Host Response to Trauma Large Scale Collaborative Research Program: Whole Blood and Leukocyte RNA Isolation for Gene Expression Analyses. *Physiol Genomics* 19:247-54, 2004.
4. Cuschieri J, Gourlay D, Garcia I, Jelacic S, Maier RV: Implications of Proteasome Inhibition: An Enhanced Macrophage Phenotype. *Cell Immunol*, 227(2):140-7, 2004.
5. Nathens AB, Brunet PF, Maier RV: Development of trauma systems and effect on outcomes after injury. *Lancet*, 363(9423):1794-801, 2004.
6. Cuschieri J, Gourlay D, Bulger E, Garcia I, Jelacic S, Maier RV: Calcium/calmodulin-dependent kinase II is required for platelet activating factor priming. *Shock* 23(2):99-106, 2005.
7. Rosengart MR, Cummings P, Nathens AB, Heagerty P, Maier RV, Rivara FP: An Evaluation of State Firearm Regulations and Homicide and Suicide Death Rates. *Injury Prevention*, 11:77-83, 2005.
8. Staudenmayer KL, Maier RV, Jelacic S, Bulger EM: Hypertonic saline modulates innate immunity in a model of systemic inflammation. *Shock*, 23(5):459-63, 2005.

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· Pathophysiology of Post-Injury Infection and Organ Failure



FUNDING

National Institute of General Medical Sciences

Severe traumatic injury results in biochemical and physiological changes that often lead to the development of nosocomial infection (pneumonia, wound infections, etc) and remote organ (lung, kidney, liver) failure. Excluding those patients who succumb to their injuries and die in the immediate (≤ 1 hour) or early (≤ 24 hours) post-injury period, infection and organ failure (MODS; multiple organ dysfunction syndrome) are leading causes of death. Furthermore, infection and organ failure contribute to prolonged and resource intensive hospital stays. However, if these complications are not lethal, they do not appear to result in major long-term disabilities.

Despite considerable progress in the understanding of the pathophysiology of post-injury infection and organ failure, it has been difficult to translate the observations made in well-designed animal experimentation into effective therapeutics in humans. Two possibilities exist that are, in part, responsible for this inability to clearly influence the course of post-injury infection and organ failure. First, it is likely that our understanding of the problem is incomplete, not from an informational perspective, but rather a conceptual oversimplification in an attempt to force a simple linear “cause – effect” model on a condition that represents a complex biological system with numerous inputs and multiple possible outputs or phenotypic expressions. Second, failure to consider individual variability, in the form of gene polymorphisms, may have reduced our ability to detect beneficial effects of novel therapies.

We are interested in both of these related phenomena and our research program aims to characterize genetic influences on the risk for and outcome from injury-related nosocomial infection and organ failure and to better characterize the nature of the inflammatory response to tissue injury. Our research program is directed at understanding

the genetic basis for human variation in inflammatory responses and how these differences influence the clinical course of sepsis. We are also focusing on pathways that have traditionally been not considered “inflammation-related”, but appear to have important influences on how the inflammatory and innate immune responses are regulated in humans.

The TLR4 +896 polymorphism is not associated with lipopolysaccharide hypo-responsiveness in leukocytes

Genetic variation in the innate immune response likely contributes to the marked variation seen in the risk for and outcome from infectious diseases, including sepsis. Epidemiologic studies have demonstrated a strong familial association with death from infectious disease in general and, more specifically, an association between a familial “anti-inflammatory” response and death from meningococcal sepsis. The role of specific genetic differences in conferring risk is less certain, with many examples of discordant observations regarding numerous genetic variants. Examples of conflicting observations have primarily concerned single nucleotide polymorphisms (SNPs) in genes involved in the innate immune response, such as tumor necrosis factor – alpha (TNF α), lipopolysaccharide binding protein (LBP) and CD14. LPS is a major component of the outer wall of gram-negative bacteria, serving as the key ligand for immune cell recognition and activation in response to infection. Innate immune cells, such as macrophages and monocytes, recognize endotoxin by a specific receptor complex, which contains CD14, LPS binding protein (LBP), and Toll-like receptor-4 (TLR4). Recognition by this receptor complex leads to the activation of specific mitogen-activated kinases (MAPK), including p38, and the synthesis and release of pro-inflammatory cytokines, including tumor necrosis factor α (TNF α),

interleukin-1 β (IL-1 β), and IL-6. TLR4 is central to LPS signaling. Its role is highlighted by animal and in vitro studies that have identified mutations in the TLR4 gene associated with hypo-responsiveness to LPS and hyper-susceptibility to infection by gram-negative bacteria (8-11).

Polymorphisms within the Toll-like receptor-4 (TLR4) gene may influence inflammatory responses in important ways. In particular, a biallelic SNP in the human TLR4 gene has been identified with a frequency approaching 10% in Caucasian populations (12) located in exon three at position +896 base pairs (bp) from the transcriptional start site. This polymorphism represents an A-G base transition resulting in an aspartic acid to glycine exchange at position 299 in the amino acid sequence (referred to as Asp299Gly or A+896G) and often co-segregates with another mutation at +1196. This second SNP is a non-synonymous C-T transition, replacing threonine with isoleucine at position 399 (referred as Thr399Ile or C+1196T). The TLR4 +896 variant (G allele) confers an alteration to the extra-cellular domain of the TLR4 receptor. Carriers have been reported to have an impaired response to bacterial endotoxin exposure compared to wild-type controls (13-16), and they may be at increased risk for gram-negative infections and septic shock, and mortality from systemic inflammatory

response syndrome (SIRS) (17-19). However, other in vivo and clinical studies have shown inconsistencies in the link between coding SNPs in the TLR4 gene and the inflammatory response (20-22).

We sought to determine the effect of the A+896G polymorphism within the TLR4 gene on the response to lipopolysaccharide in a population of healthy donors. We assessed the variability of wild-type and carriers with respect to LPS induced ex vivo PBMC activity of MAPK p38. Next, we analyzed the effect that the variant allele may have on LPS induced whole blood production of the inflammatory cytokines, TNF α , IL-1 β , and IL-6.

Venous whole blood samples were drawn on three occasions from 12 healthy subjects (8 wild-type and 4 G-allele carriers). For each subject, a dose-response curve was generated, plotting supernatant cytokine concentration against LPS concentration (Figure 1a). The LPS concentration that resulted in a half-maximal response (EC₅₀) was calculated as an estimate of leukocyte sensitivity to LPS and compared between +896 genotypes. There was no difference in log EC₅₀ for IL-6 production when comparing carriers of the TLR4 +896G allele and wild-type allele controls (Figure 1b). We also observed no difference in the EC₅₀ in TNF α and IL-1 β production in these same experiments (Figure 1c & 1d).

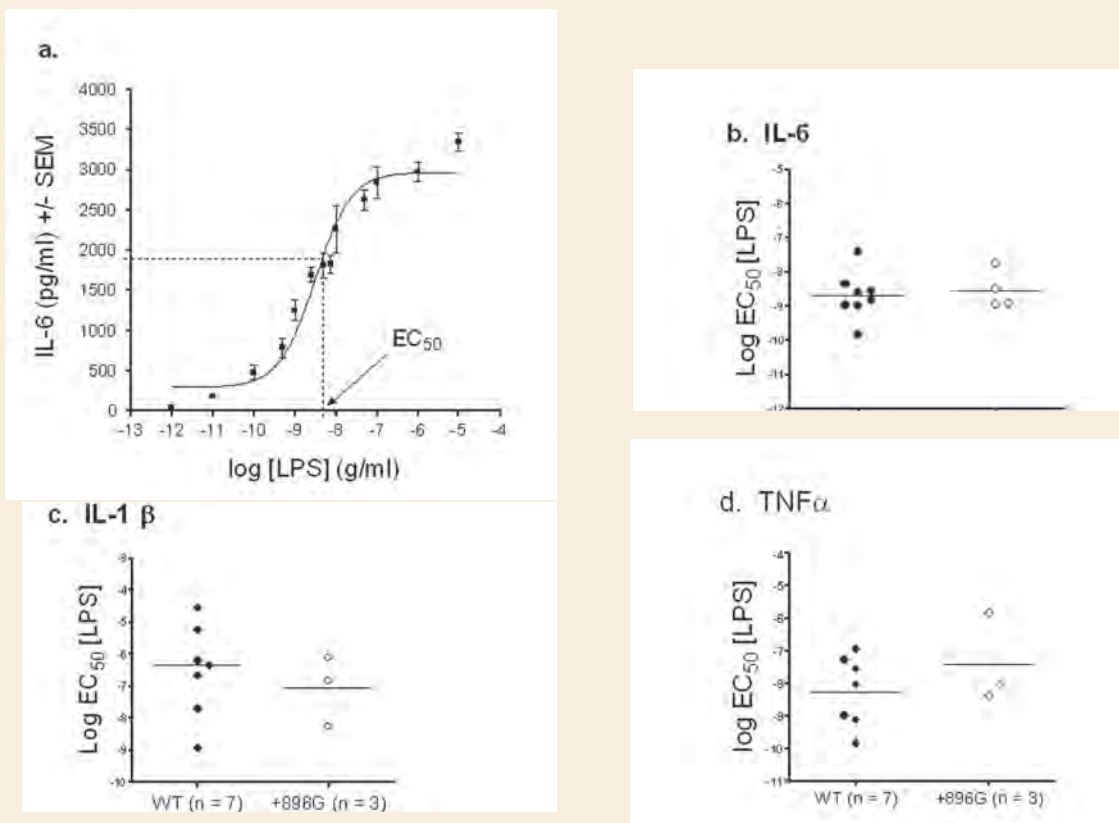


FIGURE 1

While the effects on inflammatory cytokines are well-documented, the intracellular mechanisms are not clear. As a model for epinephrine's effect on inflammatory signaling, we focused on the mechanisms behind TNF α signaling.

Pathway analysis identifies MAPK phosphatase (MKP-1/DUSP1) as possible mediator of epinephrine induced immune suppression

Cyclic AMP (cAMP) is a prototypic intracellular second messenger with a range of effects. It is a common final pathway for a number of extracellular signaling molecules that transmit their signal through the activation of G-protein coupled receptors. Epinephrine is one such signaling molecule that increases levels of cAMP via the G-protein-coupled β -adrenergic receptor. Sympathetic activation with local and systemic release of adrenergic mediators such as epinephrine is an important component of the immediate stress response that leads to increased intracellular cAMP in those cells and tissues expressing the β -adrenergic receptor.

Data indicates that stimulation of β_2 adrenergic receptors (β_2 AR) increases intracellular cAMP and decreases production of proinflammatory cytokines, such as TNF- α , while increasing production of others, such as the anti-inflammatory cytokine IL-10. These changes in the balance of inflammatory responses may have important implications for an individual's ability to respond to infection during times of stress, such as acute traumatic injury.

While the effects on inflammatory cytokines are well-documented, the intracellular mechanisms are not clear. As a model for epinephrine's effect on inflammatory signaling, we focused on the mechanisms behind TNF α signaling. TNF α is an important pro-inflammatory cytokine known to play a role in the local and systemic responses to injury and infection. Various stimuli, including endotoxin and other cytokines, induce TNF α production which is mediated, at least in part, by the nuclear factor NF- κ B. There are conflicting data regarding how cAMP influences TNF α production and NF- κ B activation.

Using Affymetrix GeneChips, and applying Ingenuity Pathway Analysis we have identified a potential role for the MAP kinase phosphatase MKP-1 (also known as DUSP1). Shown in figure 2, are the results of the pathway analysis

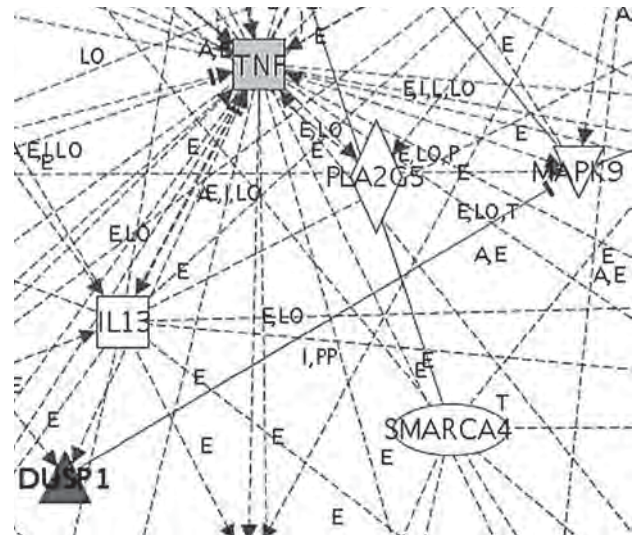


FIGURE 2

as applied to our experiments in which human monocytes were treated with endotoxin and epinephrine. After 15 minutes of exposure mRNA was extracted and analyzed. Note the red triangle indicated by "DUSP1". This analysis indicates an increase in DUSP1 in response to epinephrine and links this response to decreases in TNF gene transcription, possibly through MAPK9.

Given the frequent use of drugs (β -agonists and β -antagonists) which directly influence this pathway in critically ill patients, a better understanding of the intracellular mechanisms may facilitate more knowledgeable use of these drugs in regard to their influence on inflammation. Furthermore, it is conceivable that manipulation of inflammatory signaling by epinephrine (by blocking or enhancing) can be exploited to restore homeostasis in critically ill patients and minimize complications such as septic shock and remote organ failure. It is possible that the phosphatase pathway represents an important regulator of innate immunity that is related to epinephrine and cyclic AMP.

RELATED PUBLICATIONS

1. Rivera-Chavez FA, Munford RS, Wheeler HT, Lindberg G, O'Keefe GE. The Cytokine Response to Acute Inflammation of the Vermiform Appendix. *Annals of Surgery*, 2003; 237 (3): 408 – 416.
 2. Peters DL, Barber RC, Flood E, Garner HR, O'Keefe GE. Genotyping Methods and Bias in Studies of the TNF –308 SNP and Bacterial Sepsis. *Critical Care Medicine*. 2003; 31: 1691 – 1696.
 3. Barber RC, O'Keefe GE. Characterization of a SNP in the Lipopolysaccharide Binding Protein Locus and its Association with Sepsis. *American Journal of Respiratory and Critical Care Medicine*. 2003; 167: 1316-1320.
 4. Rothenbach P, Dahl B, Schwartz J, O'Keefe GE, Yamamoto M, Lee W, Horton J, Yin H, Turnage R. Recombinant Plasma Gelsolin Infusion Attenuates Burn-induced Pulmonary Microvascular Dysfunction. *Journal of Applied Physiology*. Published online May 2, 2003.
 5. Critical illness accelerates lipoprotein-mediated neutralization of bacterial lipopolysaccharide in both hypocholesterolemic and hypertriglyceridemic human serum. Kitchens RL, Thompson P, Munford RS, O'Keefe GE. *Journal of Lipid Research* published electronically ahead of print in August 2003.
 6. Rivera-Chavez FA, Peters-Hybki DL, Barber RC, Wheeler H, O'Keefe GE. Interleukin-6 promoter haplotypes and IL-6 cytokine responses. *Shock* 2003; 20(3): 218 – 223.
 7. Cobb JP, O'Keefe GE. Sepsis, Organ Failure and the Future of Trauma Care. *Lancet*. 2004; 363 (9426): 2076-83.
 8. Imahara SD, Jelacic S, Junker CE, O'Keefe GE. The influence of gender on human innate immunity. *Surgery*. 2005;138(2):275-82.
 9. Imahara SD, Junker CE, Jelacic S, O'Keefe GE. The TLR4 +896 Polymorphism Does Not Influence Endotoxin Responses. *Genes and Immunity*. 2005; 6(1): 37-43.
 10. Jialal I, O'Keefe GE, Devaraj S. Defining the Pro-Inflammatory Phenotype Using High Sensitive C-Reactive Protein Levels as the Biomarker. *The Journal of Clinical Endocrinology & Metabolism*. 2005; 90(8):4549-54.
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PEDIATRIC SURGERY

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• Tissue Engineering and Regenerative Medicine



AWARDS

Charles B. Huggins Research Conference Award
Chicago Surgical Society Research Award
Korean American Medical Association
Research Award

Steering Committee Award, Seattle Children's
Hospital Research Institute

Tissue loss and end-stage organ failure continue to be devastating and costly healthcare problems. This is especially true in the pediatric patient population, where appropriate replacement structures are not readily available. Despite advances in biomaterials and techniques in tissue transplantation, current treatment strategies continue to be plagued by issues such as infection, limited durability, absent growth capacity, and donor organ shortage. To address these limitations, tissue engineering has recently emerged as an interdisciplinary field that works toward the development of biological substitutes that restore, maintain, or improve tissue function.

Tissue-specific cells constitute a critical component of our investigation. One of the major limitations in advancing the understanding of intestinal epithelial differentiation and proliferation has been the difficulty in maintaining primary cultures of normal gut epithelium. Recent studies have begun to decipher the critical interactions that exist between cells of the epithelium and the underlying mesenchymal tissue. It has been shown that mesenchymal cells play an important role not only in influencing epithelial differentiation, but also in maintaining cellular proliferation. Intestinal crypt cells have been shown to be dependent on mesenchymal interaction for proliferation.

Our laboratory applies the principles of tissue engineering to develop novel therapies for patients with intestinal failure and other tissue deficits in the gastrointestinal tract.

Virtually every tissue type in the human body has been investigated and several tissue engineering products are currently being used in clinical application. Our laboratory applies the principles of tissue engineering to develop novel therapies for patients with intestinal failure and other tissue deficits in the gastrointestinal tract. The approach used in our laboratory involves combining isolated cells with highly porous biodegradable polymer matrices to fabricate new living tissue that can ultimately be implanted. As this is fundamentally a multidisciplinary effort, our laboratory collaborates with scientists in bioengineering and other disciplines to advance our goal.

This has led to the development of methods in isolating intestinal epithelial cells with the underlying mesenchymal components as a unit (organoid units) while retaining their morphological integrity. We have utilized these methods to reliably isolate intestinal epithelial organoid units in a small rodent model. We have shown that these intestinal units can attach to various polymer substrates and survive after implantation. We continue to investigate various ways to optimize cell isolation, characterization, and engraftment. With the recent advancements in stem cell biology, there is tremendous potential for utilizing cells that possess considerable regenerative capacity.

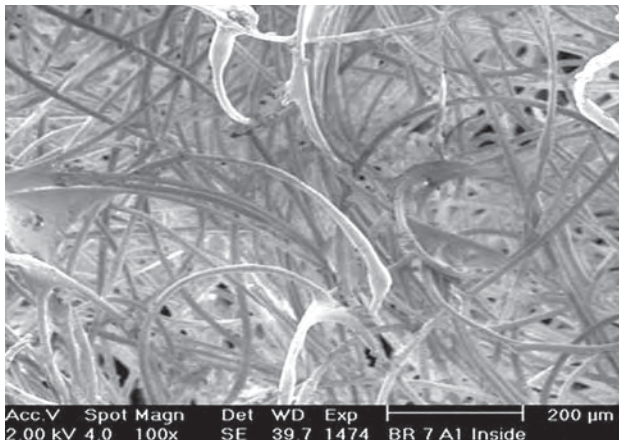


FIGURE 1: Scanning electron micrograph of different polymer matrices. a). Crystalline-based polymer matrix, b). Fiber-based polymer matrix, and c). Tubularized polymer matrix.

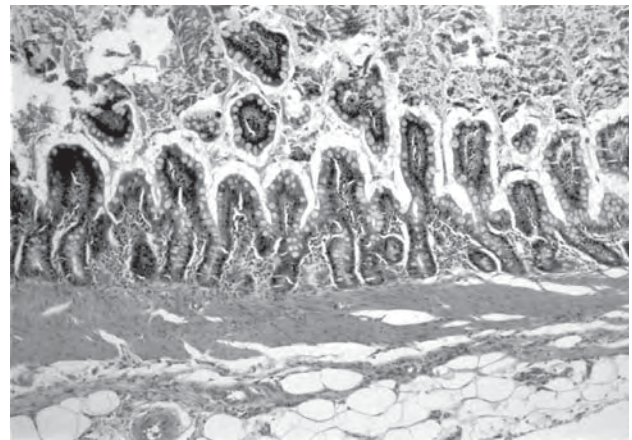


FIGURE 2: Histological section of the regenerated neomucosa.

The polymer matrices used in tissue engineering serve many important functions. They provide a substrate for cell attachment and delivery, and serve as a 3-dimensional template to guide organization. The ideal polymer matrix should be completely biocompatible, biodegradable, easily and reliably manufactured in any desired shape, provide mechanical and structural cues to guide regeneration, and possess surface chemistry that can be modified to regulate cell attachment, morphology, proliferation, and function. Many natural and synthetic matrices are currently under investigation for various tissues. Figure 1 shows representative scanning electron micrographs of different polymer matrices. For studies in intestinal engineering, highly porous tubular biodegradable polymer scaffolds have been successfully fabricated and are currently being optimized in our laboratory. We are looking at ways to modify the polymer surface in different ways to enhance cellular attachment, organization, and long-term function.

Most of the early work in tissue engineering utilized the standard static cell-culture techniques for *in vitro* studies. While this approach has been adequate for some tissues, it has been insufficient for others, especially with tissues that have high metabolic requirements. The use of perfusion culture systems (bioreactors) has been shown to improve cell survival and function *in vitro*. Bioreactors provide flow and mixing of culture media to enhance transfer of gases and nutrients to the cells and removal of waste products from the cells. Bioreactors can also be used to create an *in vitro* environment that more closely mimics the natural

milieu within the body. This provides an opportunity for developing tissues to undergo a conditioning period prior to implantation. We are currently developing a perfusion culture system for our intestinal cell-polymer constructs for long-term *in vitro* analysis and conditioning. The ability to maintain cells *in vitro* for extended periods of time will enable more accurate investigation into the mechanisms involved in intestinal epithelial development, proliferation, and organization.

The goal of our laboratory is to fabricate new living intestinal tissue that can be implanted to replace or enhance function. To this end, we have developed a reliable small rodent model for *in vivo* investigation. We have demonstrated that isolated intestinal epithelial organoid units attach and survive on our tubular biodegradable polymer matrices. After implantation, these constructs regenerate into tissues with a neomucosa that morphologically recapitulates the normal gut epithelium. Figure 2 shows a representative histological section of the neomucosa demonstrating a columnar epithelium containing goblet and paneth cells, and invaginations resembling normal crypt-villus structures. Our current investigations are focused on elucidating the mechanisms involved in tissue regeneration and exploring different strategies to augment this phenomenon. Future studies will be directed toward investigating the relevant function in the tissue engineered neointestine.

RELATED PUBLICATIONS

1. Kim SS, Utsunomiya H, Koski JA, Wu BM, Cima MJ, Sohn J, Mukai K, Griffith LG, Vacanti JP. Survival and Function of Hepatocytes on a Novel Three Dimensional Synthetic Biodegradable Polymer Scaffold with an Intrinsic Network of Channels. *Ann Surg*, 1998; 228:8-13.
 2. Kaihara S, Kim SS, Benvenuto MS, Choi R, Kim BS, Mooney DJ, Vacanti JP. Successful Anastomosis Between Tissue-Engineered Intestine and Native Small Bowel. *Transplantation*, 1999; 67:241-245.
 3. Kaihara S, Kim SS, Benvenuto M, Kim BS, Mooney D, Vacanti JP. End-to-end Anastomosis Between Tissue-Engineered Intestine and Native Small Bowel. *Tissue Eng*, 1999; 5:339-346.
 4. Kim SS, Kaihara S, Benvenuto M, Choi R, Kim BS, Mooney DJ, Taylor G, Vacanti JP. Regenerative Signals for Intestinal Epithelial Organoid Units Transplanted on Synthetic Biodegradable Polymer Scaffolds for the Tissue Engineering of Small Intestine. *Transplantation*, 1999; 67:227-233.
 5. Kim SS, Kaihara S, Benvenuto MS, Choi R, Kim BS, Mooney DJ, Vacanti JP. Effects of Anastomosis of Tissue-Engineered Neointestine to Native Small Bowel. *J Surg Res*, 1999; 87:6-13.
 6. Kaihara S, Kim SS, Kim BS, Mooney D, Vacanti JP. Long-term Follow-up of Tissue Engineered Intestine after Anastomosis to Native Small Bowel. *Transplantation*, 2000; 69:1927-1932.
 7. Kim SS, Sundback CA, Kaihara S, Benvenuto MS, Kim BS, Mooney DJ, Vacanti JP. Dynamic-Seeding and In-Vitro Culture of Hepatocytes in a Flow Perfusion System. *Tissue Eng*, 2000; 6:39-44.
 8. Penkala RA, Kim SS. Gastrointestinal Tissue Engineering. *Expert Rev Med Devices*, 2007; 4(1):65-72.
-

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Robert S. Sawin, M.D.

• Neuroblastoma in the pediatric patient



AWARDS

Robert E. Condon Surgical Resident Competition
Wisconsin Surgical Society

FUNDING

U.S. Army, Madigan Army Medical Center
• Dept. of Clinical Investigation

Neuroblastoma is the most common solid malignancy affecting children. Despite treatments involving aggressive regimens of chemotherapy, and even bone marrow transplantation, the mortality for neuroblastoma remains 40 to 50%. The biology of an individual neuroblastoma tumor varies, with advanced stage tumors manifesting very different molecular and genetic features than those with early stage disease.

Perhaps the most intriguing feature of neuroblastoma is the well-documented spontaneous maturation of highly malignant tumors to a more differentiated benign variant, called ganglioneuroma. An understanding of this maturation process, including the molecular signals that trigger that change, might engender therapeutic methods that harness that maturation process.

Our laboratory effort has focused on a particular peptide growth factor, gastrin releasing peptide (GRP), that is expressed in both adult and pediatric tumors that are derived from neural crest cells. Our work has shown that GRP and its receptor, GRP-R, are both expressed in abundance by neuroblastoma cells in culture and by tumor cells removed from children. Our cell culture studies have also shown that inhibitors of GRP retard neuroblastoma growth.

We are presently working collaboratively with the Clinical Research Institute at Madigan Army Medical Center to define the quantitative differences of GRP and GRP-R expression in neuroblastoma as compared to ganglioneuroma. Our hypothesis is that these differences account for the virulence of the behavior of a given tumor. If verified, this observation would suggest that GRP antagonists might be useful clinically to stimulate maturation of neuroblastoma cells.

Our laboratory effort has focused on a particular peptide growth factor, gastrin releasing peptide (GRP), that is expressed in both adult and pediatric tumors that are derived from neural crest cells.

RELATED PUBLICATIONS

1. Sawin, R.S., Brockenbrough, J. and Ness, J.C. Gastrin releasing peptide is an autocrine growth factor for human neuroblastoma. *Surgical Forum*, XLIII: 606 - 608, 1992.
2. Brockenbrough, J., Ness, J.C. and Sawin, R.S. Scintillation proximity assay accurately quantifies human gastrin releasing peptide. *Life Science News*, 12:14, 1993.
3. Sawin, R.S., Brockenbrough, J. Secretion of Gastrin Releasing Peptide by Retroperitoneal Tumors. *Am. J. Surgery*, 169: 550-552, 1995.
4. Trombetta I, Azarow K, Brown T, Moore K, Sawin R: Telomerase enzyme activity predicts severity of disease in neuroblastoma. *Current Surgery* 58: 413-416, 1999.
5. Sanborn C., O'Connor A., Sawin R.S., Moore K., Dehart M.J., Azarow K.S.: Comparison of telomerase levels before and after differentiation of two cell lines of human neuroblastoma cells. *J Surgical Res* 93:206-210, 2000.
6. Sebesta JA, Young A, Bullock J, Moore KH, Azarow K, Sawin RS : Gastrin-releasing peptide: a potential growth factor expressed in human neuroblastoma tumors. *Curr Surg*. 58(1):86-89, 2001.

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• Surgical Treatment Review Improves Children's Healing Process



Pediatric surgery is in general a very clinically oriented field, though there is an increased emphasis on research in our division. At CHRMC most of our research activity has been oriented toward what we do in the operating room and on the hospital ward, however new faculty have background in and are focusing on tissue engineering and outcomes research. It is important to examine the way we practice surgery and by either randomized prospective trial or by retrospective review determine how we can make changes that will benefit our patients. These studies may involve a wide spectrum of both congenital defects and problems encountered in the older child.

The treatment of Hirschsprung's disease, for example, as well as that of other congenital anomalies, has experienced a trend towards one stage surgical repair in the neonate rather than traditional delayed or multiple stage repairs. One of our recent submissions for publication detailed the technique and reported the results of our use of the transanal Swenson performed in the first several days of life. This technique, in which the Swenson is performed through the anus, thus avoiding a large abdominal dissection, had not previously been described. There are several advantages of the one stage repair. Colostomy is avoided, along with its potential complications, which in the infant may approach a rate of 20%. The length of hospital stay is decreased and hospitalization for colostomy closure is avoided entirely. In theory, long term function may be improved by earlier development of neural connections controlling anal sphincter function.

Minimally invasive surgery (MIS) is becoming an increasingly important technique in the treatment of pediatric surgical disease. MIS has often been advocated in both adult and pediatric patients based on its appeal to the

patient or consumer rather than by any rigorous trial. In one attempt to correct this problem, several years ago an attempt was made at a national level with NIH funding to examine the efficacy of MIS in the pediatric oncology patient. The questions asked dealt with safety and accuracy in obtaining tissue for histologic diagnosis. Though this study never came to fruition at a national collaborative level, we examined our own results at CHRMC to determine whether both laparoscopy and thoracoscopy were useful, accurate ways to obtain tissue. We examined patient outcome and treatment of disease based on decisions made from tissues obtained by MIS techniques. MIS was found to be an excellent, accurate method with no adverse or inappropriate clinical decisions made based on the tissues obtained.

Many MIS procedures take special skills and advanced training in order to become proficient. Often these techniques are espoused to the surgical community with little regard as to what experience is needed to be able to reasonably perform the operation. Few MIS procedures in children are encountered as often as some of those in adults, so that the ability for any one pediatric surgeon to become very experienced may be limited. Some of our studies helped to establish a learning curve with laparoscopic splenectomy and pyloromyotomy so that other surgeons learning how to do the operation might know what to expect in the early stages of learning the procedure. We have also recently examined outcomes and results of both open and laparoscopic pyloromyotomy in order to determine the efficacy of the laparoscopic approach. Future projects will involve outcomes research in pediatric gastroesophageal reflux and Nissen fundoplication.

A joint effort with orthopedics and pulmonary medicine has allowed us to be part of a national collaborative study on the use of the expandable titanium rib, used to treat children suffering from thoracic insufficiency syndrome.

Other studies have answered simple questions about every-day clinical situations such as whether a period of water seal is needed to safely remove chest tubes in children. We have evaluated our use of ERCP in children when symptoms or studies suggested common duct gallstones and tried to discern useful protocols or pathways to help determine when ERCP should be performed preoperatively rather than after cholecystectomy and intraoperative cholangiogram. Our goal was to avoid unnecessary ERCP and the general anesthetic needed to perform it in children.

Ongoing collaborative efforts with colleagues in other divisions such as orthopedics have enabled us to expand the use of minimally invasive surgery for conditions such as pediatric scoliosis by doing thoracoscopic exposures as well as thoracoscopic anterior fusion and instrumentation. A joint effort with orthopedics and pulmonary medicine has allowed us to be part of a national collaborative study on the use of the expandable titanium rib, used to treat

children suffering from thoracic insufficiency syndrome. Prior to the development of this device no good method existed for the treatment of this condition. It is hoped that the use of the expandable rib will allow us over time to expand the thorax of children with Jeune's syndrome or thoracic insufficiency from other congenital problems such as scoliosis, fused ribs or congenital diaphragmatic hernia. Children's was an FDA study center for the evaluation of this device and we are taking lead roles in determining the efficacy of this treatment.

Each of us in pediatric surgery does a high volume of clinical work and it is important to step back on occasion to examine how well one is doing and to question whether something could be done better. This has been our primary focus and the underlying intent of these and many other projects conducted in our division.

RELATED PUBLICATIONS

1. Kim SS, Lau S, Lee S, Waldhausen JHT. The learning curve for laparoscopic pyloromyotomy. *J Laparoendosc Adv Surg Tech A* 15:474-7, 2005
 2. Waldhausen JHT. Surgical Management of Gastroschisis. *Neoreviews* 6:e1-e7, 2005
 3. Waldhausen JHT. Branchial Cleft and Arch Anomalies in Children. *Sem Pediatr Surg* 15:64-69, 2006
 4. TD Beyer, SL Lee, SS Kim, JHT Waldhausen, PJ Healey, RS Sawin, DJ Ledbetter. Initial Nonoperative Management and Delayed Closure for Treatment of Giant Omphaloceles. *J Pediatr Surg* 41:1846-1850, 2006
 5. Waldhausen JHT, Redding G, Song K. Vertical Expandable Prosthetic Titanium Rib (VEPTR) for Thoracic Insufficiency Syndrome (TIS). *J Pediatr Surg* 42:76-80, 2007
 6. Acierno SP, Waldhausen JHT. Congenital Cervical Cysts Sinuses and Fistula. *Surg Clin NA*, G Isaacson (ed). *Otolaryngol Clin N Am* 40:161-176, 2007
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PLASTIC SURGERY

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RICHARD A. HOPPER, M.D., M.S.

MATTHEW B. KLEIN, M.D.

Loren H. Engrav, M.D.

- Laboratory Research
- NIDRR Burn Model System Research
- NIDRR Field Initiated Project



FUNDING

International Association of Fire Fighters
National Institutes of Health
National Institute on Disability and
Rehabilitation Research
• Department of Education

Northwest Burn Foundation
Washington State Council of Firefighters

Laboratory Research

Hypertrophic scarring (Fig. 1) is perhaps the most significant negative outcome of a burn injury. Scarring affects one's quality of life through disfigurement, which in turn, can lead to lowered self-esteem, social isolation, prejudicial societal reactions and job discrimination. Scarring also has profound rehabilitation consequences including loss of function, impairment, disability, and difficulties pursuing recreational and vocational pursuits. Children, young adults and people with pigmented skin are particularly vulnerable to scarring. There is essentially no known early treatment, leaving the only option to be reconstructive plastic surgery. It is clear that new, prospective approaches to this devastating problem, which allow us to intervene before permanent scarring occurs, are necessary. In fact, the impact of scarring is so profound that until steps are taken to greatly reduce or eliminate scarring all together, efforts to enhance rehabilitation of burn survivors will remain palliative at best.

Hypertrophic scars are hard, raised, red, itchy, tender, and contracted. They are ugly and uncomfortable and may regress, but never totally go away. Histologically, increased fibroblasts, collagen and other extracellular proteins characterize hypertrophic scars.

We have clarified the histological anatomy of the cones of skin (Fig.2) in normal uninjured skin, burn-injured skin, mature and hypertrophic scars, fetal skin, rat, rabbit, and pig skin and hope to use these structures as a window to further our understanding of hypertrophic scarring. We have also validated the female, red Duroc pig as an animal model of fibroproliferative scarring (Fig. 3) and the Yorkshire pig will serve as the control. With funding from

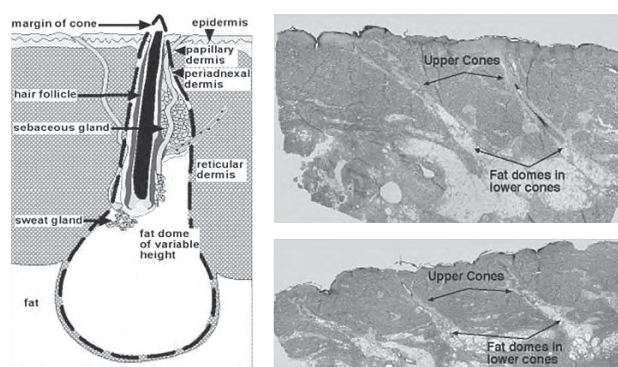


FIGURE 1: The Cones of Skin, Schematic and in vivo

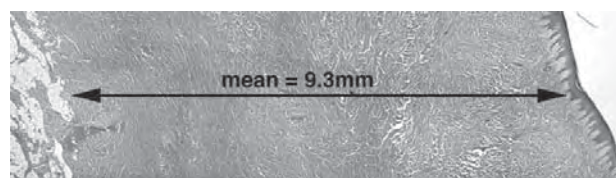


FIGURE 2: Thick Scar in Female, Red Duroc Pig

the National Institutes of Health, the National Institute on Disability and Rehabilitation Research, Department of Education (NIDRR), the Washington State Council of Fire Fighters Burn Foundation (WSCFFBF) and the Northwest Burn Foundation, we are now attempting to characterize the gene expression profile of fibroproliferative scarring. Drs. Zhu and Gibran are significantly involved in these activities. The Visiting Scientists listed played major roles in these projects. The Specific Aim page of the recent NIH application is below.

SPECIFIC AIM 1

The broad, long-term objective is to develop methods of prevention and treatment of hypertrophic scarring.

Hypertrophic (fibroproliferative) scars “represent an exaggerated proliferative response to wound healing that stays within the boundaries of the original wound.”²¹ Hypertrophic scarring regularly occurs after deep, partial-thickness wounds including burns, abrasions and skin graft donor sites and does not occur after shallow wounds that heal rapidly within two weeks. It may also occur after surgical wounds and lacerations. Such scarring is just as common, emotionally devastating and uncontrolled today as it was in the 1940s. The process occurs in up to 75% of burn survivors.²⁻⁵ This sequela can demolish self-esteem and result in social isolation and job discrimination.⁶⁻¹³ We have little understanding of the pathophysiology of hypertrophic scarring, and clinical prevention and treatment are marginal at best. There are several reasons:

1. In the past there has been no accepted and validated animal model of hypertrophic scarring.
2. It is extremely difficult to obtain human tissue early and serially after the injury in the same patient. As a result, studies of scar formation have usually analyzed human tissues obtained months or years after the injury, long after the initial causes of the process were apparent.
3. These same studies have utilized homogenized samples of scar tissue without regard for the histologic anatomy of skin and scar and this has probably obfuscated the causal mechanisms.
4. Hypertrophic scarring is probably multifactorial and therefore single gene analysis, as has been done for decades, is not only slow but is likely incomplete.
5. Given the extreme difficulty in obtaining serial, early human burn tissue, the laboratory findings that do exist have not been translated back to the human situation.
6. Prior studies of hypertrophic scarring have been carried out more in the domain of wound healing than the domain of skin disease, which includes epidermolysis bullosa and scleroderma. Since the skin has a limited number of ways of responding, it is possible that this assignment to the domain of wound healing has deprived researchers of insights necessary to understand the events. Hypertrophic scarring is thought to follow prolonged inflammation as is cutaneous fibrosis in scleroderma, suggesting a common origin. Hypertrophic scarring includes blistering and fibrosis, suggesting events in common with epidermolysis bullosa. Of the many variants of epidermolysis bullosa, some of the junctional variants and the recessive dystrophic type most resemble the deep, partial thickness wounds we are focusing on. As suggested in 2000 by Uitto¹⁴, the bodies of knowledge should be combined, the domains merged.



FIGURE 3: Hypertrophic Scar

We now have available and expertise with:

1. the Duroc(fibroproliferative)/Yorkshire(nonfibroproliferative) model of scarring, which is validated and permits us to study the process early and serially with an appropriate control¹⁵⁻²⁴;
2. laser capture microdissection, which permits us to study anatomic components of the skin and scar; and
3. the Affymetrix Porcine GeneChip containing 23,937 probe sets, which interrogate 20,201 genes.

We have used this system to obtain differential gene expression data from 3 Durocs and 3 Yorkshire pigs in shallow and deep wounds at 1, 2, and 3 weeks and 3 and 5 months post-injury.

We hypothesize that:

1. individual anatomic components of skin are involved in the scarring process; and
2. comparison of the porcine expression data; human, burn wound expression data; gene expression in scleroderma; and the molecular genetics of epidermolysis bullosa will identify a global gene expression profile of hypertrophic scar and cutaneous fibrosis.

SPECIFIC AIM 2

To develop an overarching gene expression profile of cutaneous fibrosis, we will determine gene expression in laser capture microdissected serial biopsies of early and late human burn wounds and compare and contrast the following:

1. gene expression in the Duroc/Yorkshire porcine scar model, the serial biopsies of early and late, human, burn wounds, and scleroderma; and
2. the molecular genetics of epidermolysis bullosa (particularly junctional and recessive dystrophic)

We will test the hypothesis that virtual reality will allow patients to tolerate greater stretching during physical therapy compared to no distraction, and that in spite of achieving greater range-of-motion, patients will still experience lower pain levels while in virtual reality.

NIDRR Burn Model System Research

UW BURN INJURY REHABILITATION MODEL SYSTEM

There are very few data available on the long-term outcome of burn injury. In 1993, 1997 and 2002, the National Institute on Disability and Rehabilitation Research (NIDRR) of the Department of Education funded burn model systems in order to obtain related outcome data. The UW Burn Center was awarded funding at all three time points and now we have a fourteen-year history of burn model system research matched only by the Burn Center at UT Southwestern. Current funding is \$300,000 per year for five years. A large portion of this money funds UW personnel that gather and process clinical research data. The model system research conducted at the UW Burn Center at Harborview covers burn care from injury to discharge from outpatient care with particular attention to rehabilitation and outcomes.

Our Model System grant includes five projects managed by Drs. Engrav, Patterson, Esselman and Wiechman. The Research Nurse Supervisor is Gretchen Carrougner, RN, MN. Drs. Kowalske, Fauerbach, Herndon and Lezotte are the other NIDRR Burn Rehabilitation Model System PIs. All of the projects are now essentially complete and being prepared for publication.

PROJECT 1 is titled “A New Approach to the Etiology of Hypertrophic Scarring.” The general aim for this project is to develop an increased understanding of hypertrophic scarring. To accomplish this objective, this project will focus on confirming that scarring in the red Duroc pig is similar to human hypertrophic scar and that the hypertrophic scarring process involves the cones of the skin. The results were presented above.

PROJECT 2 is titled the “Effect of Virtual Reality on Active Range-of-Motion During Physical Therapy.” At this institution our team of investigators has originated the use of distraction via immersive virtual reality as an adjunctive non-pharmacologic analgesic. Within this study, we will test the hypothesis that virtual reality will allow patients to tolerate greater stretching during physical therapy compared to no distraction, and that in spite of achieving greater range-of-motion, patients will still experience lower pain levels while in virtual reality.

INTRODUCTION: Preliminary evidence suggests that cognitive distraction with interactive, immersive virtual reality (VR) is an effective adjunctive non-pharmacologic analgesic for post-burn physical therapy. The effect on range of motion when distracted is unknown. Therefore, we performed a prospective, randomized controlled study of adjunctive VR to standard therapy in adults receiving passive range of motion (ROM) physical therapy, by assessing pain scores and maximal joint ROM immediately before and after therapy.

METHODS: Thirty-two inpatients age 21-53 years (mean 34 years) with a mean TBSA burn of 17% (range 5-50%) were studied using a within-subjects design. Each patient received his or her regular pre-therapy analgesic regimen. During physical therapy sessions on 2 consecutive days, each patient provided 0-100 Graphic Rating Scale (GRS) assessments of pain following each 10-minute treatment condition (order randomized) - “No VR” and “VR.” During the VR condition, patients wore a head-position-tracking, environment-excluding VR helmet with stereophonic sound. Patients floated through and interacted with an icy 3-D canyon by shooting snowballs at virtual snowmen, igloos, and penguins. Maximal passive ROM was recorded for all joints exercised before and after each physical therapy session. Using a mixed model analysis, we tested for significant residual effects due to receiving VR on the first or second day and for every measure evaluated, we found no significant differences. Consequently, we analyzed the data as simple paired data using Paired T-tests.

RESULTS: As shown below, VR reduced GRS pain scores, relative to the No VR condition. Average ROM difference was slightly greater with the VR condition, however, this difference failed to reach statistical significance ($p = .106$).

CONCLUSIONS: Adult inpatients reported lower pain scores during passive ROM with VR distraction than without distraction. While virtual reality did not demonstrate a statistically significant increase in range of motion, it did result in a significant reduction in pain.

PROJECT 3 is titled “Determination of Reasons for Distress in Burn-Injured Adults.” This study will identify reasons behind a burn survivor’s distress at various time-points after hospital discharge. Results of the study will allow us to better devise and implement interventions to improve the quality of life for burn survivors.

INTRODUCTION: Past research findings suggest that over half of persons with burn injuries report significant distress in the month following discharge. However, no study has looked at what exactly is causing their distress.

METHODS: This study employed a unique and underutilized qualitative technique known as a Q-Sort task to determine the top reasons for distress one month following discharge. The local burn survivor advisory group was used to develop a list of 50 possible reasons for distress that a burn survivor may be experiencing after discharge. A Q-Sort task was then developed, whereby each symptom of distress was placed on a laminated game card. In compliance with Q methodology, a game board was developed that allowed patients to rank each reason from “not causing distress” to “causing significant distress.” Only patients who scored in the top 33% on the Brief Symptom Inventory or reported their distress levels as a 3 or higher on a 10-point Graphic Rating Scale were selected for this study.

RESULTS: A total of 35 patients consented for the study, and 25 scored high enough on the screening questions to complete the Q-Sort task. The majority of the sample were Caucasian males and the mean age was 44 (s.d. = 14), the mean length of stay was 38 days (s.d. = 27) and the mean TBSA was 20% (s.d. = 14). There were no differences in gender, age or TBSA between those who consented for the study and those that did not. Each symptom was assigned a score based on the total of rankings from all 25 patients. The top 10 symptoms in order of distress include: Pain, decreased range of motion, itching, sleep disturbance, temperature changes, decreased strength, changed appearance, change in skin color, uncomfortable scars, financial concerns.

DISCUSSION: Future research will focus on extending the time points to one year and two years to determine if the nature of distress changes over time. By knowing the most common reasons that patients identify as causing distress, we are able to better prepare them for discharge and intervene more effectively.

PROJECT 4 (collaborative) is titled “Barriers for Return to Work.” This project will identify specific barriers to return to work for burn survivors. Recognition of such barriers is the first step in addressing the educational needs of survivors, medical rehabilitation professionals, employers, governmental agencies, and third-party payers.

OBJECTIVE: Identification of barriers to return to work after burn injury as identified by the patient.

DESIGN: A cohort of 154 patients followed via telephone interview up to one year.

SETTING: Hospital based burn centers at three national sites.

PARTICIPANTS: Hospitalized patients meeting the American Burn Association criteria for major burn injury, employed at least 20 hours per week at the time of injury with access to a telephone after discharge.

INTERVENTIONS: Patients were contacted via telephone every 2 weeks up to 4 months then monthly up to one year after discharge.

MAIN OUTCOME MEASURES: A Return to Work Survey was used to identify barriers that prevented patients from returning to work. A Graphic Rating Scale determined the impact of each barrier.

RESULTS: By one year, 79.7% of the patients returned to work. Physical and wound issues were barriers early after discharge. While physical abilities continued to be a significant barrier up to one year, working conditions and psychosocial factors became important issues in those with long-term disability.

CONCLUSIONS: The majority of patients return to work after a burn injury. While physical and work conditions are important barriers, psychosocial issues need to be evaluated and treated to optimize return to work.

PROJECT 5 is participation in the national burn rehabilitation database. The Burn Center staff listed above play a major role in gathering these data and have now entered 1,698 patients into the national database.

The UW Burn Rehabilitation Model System Web page may be viewed at <http://depts.washington.edu/uwnidrr/index.html>.

NIDRR Field Initiated Project

EFFICACY OF PRESSURE GARMENT THERAPY AFTER BURNS

PURPOSE: To conduct a randomized, controlled trial to determine the efficacy of custom-fit pressure garment therapy in the prevention of hypertrophic scarring in healed burns so that the garments may be prescribed based upon sound data or discontinue their use in burn care.

BACKGROUND: Approximately one million people are burned each year in the United States. The most devastating outcome following burns is the ugly, itchy, hypertrophic scar that interferes with work and all other aspects of life. Pressure garment therapy is routinely used to minimize hypertrophic scarring even though there is no scientifically valid data that this therapy is efficacious. Pressure garments are extremely unattractive, expensive and uncomfortable and their use needs to be based upon valid data.

TARGET POPULATIONS: The target populations include burn survivors, many of whom are of a minority group or poor, and healthcare providers who prescribe pressure garment therapy after burns. Burn survivors have been targeted in order to determine the efficacy of pressure garment therapy. Providers are targeted in order to alter burn care based upon the measured efficacy.

GOALS AND OBJECTIVES: We plan to determine the efficacy of pressure garment therapy in the prevention of hypertrophic scarring in healed burns so that prescription of them may be based upon sound data or discontinued.

Innovative Strategies Utilized:

1. The I-Scan® device was designed to measure pressure at the body/environment interface and allows clinicians to address pressure-related problems for at-risk patients. It has been widely used in rehabilitation medicine but not with burn survivors. We will use this device to measure the pressure at the garment/skin interface.
2. The few studies that have been attempted to determine efficacy have used between-subjects designs. Since burn depth is extremely variable from patient to patient and since hypertrophic scarring is greatly influenced by age and race/origin, the between subjects design requires very large numbers of subjects. We will use a within-subjects design randomized, controlled trial to study forearm burns and apply pressure to half of the wound and no pressure to the other half.

PROJECT OUTCOMES: The short-term outcome will be increased knowledge regarding the efficacy of pressure garment therapy. The intermediate-term outcome will be either prescription of garments based upon valid data or their use discontinued at the University of Washington Burn Center.

DISSEMINATION: The research results will be published and made available to the burn community by presentation at scientific meetings and by publication in peer-reviewed scientific journals. These findings will also be made available to laypersons and burn survivors through the University of Washington Burn Injury Rehabilitation Model System Web site.

The purpose of this study was to identify specific premorbid factors and injury characteristics associated with intentional burn injuries and to compare outcomes for individuals injured by assault and those with unintentional injuries. Participants sustaining major burns from May 1994 to August 2005 and consenting to a multi-site, prospective, longitudinal outcome study were included. Etiology of the injury was classified as intentional (i.e., assault) or unintentional. Subjects <18 years old or with self-inflicted burns were excluded. Statistical analysis was performed with t-tests, [chi]² tests, and analysis of variance. Eighty patients sustained intentional burn injuries and 1,982 subjects sustained non-intentional burn injuries. Compared to patients with non-intentional burns, those with burns related to assault were more likely to be female, black, and unemployed and to have higher rates of premorbid substance use.

Between the groups, there were no significant differences in pre-injury living situation, education level, history of psychiatric treatment, or hospital length of stay. The intentional-burn group had larger burns and a greater in-hospital mortality rate, and these patients were less likely to be discharged to home. They also demonstrated significantly greater levels of psychological distress during the acute hospitalization but not at follow-up. Understanding the unique characteristics and needs of patients with intentional burn injuries is important because these individuals are less likely to have a steady income and more likely to rely on community social services. Affordable and accessible community-based health services are necessary in order to improve their outcomes.

Advances in critical care and surgical management have significantly improved survival after burn injury over the past several decades. However, today, survival alone is an insufficient outcome. In 1994, the National Institute on Disability and Rehabilitation Research (NIDRR) created a burn model system program to evaluate the long-term sequelae of burn injuries. As part of this multicenter program, a comprehensive demographic and outcome database was developed to facilitate the study of a number of functional and psychosocial outcomes after burns. The purpose of this study is to review the database design and structure as well as the data obtained during the last 10 years.

This is a descriptive study of the NIDRR database structure as well as the patient data obtained from the four participating burn centers from 1994 to 2004. Data obtained during hospitalization and at 6, 12, and 24 months after discharge were reviewed and descriptive statistics were calculated for select database fields. The database is divided into several subsections, including demographics, injury complications, patient disposition, and functional and psychological surveys. A total of 4,600 patients have been entered into the NIDRR database. To date, 3,449 (75%) patients were alive and discharged and consented to follow-up data collection.

The NIDRR database provides an expansive repository of patient, injury, and outcome data that can be used to analyze the impact of burn injury on physical and psychosocial function and for the design of interventions to enhance the quality of life of burn survivors.

REFERENCES CITED

1. Zurada JM, Kriegel D, Davis IC. Topical treatments for hypertrophic scars. *J Am Acad Dermatol* 2006;55:1024-1031.
2. Deitch EA, Wheelahan TM, Rose MP, Clothier J, Cotter J. Hypertrophic burn scars: Analysis of variables. *J Trauma* 1983;23:895-898.
3. McDonald WS, Deitch EA. Hypertrophic skin grafts in burned patients: a prospective analysis of variables. *J Trauma* 1987;27:147-150.
4. Spurr ED, Shakespeare PG. Incidence of hypertrophic scarring in burn-injured children. *Burns* 1990;16:179-181.
5. Bombaro KM, Engrav LH, Carrougier GJ, Wiechman SA, Faucher L, Costa BA, et al. What is the prevalence of hypertrophic scarring following burns? *Burns* 2003;29:299-302.
6. Engrav LH, Heimbach DM, Reus JL, Harnar TJ, Marvin JA. Early excision and grafting vs. nonoperative treatment of burns of indeterminate depth: A randomized prospective study. *J Trauma* 1983;23:1001-1004.
7. Abdullah A, Blakeney P, Hunt R, Broemeling L, Phillips L, Herndon DN, et al. Visible scars and self-esteem in pediatric patients with burns. *J Burn Care Rehabil* 1994;15:164-168.
8. Fauerbach JA, Heinberg LJ, Lawrence JW, Munster AM, Palombo DA, Richter D, et al. Effect of early body image dissatisfaction on subsequent psychological and physical adjustment after disfiguring injury. *Psychosom Med* 2000;62:576-582.
9. Brych SB, Engrav LH, Rivara FP, Ptacek JT, Lezotte DC, Esselman PC, et al. Time off work and return to work rates after burns: systematic review of the literature and a large two-center series. *J Burn Care Rehabil* 2001;22:401-405.
10. Fauerbach JA, Heinberg LJ, Lawrence JW, Bryant AG, Richter L, Spence RJ. Coping with body image changes following a disfiguring burn injury. *Health Psychol* 2002;21:115-121.
11. Van Loey NE, Van Son MJ. Psychopathology and psychological problems in patients with burn scars: epidemiology and management. *Am J Clin Dermatol* 2003;4:245-272.
12. Herndon DN, LeMaster J, Beard S, Bernstein N, Lewis SR, Rutan TC, et al. The quality of life after major thermal injury in children: an analysis of 12 survivors with greater than or equal to 80% total body, 70% third-degree burns. *J Trauma* 1986;26:609-619.
13. Engrav LH, Covey MH, Dutcher KD, Heimbach DM, Walkinshaw MD, Marvin JA. Impairment, time out of school, and time off from work after burns. *Plast Reconstr Surg* 1987;79:927-934.
14. Uitto J, Eady R, Fine JD, Feder M, Dart J. The DEBRA International Visioning/Consensus Meeting on Epidermolysis Bullosa: summary and recommendations. *J Invest Dermatol* 2000;114:734-737.
15. Zhu KQ, Engrav LH, Gibran NS, Cole JK, Matsumura H, Piepkorn M, et al. The female, red Duroc pig as an animal model of hypertrophic scarring and the potential role of the cones of skin. *Burns* 2003;29:649-664.
16. Zhu KQ, Engrav LH, Tamura RN, Cole JK, Muangman P, Carrougier GJ, et al. Further similarities between cutaneous scarring in the female, red Duroc pig and human hypertrophic scarring. *Burns* 2004;30:518-530.
17. Liang Z, Engrav LH, Muangman P, Muffley LA, Zhu KQ, Carrougier GJ, et al. Nerve quantification in female red Duroc pig (FRDP) scar compared to human hypertrophic scar. *Burns* 2004;30:57-64.
18. Gallant CL, Olson ME, Hart DA. Molecular, histologic, and gross phenotype of skin wound healing in red Duroc pigs reveals an abnormal healing phenotype of hypercontracted, hyperpigmented scarring. *Wound Repair Regen* 2004;12:305-319.
19. Zhu KQ, Engrav LH, Armendariz RT, Muangman P, Klein MB, Carrougier GJ, et al. Changes in VEGF and nitric oxide after deep dermal injury in the female, red Duroc pig-further similarities between female, Duroc scar and human hypertrophic scar. *Burns* 2005;31:5-10.
20. Harunari N, Zhu KQ, Armendariz RT, Deubner H, Muangman P, Carrougier GJ, et al. Histology of the thick scar on the female, red Duroc pig: Final similarities to human hypertrophic scar. *Burns* 2006;32:669-677.
21. Gallant-Behm CL, Hart DA. Genetic analysis of skin wound healing and scarring in a porcine model. *Wound Repair Regen* 2006;14:46-54.
22. Gallant-Behm CL, Olson ME, Hart DA. Cytokine and Growth Factor mRNA Expression Patterns Associated with the Hypercontracted, Hyperpigmented Healing Phenotype of Red Duroc Pigs: A Model of Abnormal Human Scar Development? *J Cutan Med Surg* 2006;9:165-177.
23. Gallant-Behm CL, Tsao H, Reno C, Olson ME, Hart DA. Skin wound healing in the first generation (F1) offspring of Yorkshire and red Duroc pigs: evidence for genetic inheritance of wound phenotype. *Burns* 2006;32:180-193.
24. Liang Z, Xie CY, Lin HB, Guo ZD, Yang WG. [Pathomorphological observation of the hypertrophic scar induced by injury to conical structure in female red Duroc pig]. *Zhonghua Shao Shang Za Zhi* 2006;22:29-32.

RELATED PUBLICATIONS

1. Klein, M.B., Lezotte, D.L., Fauerbach, J.A., Herndon, D.N., Kowalske, K.J., Carrougner, G.J., deLateur, B.J., Holavanahalli, R., Esselman, P.C., San Agustin, T.B., Engrav, L.H. The NIDRR Burn Model System Database: A Tool for the Multicenter Study of the Outcome of Burn Injury. *Journal of Burn Care and Research* 28(1):84-96, 2007.
 2. Kaufman, M.S., Graham, C.C., Lezotte, D., Fauerbach, J.A., Gabriel, V., Loren H. Engrav, L.H., Esselman, P. Burns as a Result of Assault: Associated Risk Factors, Injury Characteristics and Outcomes. *Journal of Burn Care and Research* 28(1):21-28, 2007.
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• Wound Healing



FUNDING

National Institutes of Health

- National Institute of Arthritis and Musculoskeletal and Skin Diseases
- National Institute of General Medicine

Much of what we see in surgical practice involves and relies on the tissue's response to injury. When the response to injury is normal, wounds heal without complication. However, a multitude of factors such as neoplasms, infection, and radiation injury disrupt normal responses to injury and often necessitate reconstructive surgery to transfer healthy tissue.

Wound healing is a complex process requiring the coordination of inflammation, angiogenesis and epithelialization and tissue remodeling. In our effort to understand the mechanism of wound repair, our laboratory is focused on determining the role of bone marrow derived cells in wound healing and on elucidating the function of the Wnt signaling pathway during normal adult wound healing. Understanding normal wound healing will help us better understand and treat aberrant healing processes.

Origin of Cells in a Healed Wound: Bone Marrow

Normal wound repair has been thought to involve the proliferation and migration of local terminally differentiated cell types into the wound from the adjacent uninjured tissue. However, recent evidence suggests that cutaneous repair also involves recruitment of non-resident, undifferentiated cells from distant sources, such as the bone marrow. Populations of progenitor cells have been identified as valuable sources of uncommitted cells that are capable of reconstituting multiple cell types in various tissues, including skin. This population of cells may play a critical role in the induction of tissue regeneration at sites of injury. The ability to manipulate these cells may provide a previously unrecognized means of therapeutic intervention in patients with non-healing wounds.

The most studied progenitor cell type is the hematopoietic stem cell (HSC) from the bone marrow. By creating chimeric mice that express green fluorescent protein (GFP) only in their bone marrow cells, we have found that HSCs migrate to sites of dermal injury, differentiate into several cell phenotypes, and incorporate into the cutaneous wound for the long term. The majority of these bone marrow derived cells resemble undifferentiated dermal fibroblasts with occasional dendritic type cells and endothelial cells (Figure 1). These findings suggest that bone marrow derived cells in the wound not only participate in the inflammatory response, but are an important source of cells for reconstituting the dermis. We are currently investigating this unique role of bone marrow-derived cells in wound repair and are also interested in identifying the signaling pathway responsible for the differentiation of the progenitor cells in the wound.

Gene Expression Profiling of Normal Human Wound Healing

Response to acute cutaneous injury is dependent on the temporal activation and silencing of thousands of genes. Gene expression profiling using cDNA microarrays allows for simultaneous comparison of thousands of genes. Using cDNA microarrays, we analyzed the gene expression profile of human skin during the first few hours following cutaneous wounding.

We observed significant up-regulation of gene expression at thirty minutes after wounding: expression of 334/4,000 genes was increased >3 fold. Expression of genes involved in the inhibition of cell signaling including SOCS and the suppressor of ras-1 were up-regulated. In addition, expression of genes encoding regulators of the cell cycle (e.g. Rb) and proteases (e.g. uPA) were down-regulated. At

Our data demonstrate the complexity of the gene activation/suppression processes that occur early in the normal human wound healing process.

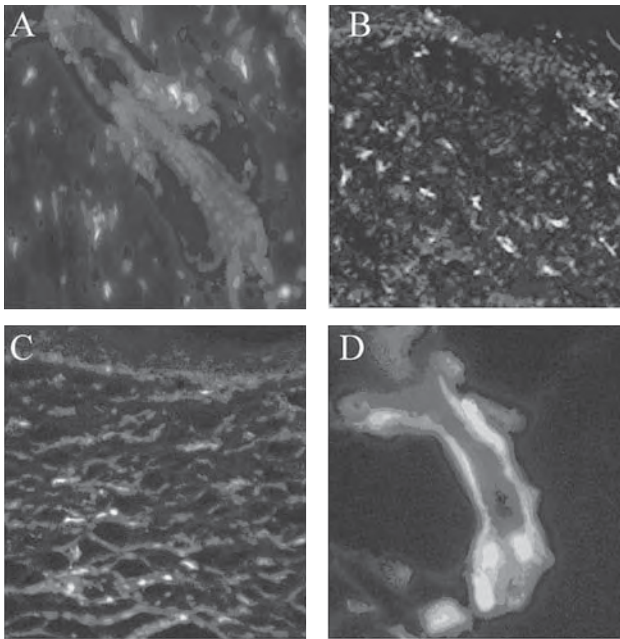


FIGURE 1 A: Normal skin showing numerous green cells of bone marrow origin in normal mouse skin.

FIGURE 1 B: At 30 days after wounding, healing skin shows a large number of dermal green cells, which (C) persist at 90 days after the wound has healed (D). Note the numerous green cells that line a blood vessel in a healed wound, demonstrating the potential for bone marrow cells to form microvessels (brighter signal seen in black & white).

one hour post wounding, 471/4,000 genes were increased > 3 fold. We observed down-regulation of transcriptional and signaling inhibitors, and up-regulation of multiple transcriptional activators. A searchable Web site has been constructed to disseminate these data (<http://faculty.washington.edu/isik/research.html>).

Our data demonstrate the complexity of the gene activation/suppression processes that occur early in the normal human wound healing process. Most of these genes have never been examined in wound healing research. Using this database, new targets have emerged that provide further insight into the study of normal response to injury. Analyzing our microarray database has resulted in a new direction for our laboratory, by identifying a cluster of genes encoding key components of the Wnt signaling pathway that are up-regulated in wound repair.

Developmental Genes Reintroduced in Adult Wound Healing: Wnt Genes

The Wnt signaling pathway plays an important role during embryonic development. Wnt signal transduction is involved in axis specification, mesoderm patterning, nervous system development and organogenesis. Less is known about the function of Wnt signaling in the adult organism. Although it is clear that Wnt signaling in the adult organism regulates cell proliferation in the crypts of the intestine, recent studies have also investigated the role of Wnt signaling on the self-renewal and differentiation of adult stem cells. Blocking Wnt signaling results in inhibition of growth and reduced reconstitution of hematopoietic stem cells in vivo. Wnt signaling also induces differentiation of adult stem cells into myoblasts during muscle regeneration. Uncontrolled Wnt signaling has also been implicated in oncogenesis. Mutations in key components of the pathway have been identified in a number of cancers including colorectal, hepatocellular, ovarian and prostate cancer.

Several mechanisms for transduction of the Wnt signal have been elucidated. The Wnt/ β -catenin pathway is the most well characterized. Activation of the Wnt/ β -catenin signaling pathway promotes the stabilization of β -catenin in the cytoplasm. This pool of β -catenin is now available to translocate into the nucleus where it interacts with the LEF/TCF transcription factors and activates target gene expression. Target genes of Wnt/ β -catenin signaling include cyclinD1 and c-myc. It is apparent that not all Wnts signal through this canonical pathway, for example Wnt5a does not promote stabilization of β -catenin. Instead, Wnt5a signaling stimulates intracellular calcium release. This pathway has been called the Wnt/calcium pathway. Other mediators of non-canonical Wnt signaling include JNK, heterotrimeric G proteins and the small GTPases of the Rho family. Our understanding of the mechanisms of non-canonical signaling is incomplete, as it remains unclear whether there is a single discrete pathway or several different pathways.

The role of Wnt signal transduction during wound healing remains unexplored. However, it is clear that the Wnt signaling pathway can play an important role in the skin. Genes encoding Wnts and other components of the pathway are expressed in skin during embryonic development. Activation of Wnt/ β -catenin signaling is required for hair follicle morphogenesis and recent data also indicate that inhibition of Wnt/ β -catenin signaling may be necessary for basal epidermal cell specification. Our microarray data revealed that components of the Wnt pathway, including TCF-4, β -catenin, TCF-1, Dvl2, Wnt5a and Wnt1, are up-regulated after wounding, but only transiently and early on. The induction of Wnts during wound healing was confirmed by RT-PCR and Western Blot analysis. In order to determine the contribution of Wnt signal transduction to wound repair, we applied Wnt5a retrovirus containing media to wounds of mice.

The healed wound treated with the Wnt5a had a distinct histology compared with controls. At day 40 post injury, sebaceous units along with hair follicles are found in the deep dermis of the healed wound of Wnt5a treated mice, whereas the control mice never develop epidermal regeneration (Figure 2). This histology suggests that Wnt5a has a potential role in promoting epidermal and dermal regeneration. Current work in our laboratory is comparing the histology of healed wounds treated with either Wnt1 (Wnt/ β -catenin pathway) or Wnt5a (Wnt/calcium pathway). We are also investigating how Wnt signaling can induce the regenerative elements such as hair follicles and sebaceous glands missing in wound repair in adult organisms (Figure 3). Finally, we are interested in determining if the Wnt signaling pathway is responsible for the homing and differentiation of the bone marrow derived stem cells in the wound.

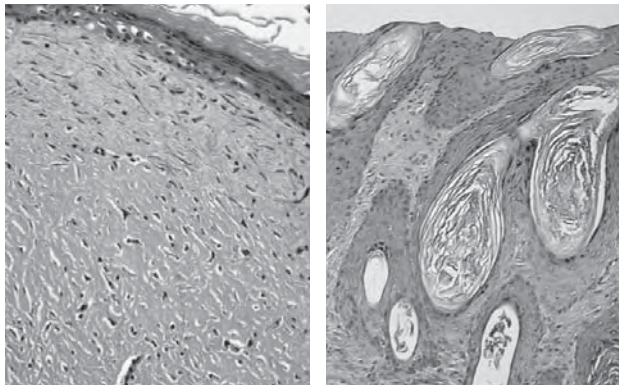


FIGURE 2: Figure on left shows a normal scar with simple stratified squamous epithelium over a collagen-rich dermal matrix. On right, a similar 40 day wound that overexpressed Wnt 5A retrovirus. Note the numerous epidermal cysts invaginating into the dermis, which later have structures resembling sebaceous glands and hair follicles. There is no evidence of tumor formation even at 90 days.

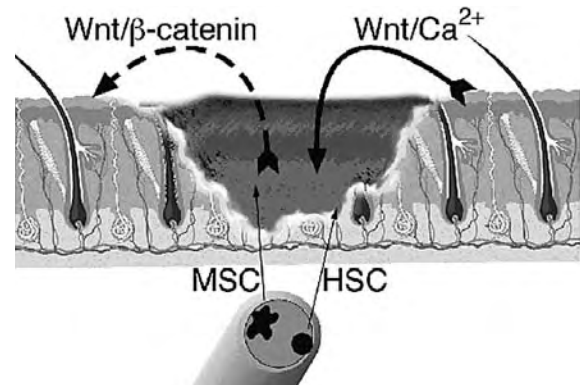


FIGURE 3: Graphic demonstration of our hypotheses. We think stem cells in the epidermis and stem cells from bone marrow can provide the missing cell types following loss due to injury. However, we think that the lack of morphogens in the wound may account for the lack of regeneration seen in wound repair. We propose that the epidermal stem cells regulate the deeper dermal stem cells' fate, and that the deeper dermal cells regulate the epidermal stem cells' fate, based on the two Wnt signaling pathways.

RELATED PUBLICATIONS

1. Hocking, A.M., Shinomura, T. and McQuillan, D.J. 1998 Leucine-rich repeat glycoproteins of the extracellular matrix. *Matrix Biology* 17:1-19.
2. Krishnan, P., Hocking, A.M., Holik, K., Scholtz, J.M., Pace, C.N., and McQuillan, D.J. 1998 Distinct secondary structures of the leucine-rich repeat proteoglycans, decorin and biglycan: glycosylation-dependent conformational stability. *Journal of Biological Chemistry* 274:10945-10950.
3. Miller, J.R., Hocking, A.M., Brown, J.D and Moon, R.T. 1999 Mechanism and function of signal transduction by the Wnt/b-catenin and Wnt/Ca²⁺ pathways. *Oncogene* 18:7860-7872.
4. McQuillan, D.J., Seo, N.S., Hocking, A.M., and McQuillan, C.I. 2001. Recombinant expression of proteoglycans in mammalian cells. Utility and advantages of the vaccinia virus/T7 bacteriophage hybrid expression system. *Methods in Molecular Biology* 171:201-219.
5. Fathke, C., Wilson, L., Hutter, J., Kapoor, V., Smith, A., Hocking, A., and Isik, F. 2004. Contribution of bone marrow-derived cells to skin: Collagen deposition and wound repair. *Stem Cells* 22:812-822.
6. Waxman, J.S., Hocking, A.M., Stoick, C.L. and Moon, R.T. 2004 Zebrafish Dapper1 and Dapper2 play distinct roles in Wnt-mediated developmental processes. *Development* 131:5909-5921.
7. Seo, N.S., Hocking, A.M., Hook, M. and McQuillan, D.J. 2005 Decorin core protein secretion is regulated by N-linked oligosaccharides and glycosaminoglycan addition. *Journal of Biological Chemistry* 280:42774-42784
8. Fathke, C., Wilson, L., Shah, K.V., Kim, B., Hocking, A.M., Moon, R. and Isik, F. 2006 Wnt signaling induces epithelial differentiation during cutaneous wound healing. *BMC Cell Biology* 7:4-13

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- Cleft Lip and Palate
- Syndromic Severe Midface Hypoplasia
- Craniosynostosis



FUNDING

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KLS Martin Ltd.

Craniofacial surgery is a relatively new subspecialty of Plastic Surgery, being officially initiated at the 4th Congress of the International Confederation for Plastic and Reconstructive Surgery in Rome in 1967. Since then it has become an active field of clinical and basic science research with the goal of improving the treatment of a broad spectrum of reconstructive procedures of the cranium and face. Our research is focused on the treatment of three specific birth defects affecting children: cleft lip and palate, syndromic midface hypoplasia, and craniosynostosis.

we have identified a sub-group of infants with cleft lip and palate who do not gain weight and grow appropriately, despite standard of care feeding and nutritional intervention. If these infants can be identified before they demonstrate failure to grow, their diets could be tailored to prepare them for surgery.

We have initiated a study to measure the metabolic rates of infants with cleft lip and/or cleft palate using indirect calorimetry, and to compare these with clinical measurements such as weight gain, growth, and diagnosis. The study

Children born with a cleft lip and/or palate require intensive multi-disciplinary care from the day they are diagnosed to the time they stop growing. The goal of research in this field is to optimize these two operations so that the need for multiple secondary surgeries during early childhood and adolescence is minimized.

Clinical Research

CLEFT LIP AND PALATE

Children born with a cleft lip and/or palate require intensive multi-disciplinary care from the day they are diagnosed to the time they stop growing. In the first year of life the children undergo two fundamental operations; repair of their cleft lip and nose deformity at age three months, and repair of their palate at age one year. The goal of research in this field is to optimize these two operations so that the need for multiple secondary surgeries during early childhood and adolescence is minimized.

One way to optimize surgeries is to ensure that the infants are well nourished in preparation for the stress and post-operative healing of the procedure. From clinical experience

is taking place at the Craniofacial Center at Children's Hospital and Regional Medical Center. It will enroll 30 children a year in the study and follow them during the first year of their life, before and after each of their surgeries. The goal of the study is to create new guidelines for the nutritional care of infants with cleft lip and palate based on their individual needs.

SYNDROMIC SEVERE MIDFACE HYPOPLASIA

Children born with syndromes such as those described by Apert, Crouzon, and Pfeiffer can have such poor growth of their upper facial skeleton, or midface, that it compromises the closure of their eyelids and therefore the protection of their vision, the airway of their nose and therefore their ability to sleep, and the relationship between their upper and

lower jaws and their ability to chew. The recognized surgical treatment of these children is to separate the upper facial skeleton from the rest of the skull, known as a LeFort III osteotomy, then to move the upper face forward and secure it in place with bone graft harvested from the child's ribs.

The limitations of this traditional Lefort III advancement are that some of the child's ribs need to be removed and, because of the tightness of the skin and muscle overlying the upper facial skeleton, the face can usually only be moved forward around one centimeter. Repeat Lefort III operations, or inadequate advancements were therefore not uncommon in children with severe midface hypoplasia, or restricted growth.

Over the past ten years, a technique known as distraction osteogenesis has been used to treat severe midface hypoplasia (Figure 1). This involves performing a Lefort III osteotomy, but instead of advancement and bone grafting, the incisions are closed and a skull based distraction device is attached to the upper facial bones with wires. Over the next two to three weeks, the midface is slowly moved forward at a rate of 1 mm a day. This slow advancement allows the skin and muscle to adjust, such that advancements of up to three centimeters are possible. Once the advancement is complete, the device remains in place for two months while the fibrous tissue that has formed in the bone gap turns into solid bone. Bone grafts are therefore not needed.

Since midface distraction osteogenesis is a relatively new technique in evolution, we are actively researching ways to improve the process at the Craniofacial Center of Children's Hospital. A prospective Institutional Research Board (IRB)

approved study is underway to examine the psychosocial impact of the three month long procedure on the patients and their families, and to suggest interventions to minimize the stress. Pre- and post-operative extensive sleep studies are being performed on all the children undergoing the procedure to examine the effect on quality of sleep. Sequential radiographic imaging is being used to learn how the facial bones adjust, remodel, and grow after they have been advanced such a large distance. Timing of how long it takes the new bone to form behind the advanced facial bones is also being studied to determine the optimum time to remove the distraction device.

Basic Science Research

CRANIOSYNOSTOSIS

Craniosynostosis is early fusion of one or more of the growth sutures of an infant's skull, resulting in a progressive deformity of the child's skull shape. In some cases craniosynostosis can also result in deviation of the position of the eyes and face, or can restrict the expansion of the brain as it grows. The majority of affected infants have isolated craniosynostosis with no family history of the birth defect and no other medical problems. Unfortunately, the current treatment of craniosynostosis is to subject these otherwise healthy infants to a joint neurosurgery and craniofacial plastic surgery operation with the need for blood transfusions and the risks of severe morbidity, or in rare cases, mortality. The ideal treatment of isolated craniosynostosis would be to prevent the suture fusion from occurring by blocking the responsible abnormal molecular pathway.

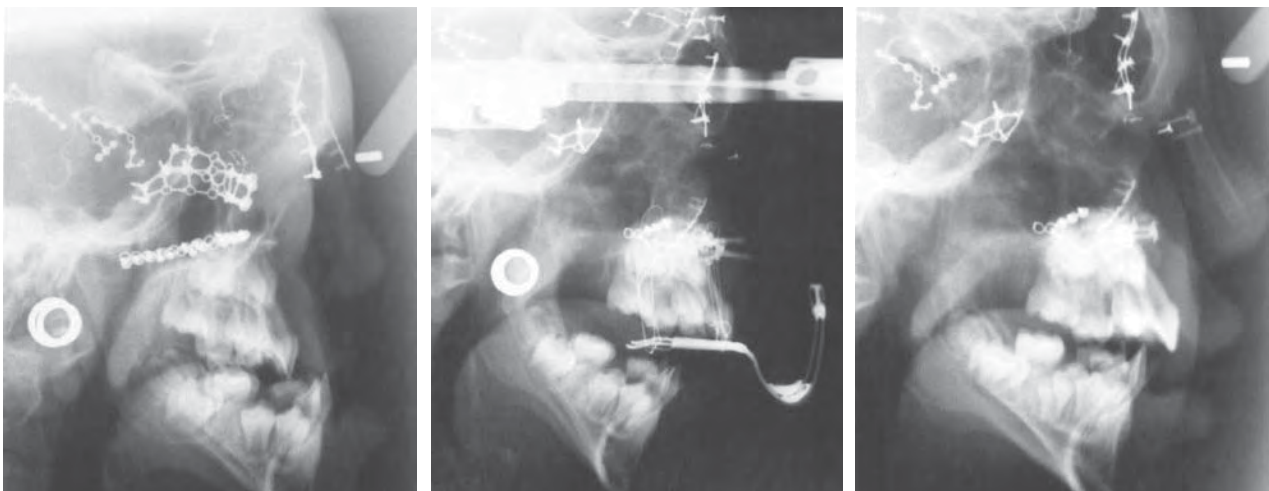


FIGURE 1: Lateral Cephalograms of a child undergoing midface distraction osteogenesis with an external device (Left) Before the operation, the child is having problems sleeping due to constriction of her nasopharynx, problems with dry eyes due to lack of cheek protection, and a problem chewing due to her upper jaw being well behind her lower jaw. (Middle) The facial bones have been separated from the skull and the external distraction device has slowly advanced them over a period of two weeks. This process is not painful, but involves frequent follow-up visits and parent support. (Right) After removal of the device, the advanced bone has healed in a favorable position, with a small over correction to allow for future mandible growth.

There is a reliable sex ratio to the presentation of isolated craniosynostosis that has not been explained. Early closure of the sagittal or metopic sutures, both midline sutures, occurs predominantly in males. In contrast, coronal suture fusion is more common in females. Our theory is that there is a subgroup of individuals with craniosynostosis whose bone cells, or osteoblasts, are more susceptible to the *in utero* effects of sex hormones. Both testosterone and estrogen are present in the uterine environment, and from research on osteoporosis in the elderly, both are known to increase osteoblast differentiation into mineralized bone.

With IRB approval, we have been collecting bone samples from children undergoing craniofacial surgery for craniosynostosis and creating osteoblast cultures from them. Now that we have established primary cell lines representing different types of craniosynostosis and different sexes, we are examining the effect of different concentrations of sex hormones on osteoblast growth, differentiation and selective gene upregulation. Our goal is to identify patients whose osteoblasts have an increased susceptibility to the effects of sex hormones and to determine the molecular reason for this susceptibility.

Osteoblasts cultured from fused sutures grow faster than osteoblasts cultured from open, or patent, sutures. The prevailing theory is that osteoblasts around fused sutures are abnormal, however our alternate theory is that there are cells within normal sutures that serve to inhibit the growth of surrounding osteoblasts to prevent premature suture closure. In craniosynostosis, this normal inhibitory mechanism is lost, and fusion occurs. To test this theory, we have cultured sub-populations of cells grown from fused and open sutures in the same individual. We are examining differences in gene expression among these sub-populations and how one population can affect the growth of the other.

Osteoblasts do not exist in isolation in the skull. Bone healing involves a complex coordination between osteoblasts and adjacent blood vessel, or endothelial, cells. A collaborative project with Dr. Geoff Gurtner at New York University Medical Center examined the interaction of rat cranial osteoblasts with endothelial cells in the presence of pulsed electromagnetic fields (PEMF). We found that when the osteoblasts were stimulated with PEMF, they secreted a protein that increased the growth rate of endothelial cells almost five fold (Figure 2).

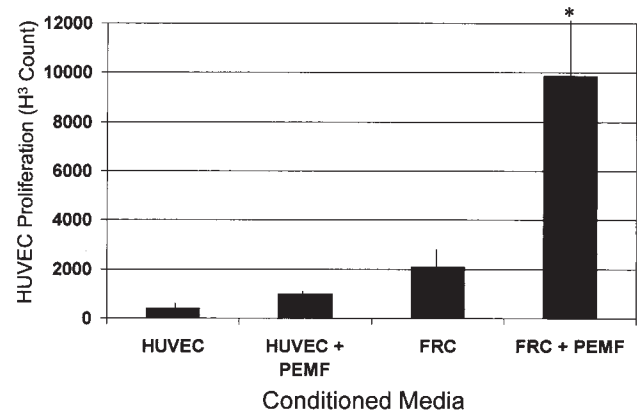


FIGURE 2: The effect of media from osteoblasts and endothelial cells stimulated with a pulsed electromagnetic field (PEMF) on endothelial cell proliferation. When human endothelial cells (HUVEC) were grown in media from fetal rat osteoblasts stimulated with PEMF (FRC+PEMF), their rate of proliferation increased significantly (* $p < 0.05$) compared to media from unstimulated osteoblasts (FRC) and stimulated or unstimulated HUVEC.

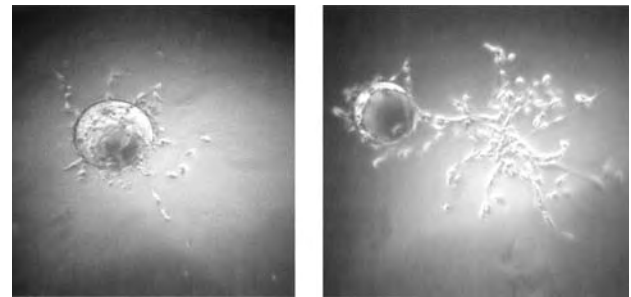


FIGURE 3: The effect of pulsed electromagnetic fields (PEMF) on endothelial cell tubulization. Endothelial cells attached to microspheres formed significantly more tubules when exposed to PEMF (left) than when not exposed the PEMF (right).

This dramatic increase in blood vessel growth does not appear to be due to the well known vascular endothelial growth factor (VEGF), therefore the next phase of the project is to identify the protein responsible. PEMF was also shown to increase directly the formation of early blood vessels, or tubules, by the endothelial cells (Figure 3). These two observations help us to understand better the beneficial effects of PEMF on bone healing, and may eventually lead to ways to create the same effect without the use of cumbersome electromagnetic devices.

As an exciting extension of our continuing work on craniosynostosis osteoblasts, and osteoblast-endothelial cell interactions, we are collaborating with Professor Patrick Stayton of Bioengineering to use the technique of micropatterning to examine and manipulate cell-cell interactions in a controlled fashion.

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Matthew B. Klein, M.D.

- Organization and Delivery of Burn Care
- Psychosocial and Functional Outcomes of Older Adults Following Burn Injury
- The Impact of Resuscitation Fluid Volume Received on Outcome
- Development of Validated Patient Reported Outcome Measurement Tools for Burn Survivors



FUNDING

National Institutes of Health
National Institute on Disability and
Rehabilitation Research

Washington State Council of Firefighters
Burn Foundation

Advances in critical care and surgical management have significantly improved survival following burn injury. In 2007, survival following extensive burn injury has become the rule rather than the exception. Accordingly, the emphasis of burn care and research has shifted towards optimizing the outcome of burn survivors. Within the overall theme of burn injury outcomes, my research has evolved into four domains: organization and delivery of burn care; psychosocial and functional outcomes of older adults following burn injury, the impact of fluid resuscitation volumes on outcome and the development of validated instruments for assessment of burn outcomes.

Organization and Delivery of Burn Care

Burn care is a resource-intensive endeavor requiring specialized equipment and personnel. While the optimal national structure for delivery of burn care has long been debated, the need for organized systems and quality measures of burn care has received increased attention in light of recent concerns for mass casualty disaster planning. Our research in this domain has focused on the concept of regionalization of burn care; that is, a system in which a single center provides care over a defined geographic area as exists currently in the Pacific Northwest. The initial study performed on this topic was “An Outcome Analysis of Patients Transferred to a Regional Burn Center: Does Transfer Status Impact Survival?” published in the international journal *Burns* in December 2006. This was a retrospective cohort study comparing the outcomes of patients transferred to our regional burn center with those of patients admitted to the burn center directly from the field. While there have been numerous previous studies demonstrating worse outcomes for trauma patients transferred to trauma centers from preliminary care facilities,

there were previously no studies examining the outcome of transferred burn patients. In this study, we found that there was no difference in outcome between patients transferred to our burn center from a preliminary care facility and patients admitted directly from the field—a critical requisite for the delivery of burn care over a large geographic area in which patients will often receive initial care at a hospital without a burn center.

The next project in this domain examined the complications that occurred during the long-distance transport of a cohort of patients admitted to the UW Burn Center from 2000-2003, and the manuscript “An Analysis of Long Distance Transport of Burn Patients to a Regional Burn Center” has been recently published in the *Journal of Burn Care and Research*. This study demonstrated that patients can be transported safely and efficiently over long distances to a regional burn center. This finding has important implications for the organization of burn care nationally, given the decreasing number of American burn centers and the decreasing number of burn surgeons. In addition, these findings also have important implications for national disaster planning that must rely on safe and efficient triage and transport of burn injured patients in a mass casualty event.

Ongoing studies in this domain include an analysis of the geographic distribution of burn centers relative to population density and an analysis of population access to burn centers by ground and air transport utilizing two different geography databases. In addition, studies comparing the outcome of patients treated at verified burn centers (verified by the American College of Surgeons/American Burn Association Verification Committee) with those treated at non-verified burn centers utilizing data from the national Healthcare Utilization Program National Inpatient database are underway. Similar studies have been performed for non-burn trauma patients but have not been done for burn patients.

Patients can be transported safely and efficiently over long distances to a regional burn center.

This finding has important implications for the organization of burn care nationally, given the decreasing number of American burn centers and the decreasing number of burn surgeons.

Psychosocial and Functional Outcomes of Older Adults Following Burn Injury

Older adults are at increased risk for burn injury for a number of reasons and are at increased risk for adverse outcomes. The majority of the literature on elderly burn patients has focused merely on factors that influence survival, with less attention on the psychosocial and functional outcomes of those patients that survive their injuries. We have recently completed a study using data from the National Institute on Disability and Rehabilitation Research multicenter database examining the long-term functional outcome, health related quality of life and psychological distress in a cohort of burn patients age 55 and older. A second project examining the impact of extent of burn injury and pre-injury comorbidities on morbidity and outcome utilizing data from the UW Burn Registry is currently underway. The findings from these two studies will be used to design an interventional strategy aimed at improving the outcome of older adults following burn injury. In addition, we are examining national trends in older adult burn injury and outcome utilizing the American Burn Association National Burn Repository.

The Impact of Resuscitation Fluid Volume Received on Outcome

Fluid resuscitation is a critical component of the acute care of a burn patient. There has been a recent trend towards larger volumes of fluid being administered following burn injury that has been purported to increase injury complications. We have recently published two studies examining the trend over the past 30 years towards larger volumes of fluid administered to burn patients: “Is Supra-Baxter Resuscitation in Burn Patients a New Phenomenon?” and “‘Opioid Creep’ Is Real and May Be the Cause of ‘Fluid Creep.’” The first paper reported that the volume of fluids administered to a cohort of patients in 2000

was significantly higher than that administered to an age and injury-matched cohort from 1977. In the subsequent paper we examined the potential role of increased opioid administration over the same time period.

More recently, we reported on the complication of orbital compartment syndrome in patients who received large volumes of fluid resuscitation in the manuscript “Elevated Intraocular Pressure: Another Untoward Effect of Massive Fluid Resuscitation,” which was published in the *Journal of Trauma*. This clinical study demonstrates the association between large fluid volumes received and the possible development of orbital compartment syndrome in a group of severely burned patients. Detection and treatment of orbital compartment syndrome can be critical to the prevention of ocular complications including decreased vision.

To better examine the factors influencing the need for large volumes of fluid resuscitation and to verify the long hypothesized association between increased fluids received and risk for adverse outcome, we analyzed the data collected as part of the multicenter NIH-funded “Inflammation and Host Response to Injury” project. The results of this study are reported in the manuscript “The Association Between Fluid Administration and Outcome Following Major Burn Injury: A Multicenter Study,” which has been accepted for publication in the *Annals of Surgery*. This is the first manuscript in the burn literature utilizing prospectively collected multicenter data to demonstrate an association between large volumes of fluid received and increased risk of adverse outcomes including mortality.

Ongoing studies in this research domain are focused on development of better statistical models that can predict adverse outcome based on fluids received, and plans are underway to try and develop an interventional study that will utilize alternatives to narcotics in the early post-injury period which may reduce fluid volume requirements.

Development of Validated Patient Reported Outcome Measurement Tools for Burn Survivors

Effective assessment of the impact of burn injury on psychosocial and functional outcomes and development of effective intervention and rehabilitation strategies are contingent on the availability of reliable burn-specific outcome measurement tools. Traditionally, burn research studies have relied on functional and psychosocial assessment surveys, which have been developed and validated using non-burn survivor populations. The validity of these tools for burn survivors has not been assessed.

As the first step in this project, we are performing a systematic review of the literature to determine which patient reported outcome (PRO) instruments have been used for burn outcome studies and which of these have been previously validated. We will then develop a concept bank of issues that are critical to burn survivors functional, psychosocial and community integration aptitude. This concept bank will be developed from focus groups with burn survivors themselves as well as from focus groups with burn providers. It will also be used in the content validity assessment of currently available PROs and as the first step in the development of a PRO validated for burn survivors.

RELATED PUBLICATIONS

1. Sullivan SR, Amadi AJ, Singh C, Sires B, Engrav L, Gibran NS, Klein MB. Elevated Intraocular Pressure: Another untoward effect of overresuscitation. *J Trauma* 2006;60:72-6.
2. Klein MB, Silver GM, Gamelli RL, Gibran NS, Herndon DN, Hunt JL, Tompkins RG. Inflammation and the Host Response to Injury: An Overview of the Multicenter Study of the Genomic and Proteomic Response to Burn Injury. *J Burn Care Res* 2006;27:448-51.
3. Muangman P, Engrav LH, Heimbach DM, Harunari N, Honari S, Gibran NS, Klein MB. Complex Wound Management Utilizing an Artificial Dermal Matrix. *Ann Plast Surg* 2006;57:199-202.
4. Klein MB, Nathens AB, Heimbach DM, Gibran NS. An Outcome Analysis of Patients Transferred to a Regional Burn Center: Does Transfer Status Impact Survival? *Burns* 2006;32:940-5.
5. Pham TN, Neff MJ, Simmons JM, Gibran NS, Heimbach DM, Klein MB. The Clinical Pulmonary Infection Score Poorly Predicts Pneumonia in Burn Patients. *J Burn Care Res* 2007;28:76-9.
6. Klein MB, Lezotte DL, Fauerbach JA, Herndon DN, Kowalske KJ, Carrougier GJ, deLateur BJ, Holavanahalli R, Esselman PC, San Agustin TB, Engrav LH. The NIDRR Burn Model System Database: A Tool for the Multi-center Study of the Outcome of Burn Injury. *J Burn Care Res* 2007;28:84-96.
7. Klein MB, Nathens AB, Emerson D, Heimbach DM, Gibran NS. An Analysis of Long Distance Transport of Burn Patients to a Regional Burn Center. *J Burn Care Res* 2007;28:49-55.
8. Scott JR, Klein MB, Gernsheimer T, Honari S, Gibbons J, Gibran NS. Arterial and Venous Complications of Heparin-induced Thrombocytopenia in Burn Patients. *J Burn Res* 2007;28:71-5.
9. Klein MB, Ahmadi AJ, Sires B, Engrav LH. Reversible Marginal Tarsorrhaphy: An Adjunctive Tool in the Management of Severe Periocular Burns. *Plast Reconstr Surg* 2006 (In Press).
10. Klein MB, Hayden D, Elson C, Nathens AB, Gamelli RL, Gibran NS, Herndon DN, Hunt J, Silver G, Schoenfeld D, Tompkins RG. The Association Between Fluid Administration and Outcome Following Major Burn Injury: A Multicenter Study. *Ann Surg* (In Press).

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TRANSPLANT SURGERY

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JAMES D. PERKINS, M.D.

Wei Li, M.D., Ph.D.

- Mechanism of organ transplant tolerance and rejection:
The role of regulatory T cells (Treg), dendritic cells (DC),
and costimulatory molecules on tolerance induction



New immunosuppressive drugs improve the short-term survival of organ transplant recipients. However, long-term survival remains comparatively poor. This is likely due to the fact that immunosuppressive strategies are not tolerogenic. Transplant tolerance is likely to arise not from improved immunosuppressive regimens, but from improved understanding of the normal mechanisms that generate and maintain self-tolerance, and the ability to manipulate these mechanisms for the prevention and treatment of transplant rejection.

reagents to define and characterize the dominant factors involved in organ transplant tolerance induction. These factors include T cell subsets (including T regulatory cells [Treg]), the signals or pathways between antigen presenting cells (APC) (such as dendritic cells [DC]) and alloreactive T cells, both locally (in grafts) and systemically (in the spleen and lymph nodes), and the cytokines which modulate T cell activations and differentiations.

Apoptosis alone cannot explain liver-induced tolerance to subsequent other organ grafts from the same donor strain. The liver tolerance seems to be an active process and one which is mediated by regulatory T cells.

The overall goal of my research is to define mechanisms of peripheral tolerance induction in order to develop new strategies to guide clinical therapy in transplant recipients. I am currently focusing on studying the cellular and molecular basis of immune mechanisms of organ transplant tolerance and rejection using our unique mouse orthotopic liver transplant (OLTx), heterotopic heart transplant (HTx), skin transplant (STx), or islet transplant (ITx) models. Our research uses the characteristics of TCR transgenic or gene knockout mice and costimulatory molecule blocking

The goals of our research are:

- to further ascertain the mechanisms of organ transplant tolerance;
- to examine the ability of tolerogenic dendritic cells to induce Treg, *in vivo* and *in vitro*, and to study the cytokines or costimulatory molecules that modulate this activity;
- to assess and maximize the therapeutic potential of DC and Treg in promoting tolerance induction in organ transplantation.

Mechanisms of murine spontaneous liver transplant tolerance and the role of regulatory T cells

It has been previously demonstrated that murine liver grafts are accepted spontaneously across all MHC barriers and induce donor-specific tolerance without immunosuppressive therapy (hepatic tolerance). The tolerance induced by a liver allograft can further induce the tolerance of subsequent organs such as a heart or kidney from the same donor origin. The tolerance is transferable to the naïve syngeneic mice by spleen or liver graft infiltrating cells obtained from long-term liver allograft recipients. Despite *in vivo* hyporesponsiveness to the liver allografts and to subsequent grafts from the same donor, *in vitro* mixed lymphocyte response (MLR) and cytotoxic lympholysis (CTL) assays showed unimpaired antidonor reactivity (split tolerance).

By contrast, livers from donors treated with Flt3 ligand (FL), which dramatically increases hepatic functional mature DC, are rejected acutely. This switch from tolerance to rejection is associated with marked reduction in apoptotic activity of graft infiltrating T cells, enhancement in costimulation between donor APCs, major DC and recipient T cells, and increased production of IL-12, IFN- γ , and IL-10. The mechanism of liver tolerance continues to be extensively investigated and is considered by many to be due to the tolerogenicity induced by liver DC. Apoptosis of mature T cells in the liver, but with persistence of their precursors in the periphery, was suggested to be the explanation for split tolerance.

However, apoptosis alone cannot explain liver-induced tolerance to subsequent other organ grafts from the same donor strain. The liver tolerance seems to be an active process and one which is mediated by regulatory T cells. We hypothesize that inducing activated T cell apoptosis and Treg production are both critical to liver tolerance. Liver immature DC may be a key factor to induce Treg cell production and mediate activated T cell apoptosis. Co-stimulation between donor DC and recipient T-cells contribute to the T cell immune deviation, alloreactive T cell apoptosis, and function of regulatory T cells. To test our hypothesis, we treated liver donors or recipients with depleting anti-CD25 mAb. For the first time, we confirmed that depletion of recipient, but not donor, CD4⁺CD25⁺ regulatory T cells prevented spontaneous liver transplant tolerance. It was associated with enhanced anti-donor immune responses (MLR, CTL, NK activities, and Th1 cytokines IL-2 & IFN- γ production) and decreased alloreactive T cells, particularly in CD8 T cell apoptosis.

This suggests that recipient CD4⁺CD25⁺ regulatory T cells play a very important role in spontaneous liver transplant tolerance induction, and this Treg may mainly affect the indirect pathway of antigen recognition. Further studies on other potential mechanisms of CD4⁺CD25⁺ Treg on liver tolerance induction are undertaken in our laboratory.

The role of costimulatory molecules on tolerance induction

T cell activation requires two distinct signals: Signal 1 is antigen specific, mediated via the T cell receptors, and delivered in the context of donor MHC class II; Signal 2, the costimulatory signal, is not antigen specific. Costimulatory molecules, in particular the B7/CD28 super family, have recently been extensively studied. A number of new members have been discovered and characterized, including B7/ CD28, B7/ CTLA4, CD40/CD40L, and most recently PD-L/ PD-1, B7H / ICOS, OX40L / OX40, 4-1BBL/4-1BB, CD30L/CD30, and Tim3L /Tim3. It has already been known that B7/CTLA4, PD-L /PD-1, and Tim3L /Tim3 interactions provide a negative signal to the T cell, inhibit T cell activation and IL-2 production, and induce tolerance. On the other hand, B7/CD28, B7H/ICOS, CD40L/CD40, 4-1BBL/4-1BB, and OX40L/OX40 interactions provide a positive signal to the T cells, promote T cell proliferation and IL-2 production, and induce immunity. Each of these costimulatory pathways may function independently or cooperatively with each other.

To examine the mechanistic relationships among these signals and precisely assess which signal is critical for transplant tolerance induction and rejection, our approach was a comprehensive investigation of their molecular constituents and functions on the alloimmune response. Using a model of orthotopic liver transplantation and heterotopic heart transplantation in mice with a costimulatory pathway deficiency, we analyzed the expression profiles of those genes and the outcome of the allografts. These studies on the role of these new accessory molecules and their effect on tolerance induction, activated T cell apoptosis, and possible promotion of Treg may provide crucial implications for designing a target for a trial of DC, antibody, or gene based therapy in patients receiving organ transplants.

We have recently tested costimulation blockade on liver DC and T cell interaction by using CTLA4 Ig and anti-CTLA4 mAb. The results showed that blocking both B7-CD28/ B7-CTLA4 signals using CTLA4 Ig promoted liver allograft survival from FL pretreated donors. It was associated with

increased alloreactive T cell apoptosis in the liver graft and recipient spleen, and increased IL-10, decreased IFN- γ levels in the recipient serum. In contrast, blocking CTLA4 signal using anti-CTLA4 mAb, which was defined as a negative signal to the T cells, broke the liver spontaneous tolerance and induced liver allograft acute rejection. This was associated with decreased alloreactive T cell apoptosis in the liver grafts and recipient spleens, and increased IL-2, IFN- γ , decreased IL-4 production, and decreased the CD4⁺CD25⁺ regulatory T cells in the recipient spleens.

The role of dendritic cells (DC) in organ transplantation

DC, professional antigen presenting cells of the immune system, have been considered as having the potential to either stimulate or inhibit immune responses. Exploiting the immune-regulatory and tolerogenic capacities of DC holds great promise for the treatment of cancer, autoimmune disease, and prevention of transplant rejection. We have reported that liver immature DC play a critical role in the liver transplant spontaneous tolerance. We also reported that the immunoregulatory cytokine IL-10 induces Treg both *in vivo* and *in vitro* and promotes heart allograft survival in mice.

A recent report revealed that DC is capable of inducing CD4⁺CD25⁺ Treg which express CTLA4 and produce immunosuppressive cytokines IL-10 and TGF β , down-regulating alloimmune responses. Costimulation between donor DC and recipient T-cells may not only contribute to T cell immune deviation and alloreactive T cell apoptosis, but also may lead to production of regulatory T cells. Thus, treating the allograft recipient with immature donor DC in the presence of IL-10 or TGF β may drive regulatory T cell generation *in vivo* and promote organ transplant tolerance. We will challenge DC-treated recipients with allogeneic heart transplants or islet transplants (in NOD mice or STZ treated diabetes mice) to assess the therapeutic potential of DC-induced alloantigen specific tolerance.

We believe that these studies will provide better understanding of the mechanism of transplant tolerance and rejection, and facilitate novel therapeutic strategies to combat organ rejection and even autoimmune disorders such as diabetes.

RELATED PUBLICATIONS

1. Li W, Fu F, Lu L, Narula SK, Fung JJ, Thomson AW, Qian S. Systemic administration of anti-IL-10 antibodies prolongs mouse allograft survival in normal and presensitized recipients. *Transplantation* 66(12): 1587-1596, 1998.
 2. Li W, Fu F, Lu L, Narula SK, Fung JJ, Thomson AW, Qian S. Recipient pretreatment with cellular IL-10 prolongs cardiac allograft survival in mice. *Transplantation* 68:1402, 1999.
 3. Steptoe R, Li W, Fu F, O'Connell PJ, and Thomson AW. Trafficking of APC from liver allografts of Flt3L-treated donors. *Transplant Immunology* 7:51-57, 1999.
 4. Li W, Lu L, Wang Z, Wang L, Fung JJ, Thomson AW, and Qian S. IL-12 antagonism enhances apoptotic death of T cells within hepatic allografts from Flt3-treated donors and promotes the restoration of transplant tolerance. *J. Immunology*, 166:5619-5628, 2001.
 5. Li W, Lu L, Wang Z, Wang Z, Fung JJ, Thomson AW, and Qian S. Costimulation blockade promotes the apoptotic death of graft infiltrating T cells and prolongs survival of hepatic allografts from Flt3L-treated donors. *Transplantation*, 72:1423-1432, 2001.
 6. O'Connell PJ, Li W, Wang Z, Logar AJ and Thomson AW. Immature and mature CD8 α ⁺ dendritic cells prolong the survival of vascularized heart allografts. *J. Immunology*, 168:143, 2002.
 7. Li W, Chou S, Wang C, Kuhr C, Perkins J. The role of the liver in peripheral tolerance: induction through oral antigen feeding. *American Journal of Transplantation*, 4:1574-1582, 2004.
 8. Li W, Zheng XX, Kuhr CS, Perkins JD. CTLA4 engagement is required for induction of mouse liver transplant spontaneous tolerance. *American Journal of Transplantation*, 5:978-986, 2005.
 9. Li W, Carper K, Perkins JD. Enhancement of NKT cells and increase in regulatory T cells results in improved allograft survival. *Journal of Surgical Research*. 134:10-21, 2006.
 10. Li W, Carper K, Liang Y, Zheng XX, Kuhr CS, Reyes JD, Perkins DL, Thomson AW, Perkins JD. The role of Foxp3 regulatory T cells in liver transplant tolerance. *Transplant Proc*. 38:3205-3206, 2006.
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- Achieving Transplantation Goals through Basic Science Investigations, Clinical Outcomes Research, and Quality Studies



The Goal of Transplantation

Liver transplantation has progressed remarkably since the first successful human liver transplant was performed in 1963. The surgical technique for the operation was quickly mastered, but understanding how to avoid rejection of the transplanted organ has been more difficult. With the discovery of cyclosporine and other immunosuppressive drugs, patient survival has risen to a high enough level that liver transplantation has long ceased to be considered experimental. Nevertheless, the ultimate goal of transplantation has yet to be achieved, which is acceptance of the transplanted organ without compromising the patient's overall immune system. This ideal state is referred to as "tolerance," in which the body accepts the grafted organ while yet defending itself against all other "foreign" substances.

Aside from the scientific concept of tolerance, attention in transplantation needs to be given in a more general way. Tolerance is determined at the "micro" cellular level, but what factors at the "macro" level affect clinical outcomes? What donor and recipient factors lead to the highest post-transplantation survival rates? At an even higher level, how do we evaluate our protocols in order to determine our quality of care? How can we determine if our present care systems are working as well as they could be? These three areas, basic science investigation, clinical outcomes research, and quality studies, have been the areas of focus in our work, all with the goal of giving patients with organ failure an opportunity to pursue a higher quality of life.

BASIC SCIENCE RESEARCH ON PERIPHERAL TOLERANCE

There are two types of tolerance: "central" and "peripheral." Central tolerance occurs when immature lymphocytes encounter antigens and are deleted (the process of "negative selection," also called "clonal deletion," "programmed

death," or "apoptosis.") Peripheral tolerance occurs in peripheral lymph organs, such as the lymph nodes and spleen, where mature lymphocytes encounter antigens under particular conditions. Three principle mechanisms contributing to peripheral tolerance are: 1) clonal deletion, 2) clonal anergy (functional inactivation of lymphocytes without cell death), and 3) immune regulation (suppression of lymphocyte activity by regulatory T cells). These three mechanisms are not mutually exclusive.

The liver has long been known to have a positive effect on the induction of peripheral tolerance. Patients who receive a combined liver-kidney transplant experience significantly less rejection of the kidney than patients receiving a kidney transplant alone. In both animals and humans, certain vascularized allografts have improved survival with the venous drainage via the portal vein into the liver. In mice, liver allografts (unlike heart or kidney allografts) are accepted spontaneously without the need for immunosuppression. The tolerance induced by liver allografts in these animals subsequently protects future donor hearts or skin grafts from acute and chronic rejection.

The liver is a major hematopoietic organ which gives birth to all leukocyte lineages, including extrathymic T cells, natural killer (NK) cells, natural killer T (NKT) cells, dendritic cells, and granulocytes. This unique combination of leukocytes in the liver may be the major cause of liver tolerogenicity. Extrathymic T cells during their development in the liver undergo incomplete negative selection. It is unknown whether the mechanisms in the liver for clonal deletion, for selecting naïve extrathymic T cells, and for removing antigen-specific T cells to develop peripheral tolerance are linked. Studies have revealed that apoptotic cells adhere to liver sinusoidal endothelial cells (LSEC) in

Patients who receive a combined liver–kidney transplant experience significantly less rejection of the kidney than patients receiving a kidney transplant alone.

the periportal region. LSEC have been demonstrated to trap and induce apoptotic cells by an active receptor-mediated binding process. NKT cells have also been suggested as necessary for the formation of tolerance induction by portal vein injection of antigens and necessary for the induction of oral tolerance. The exact mechanism of NKT cell tolerance is unknown. Therefore, several mechanisms of peripheral tolerance may be active in the hepatic immune system.

Antigen given via a mucosal route favors the induction of peripheral tolerance. This type of induced peripheral tolerance is commonly called “oral tolerance.” The mechanisms of the liver’s role in oral tolerance induction are not clear.

We have used a murine transplant model to study the liver’s role in inducing and maintaining peripheral tolerance induced via oral antigens (Figure 1). We chose OVA (chicken albumin) in a low dose and a high dose as an agent to induce oral tolerance. Our unique model has allowed for removal and insertion of various liver combinations to facilitate study of the liver’s role in the different mechanisms of peripheral tolerance. In addition to our *in vivo* studies, we have performed *in vitro* studies to measure the cytokine levels of IL-2, IFN- γ , and IL-10 from mixed lymphocyte reaction (MLR) culture detected by ELISA.

HOW THE MODEL DEMONSTRATED INDUCTION AND TRANSFER OF TOLERANCE

To date, the results in our model can be summarized as follows:

- OVA feeding induced tolerance to OVA.
- The transplanted murine livers transferred tolerance from OVA fed mice to naïve mice.
- OVA feeding inhibited T cell proliferative activities of liver graft NPC and recipient spleen cells.
- OVA feeding inhibited IL-2 and increased IL-4 production of liver NPC and SC.
- Liver NPC from OVA-fed mice were capable of transferring tolerance to OVA to naïve mice.
- Removal of the liver from tolerant mice could not break the established tolerance.
- NKT cells play a role in tolerance.

Our experiments demonstrated that several sites, including the intestinal epithelial cells and gut-associated lymphoid tissue, are involved in peripheral tolerance induction to orally administered antigens. Furthermore, our results suggest that different mechanisms of tolerance are influenced differently by the liver depending on the dose of the antigen. Oral tolerance can be adoptively transferred by the NPC of the liver from either the low dose or high dose groups; however, only the SC from the low dose group can transfer tolerance. The high dose group is more tolerizing since the DTH response and the proliferative responses are significantly less than with the low dose group. Possibly with a lower dose, less antigen reaches the liver via the portal vein, and the gut lymph dominates the tolerance mechanisms. With the higher dose more native antigen gets to the liver, and the liver, with its relatively large size in proportion to body weight, has an increased role in tolerance induction. This could help explain some controversies regarding the liver’s role in peripheral tolerance.

Our results of the proliferative response and the cytokine profiles also suggest that the mechanisms of tolerance induction for the high dose and low dose fed livers are different. IL-10 is increased in both the NPC and SC in the low dose fed antigen group, but is not increased in the NPC of the high dose group. This indicates that the tolerance in the low dose fed group is more suggestive of a TH2 response, while that of the high dose fed group is not. Other reports have also indicated that IL-10 is enhanced in oral tolerance. Another mechanism involved with the immunologically diverse hepatic immune system is NKT cells. Our data are consistent with NKT cells being involved in the induction of oral tolerance, specifically for the high dose of antigen. Since our proliferative assay did not produce increased amounts of IFN- γ production, this suggests that different lineages of NKT cells contribute to the induction of tolerance.

Clinical Outcomes Research in Transplantation

Clinical outcomes research concerns the treatment of specific conditions. Questions are asked concerning how care protocols can lead to improve results. Improved results in the field of transplantation might take the form of higher patient survival rates, lower rejection rates concomitant with low infection rates, more effective use of immunosuppressive therapy, or shorter hospital stay. Below are two examples of clinical outcomes research we are currently pursuing in the Division of Transplantation.

USE OF DONATION AFTER CARDIAC DEATH (DCD) DONORS IN LIVER TRANSPLANTATION

The disparity between the number of patients on the waiting list for a liver transplant and the number of available donor organs compels us to explore all possible types of donor organs. The use of donation after cardiac death (DCD) hepatic allografts is an important source of organs for transplantation. We conducted a retrospective review of all DCD and donation after brain death (DBD) donor liver recipients from September 2003 through February 2006. Patient survival and graft survival, recipient demographics, and complications were compared between the two groups. There was no difference in one-year patient or graft survival rates between the two groups. There was no incidence of primary non-function (PNF) from the DCD allografts. Hepatic artery complications and anastomotic bile duct complications were equivalent in the two groups. However, there was an increased risk for the development of ischemic cholangiopathy in the DCD group (13% vs. 2%, $p=0.003$). Donor weight > 100 kg and total ischemia time > 9 hours in patients older than 50 years of age were found to be significant in developing ischemic cholangiopathy. Despite the higher incidence of ischemic cholangiopathy in the DCD group, patient and graft outcomes using DCD donors are comparable to DBD donors. Minimization of total ischemia time in older donors and improving preservation techniques in donors > 100 kg may improve results and expand utilization of DCD donors.

RECURRENCE OF HEPATOCELLULAR CARCINOMA AFTER LIVER TRANSPLANTATION

The recurrence of hepatocellular carcinoma (HCC) following liver transplantation plays a major role in recipient mortality. Pre-transplant staging is inaccurate at predicting explant staging and post-transplant survival. Identification of factors influencing survival of patients with HCC is necessary to plan surveillance strategies and

adjuvant therapies. We performed a retrospective review of 505 consecutive liver transplantations from 1/1/2001 to 12/31/2005. Mean follow-up was 817 ± 486 days. Patients with HCC on explant were identified. Review of explant pathology, donor and recipient characteristics, intra- and post-operative events was performed to determine the factors associated with survival of patients transplanted for HCC. Explant pathological analysis identified 116 patients with HCC. Twelve patients developed recurrent HCC, and 8 (6.8%) of those have died. Of recipient characteristics, age > 60 ($p=0.002$) and the presence of metabolic syndrome ($p=0.04$) decreased survival. On explant pathology, total number of tumors ($p=0.0002$), total tumor size > 8.5 cm ($p=0.0001$), tumor differentiation (well, moderate, poor) ($p=0.0002$), multi-lobulated ($p=0.003$), macro-invasion ($p=0.02$) and tumor in both lobes of the liver ($p=0.0014$), correlated with survival. No donor characteristics or intra- or post-operative events significantly influenced survival. Only 54% were accurately staged pre-operatively. Restaging via TNM classification did not significantly predict survival. A new score for predicting cancer recurrence (SPCR) was developed where tumors in both lobes of the liver and poorly differentiated tumors are given 2 points; macro-invasion, moderately-differentiated tumors, and total tumor size > 8.5 cm are given 1 point; and well-differentiated tumors, tumors in one lobe of the liver, and absence of vascular invasion are given 0 points. This SPCR significantly predicts survival at all levels ($p=0.007$); 0-1 point predicts a 3-year survival of 98%; 2-4 points predict an intermediate 3-year survival of 73%; and ≥ 5 points predict a 3-year survival of 20%. HCC recurrence impacts recipient mortality. The SPCR can direct patients' post-operative surveillance schedule or adjuvant treatment for those with a low predicted survival.

QUALITY STUDIES

Quality studies answer questions concerning *how* to deliver better care. How can we organize our transplantation efforts to achieve even better results? There are four areas in which we have been pursuing quality studies in the Division of Transplantation: 1) data analysis using our clinical patient transplant database; 2) evaluation of our written care protocols; 3) review of surgeon faculty evaluations (categorization of critical factors that lead to various outcomes, enabling surgeons to evaluate their strengths and weaknesses); and 4) root cause analysis to determine the specific causes of poor outcomes.

ROOT CAUSE ANALYSIS ENABLED INCREASE IN LIVER TRANSPLANT SURVIVAL RATE

We performed aggregate root cause analysis (RCA) to determine the underlying reasons for patient deaths in our liver transplant program from June 1, 1998 to June 30, 2000. During this period, our survivals after liver transplantation were lower than our expected rates (graft 81.89% and patient 88.3%) according to the U.S. Scientific Registry of Transplant recipients (SRTR). Of 355 patients receiving their first transplant, there were 90 deaths, with 188 root causes identified. Apportionment of deaths according to phase of the transplant process was found to be: patient selection, 50%; transplant procedure, 17%; donor selection, 15%; post-transplant care, 8%; and psychosocial issues, 10%. In response to our findings, we developed risk reduction plans and changed our care protocol in an effort to correct specific problem areas. In April 2004, SRTR data revealed that for patients transplanted between January 1, 2001 and June 30, 2003, our one-year liver graft survival rose to 90.73% ($P=0.018$), which was now significantly higher than the national expected rate of 84.48%. Our one-year patient survival rate rose to 92.66% ($P=0.285$), which was higher than the expected rate of 89.29%.

Research Opportunities and Resources

The field of transplantation is rich in possibilities for both basic science and clinical research. We are most fortunate in the Division of Transplantation at the University of Washington to have not only interesting questions to pursue, but also the resources available and an environment conducive to investigation. Our statistical expertise, together with our custom-designed clinical transplantation database, allow us to perform multiple clinical outcomes research projects per year as ideas are developed. Our transplant fellows are afforded an excellent opportunity to learn research methods and receive guidance and encouragement through faculty mentorship. Our most important resources are our *people* – our gifted faculty and fellows who ask important questions and persevere until they find answers. We look forward to the answers our research will bring in order to improve the lives of patients who can benefit from transplantation.

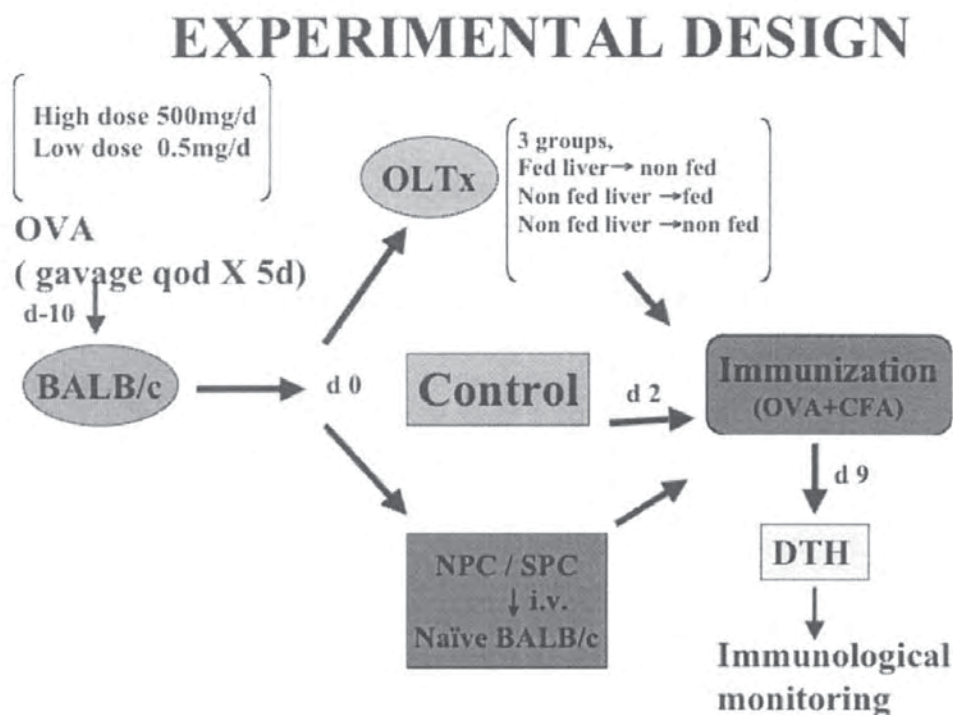


FIGURE 1: A model to study the liver's role in peripheral tolerance.

RELATED PUBLICATIONS

1. Perkins JD, Nelson DL, Rakela J, Grambsch PM, Krom RAF. Soluble interleukin 2 receptor level in liver allograft recipients: an indicator of rejection. *Transplantation Proceedings*, 1989; 21 (1 Pt 2):2275-2276.
2. Perkins JD, Rakela J, Sterioff S, Banks PM, Wiesner RH, Krom RAF. Immunohistologic pattern of the portal T-lymphocyte infiltration in hepatic allograft rejection. *Mayo Clinic Proceedings*, 1989; 64(5):565-569.
3. Li W, Lu L, Wang Z, Wang L, Fung JJ, Thomson AW, Qian S. IL-12 antagonism enhances apoptotic death of T cells within hepatic allografts from Flt3 ligand-treated donors and promotes graft acceptance. *J Immunology*, 2001; 166:5619-5628.
4. Li W, Lu L, Wang Z, Wang L, Fung JJ, Thomson AW, Qian S. Costimulation blockade promotes the apoptotic death of graft-infiltrating T cells and prolongs survival of hepatic allografts from FLT3L-treated donors. *Transplantation*, 2001; 72:1423-1432.
5. Carithers RL and Perkins JD. 69. Liver and Pancreas Transplantation. In: *WebMD Scientific American Medicine*, Editors: Dale DC and Federman DO. New York: WebMD, Inc., 2003, 866-871.
6. Li W, Zheng XX, Kuhr CS, Perkins JD. CTLA4 engagement is required for induction of mouse liver transplant spontaneous tolerance. *Am J Transplant*, 5:978-986, 2005.
7. Perkins JD, Levy AE, Duncan JB, Carithers Jr RL. Using root cause analysis to improve survival in a liver transplant program. *J Surg Res*, 129:6-16, 2005.
8. Li W, Carper K, Perkins JD. Enhancement of NKNT cells and increase in regulatory T cells results in improved allograft survival. *J Surg Res*, 134:10-21, 2006.
9. Li W, Carper K, Zheng XX, Kuhr CS, Reyes JD, Liang Y, Perkins DL, Thomson AW, Perkins JD. The role of Foxp3⁺ regulatory T cells in liver transplant tolerance. *Transplant Proc*, 12:3205-3206, 2006.
10. Li W, Carper K, Liang Y, Zheng XX, Kuhr CS, Reyes JD, Perkins DL, Thomson AW, Perkins JD. Anti-CD25 mAb administration prevents spontaneous liver transplant tolerance. *Transplant Proc*, 12:3207-3208, 2006.

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• The Breast Health Global Initiative (BHGI)



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Purpose and Specific Aims

The Breast Health Global Initiative (BHGI) is an ongoing public-private global health alliance devoted to medically underserved women, co-sponsored and co-led by the Fred Hutchinson Cancer Research Center and Susan G. Komen for the Cure. BHGI is an outcome oriented program that strives to develop, implement and study evidence-based, economically feasible, and culturally appropriate guidelines for developing countries to improve breast health outcomes. The guidelines outline a stepwise systematic approach to breast health care improvement for limited resource settings, focusing on early detection, diagnosis and treatment of breast cancer. The process of guideline development creates a hub for linkage and alliances among the clinical community, health care policy makers, advocacy groups and non-governmental organizations (NGOs) and the public health sciences research community.

BACKGROUND

Breast cancer is the most common cause of cancer-related death among women around the globe. Each year, breast cancer is newly diagnosed in more than 1.1 million women, and these cases represent more than 10% of all new cancer cases. With more than 410,000 deaths each year, breast cancer accounts for over 1.6% of all female deaths worldwide. Breast cancer already is an urgent public health problem in high resource regions, and is becoming an increasingly urgent problem in low resource regions, where incidence rates have been increasing by up to 5% per year.

Low resource countries have generally not identified cancer as a priority health care issue because infectious disease is the predominant public health threat in such settings. Nonetheless, resources are inevitably spent on cancer treat-

ment when patients seek medical care for what is typically advanced-stage disease. Cancer becomes an increasing problem in such countries as the control of communicable diseases improves and life expectancy increases. However, obstacles to improving cancer care arise from multiple sources, including deficits in public knowledge and awareness, social and cultural barriers, challenges in organizing health care, and insufficient resources.

In high resource countries, evidence-based guidelines outlining optimal approaches to early detection, diagnosis, and treatment of breast cancer have been defined and disseminated. These guidelines from wealthy countries do not consider variable resource distributions and are likely to be unworkable in the face of the ubiquitous infrastructure and resources deficits in limited-resource countries. Moreover, these guidelines are not designed to consider implementation costs or provide guidance as to how a suboptimal system can be improved incrementally toward an optimal system. As pointed out by WHO, guidelines defining ideal cancer care and services have limited utility in resource-constrained countries. Thus, there has been a lack of resource-based guidance related to strategies to reduce the burden of breast cancer in settings where ideal care is not feasible.

The Global Summits 2002, 2005 and 2007

GLOBAL SUMMIT 2002 (SEATTLE)

The first biennial Global Summit was held in Seattle, October 2002, to establish the first breast health Guidelines to address how care may best be provided in countries where significant gaps in health care resources exist. Guidelines development followed consensus panel analysis of evidence-

*With more than 410,000 deaths each year, breast cancer
accounts for over 1.6% of all female deaths worldwide.*

based breast care modeling. Panels of breast cancer experts representing 17 countries and nine world regions created Guidelines for breast cancer in countries with limited health care resources based upon definitions created by the World Health Organization (WHO) for national cancer programs. The breast health care Guidelines were published as a supplement publication in *The Breast Journal* in 2003 and have been made freely available in an unrestricted fashion on the Internet for world-wide access (<http://www.fhcr.org/science/phs/bhgi/>). To date, these are the only written consensus guidelines that specifically address issues of breast care implementation in countries of limited resources.

GLOBAL SUMMIT 2005 (BETHESDA)

To update and expand on the BHGI guidelines published in 2003, the 2005 BHGI panels outlined a stepwise, systematic approach to health care improvement in the areas of early detection and access to care, diagnosis and pathology, treatment and resource allocation, and health care systems and public policy, as they relate to breast health care in limited-resource settings. A tiered system of resource allotment was defined using four levels—basic, limited, enhanced, and maximal—based on the contribution of each resource toward improving clinical outcomes. During this analysis, a number of key points were identified and/or demonstrated:

- Early breast cancer detection improves outcome in a cost effective fashion assuming treatment is available;
- The effectiveness of early detection programs require public education to foster active patient participation in diagnosis and treatment;
- Clinical breast examination combined with diagnostic breast imaging (breast ultrasound with or without diagnostic mammography) can facilitate cost-effective tissue sampling techniques for cytological or histological diagnosis;
- Breast conserving therapy with partial mastectomy and radiation requires more health care resources and infrastructure than mastectomy, but can be provided in a thoughtfully designed limited resource setting;
- The availability and administration of systemic therapy are critical to improving breast cancer survival;

- Estrogen receptor testing allows patient selection for hormonal treatments (tamoxifen, oophorectomy) which is both better for patient care and allows proper distribution of services;
- Chemotherapy, which requires some allocation of resources and infrastructure, is needed to treat node-positive, locally advanced breast cancers, which represent the most common clinical presentation of disease in low-resource countries;
- When chemotherapy is unavailable, patients presenting with locally advanced, hormone receptor negative cancers can only receive palliative therapy.

The Guidelines Tables that delineate cancer detection, diagnosis and treatment resources and services within an organized stratification schema are published in a January/February 2006 supplement to the *Breast Journal* and are available on line (<http://www.fhcr.org/science/phs/bhgi/>). These tools can be used to communicate programmatic needs to hospital administrations, government officials and/or health care ministries. It is the thesis of the BHGI that these works create a framework for change, by defining practical pathways through which breast cancer care can be improved in an incremental and cost-effective fashion.

GLOBAL SUMMIT 2007 (BUDAPEST, HUNGARY)

The 2007 Global Summit format will fundamentally adhere to the 2005 Summit structure, bringing together some of the best minds in medicine, science, policy, public health and health economics to address “best practices with limited resources.” The American Society of Clinical Oncology is the official host organization of the meeting to be held October 1-4, 2007 in Budapest, Hungary. Preceding the Global Summit and in association with the Breast Health Global Initiative, Susan G. Komen for the Cure will host and hold a *Global Breast Cancer Advocates Summit*, September 29-30, 2007, in Budapest (www.komen.org).

BHGI Global Summit panels will address early detection, diagnosis, treatment, and health care systems. However, the 2007 summit focus will shift, from development and expansion of the 2006 *Guidelines for International Breast Health and Cancer Control*, to address effective implementation



FIGURE 1: BHGI is a structure for linkage among stakeholder groups to implement change.

and integration of breast health care interventions described in the Guidelines. Based upon reallocation of existing resources and incorporation of a breast health care program with existing programs to potentially improve outcomes in a cost-sensitive manner and infrastructure, the 2007 Global Summit will improve breast health services and ultimately breast cancer survival for women in geographic areas where resources are limited. The aim of the health systems panel discussion will be to identify effective, efficient and feasible approaches to cancer care delivery that promote high quality care in a climate of resource limitations, using breast cancer as model disease that needs to be addressed in limited resource countries. Specifically the panel will:

- Outline breast health interventions for early detection, diagnosis and treatment proven successful in the setting of limited resources;
- Define programmatic approaches that support key breast health interventions that can be replicated in communities where resources are limited ;
- Provide guidance on how to overcome obstacles to implementation of breast health interventions when resources are limited;
- Foster collaboration, coordination and integration of breast health services among clinical communities, public health researchers and advocacy and non-governmental organizations by defining optimal interventions that will effect real benefit;
- Support the testing, implementation and development of new international and national pilot research, demonstration and technology development projects for breast health care in areas with limited resources;
- Disseminate results of the conference to educate providers and patients about how breast cancer can be effectively treated in areas where resources are constrained; and
- Promote the further development and use of economic modeling tools that will benefit countries and individual communities in determining cost-effective strategies to improve breast health outcome.

BHGI Organizational Linkages

The BHGI is a structure for linkages through interdisciplinary communication, cooperation and alliance-building via the Global Summits, on-going communications, the Web site, and pilot research and demonstration projects between three core groups, (Figure 1):

1. **Clinicians and governmental health care agencies** (health care systems, physicians, scientists and government agencies);
2. **Advocacy and non-governmental organizations** (communication, patient advocacy, public education);
3. **Public health researchers** (outcomes analysis, economic modeling, demonstration projects, social impact studies)

Pilot Research & Demonstration Projects

Funded through a special \$500,000 award from Susan G. Komen for the Cure, the BHGI has created an international research arm. Guidelines do not in and of themselves improve outcome for women. Implementation is the critical step by which the value of the guidelines may be measured. An aim of the BHGI is to operate as a catalyst for international breast cancer pilot research and demonstration projects with partnering organizations. In order to implement the Guidelines, the BHGI is developing project proposals that fit with the mission of the BHGI and follow the Guidelines framework. Linked to the Guidelines to test and validate them, the international projects currently under review for development include:

- Pilot research
- Guideline demonstration
- Public education
- Special technology development

The results of pilot research projects and demonstration projects need to be studied and reported, both to determine the effectiveness of the guidelines, and to create evidence that will allow guideline implementation in other places. In this way, the BHGI endeavors to help women cope with and survive the ravages of the most common cancer and most common cancer killer among women.

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RELATED PUBLICATIONS

1. Anderson B.O., Shyyan R., Eniu A.E., Smith R.A., Yip C.-H., Bese, N.S., Chow, L.W.C., Masood, S., Ramsey, S., Carlson, R.W. Breast cancer in limited-resource countries: An overview of the Breast Health Global Initiative 2005 Guidelines. *Breast J.*, 12(s1):s3-s15, 2006.
2. Eniu A.E., Carlson, R.W., Aziz Z, Bines, J., Hortobágyi G, Bese, N.S., Love, R.R., Vikram, B., Kurkure, K., Anderson B.O.: Breast cancer in limited-resource countries: Treatment and allocation of resources. *Breast J.*, 12(s1):s38-s53, 2006.
3. Anderson B.O., Yip C.-H., Ramsey S., Bengoa R., Braun S., Fitch M., Groot, M.T., Sancho-Garnier, H., Tsu, V.D.: Breast cancer in limited-resource countries: Health care systems and public policy. *Breast J.*, 12(s1):s54-s69, 2006.
4. Groot, M.T., Baltussen, R. Uyl-de Groot, C.A., Anderson B.O., Hortobágyi, G.N.: Costs and health effects of breast cancer interventions in epidemiologically different regions of Africa, North America, and Asia. *Breast J.*, 12(1):s81-s90, 2006.
5. Anderson, B.O.: Breast health care and cancer control in limited resource countries: a framework for change. *Nature Clinical Practice Oncology*, 3(1):4-5, 2006.
6. Anderson, B.O., Khalil, E.A.: Breast conservation without mammography? Oncology practice in the real world of limited resource countries. *Breast*, 15:575-579, 2006.
7. Anderson, B.O., Caleffi, M.: The Breast Health Global Initiative: Special issues in Latin America. *Revista Médica de Clínica Las Condes*, 17(4):137-141, 2006.
8. Anderson, B.O., Eniu, A.E., Shyyan, R.: The Breast Health Global Initiative: A framework for improving breast healthcare in limited-resource countries. *Breast Diseases: A Year Book Quarterly*, 17(4):314-317, 2007.
9. Anderson, B.O., Eniu, A.E., Sullivan, L.M., Carlson, R.W.: Defining resource-level-appropriate cancer control (Chapter 4). In: *Institute of Medicine Report – Cancer Control Opportunities in Low- and Middle-Income Countries*, Sloan FA, Gelband H, editors. National Academy Press, pp. 106-137, 2007.

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· Advancing the Clinical Science of Surgery
Using Outcomes Research Tools



FUNDING

National Institutes of Health

Over the last decade “outcomes” research became a catch phrase for healthcare administrators, providers and researchers, but outcomes research means different things to different people. For some it’s viewed as a way to provide more services for fewer dollars; for others it means finding ways to regulate physician variability to improve care. Neither of these definitions fully describes the potential of this form of research. I believe outcomes research means moving beyond a research culture that shows us what can be done by surgeons, to one that emphasizes what should be done by surgeons. The “should” in that statement indicates a balance of the feasibility of an operative procedure with an assessment of the burden of that operation on the patient and society. Only by determining the impact of procedures in their totality can we understand what should be done rather than simply what can be done.

To do this we have to consider the impact of the operation on the patient’s life, both in the context of life expectancy and quality of life, while assessing the burden of that intervention for the patient and society. Since the publication of the Institute of Medicine report, “To Err is Human,” the public has focused on the “burden” of the healthcare system as it refers to adverse outcomes and medical errors. Answering the question, “What should we be doing?” requires that we address these adverse clinical outcomes in the context of system-level quality improvement.

To do this, outcomes researchers use a set of tools borrowed from health economics, decision analysis, epidemiology and biostatistics. To address this goal of system-level quality improvement for all areas of clinical interest, we use these tools to answer four necessary questions.

Can we determine the way surgical procedures impact the average patient?

Risk of adverse outcome is a component of all surgical procedures. While the informed consent process tries to address this by providing the patient with a summary of the expected risk, in fact what we really offer in the consent process are the results found in the published case series of the best practitioners in the field. For the vast majority of general surgical procedures we simply don’t know the community level risk of adverse outcome. As such, we are unable to determine what should be considered the standard, who are the outliers (both good and bad) and what techniques work out of the research environment. In the absence of a tracking system for outcomes we often rely on estimates derived from randomized trials (which for most general surgical procedures have not been completed) or administrative data. Only by understanding the real level of risk can we determine the opportunities for improvement in the system.

Research I’ve been involved with during the last year has addressed this issue of community-level risk in commonly performed general surgical procedures by using administrative data. Determining population-level risk requires the analysis of large databases. For example, in evaluating rates of misdiagnosis in appendectomy we studied 80,000 patient records and found that the rate of misdiagnosis in appendicitis has not improved in the past 13 years (~15% overall and ~25% in women of reproductive age) despite the growing availability of CT scanning. We studied over 30,000 patients undergoing cholecystectomy to describe the rates of major common bile duct (CBD) injury over time and found that rates of this outcome (0.025%) have not significantly improved with time.

There are almost always avoidable factors that are associated with adverse outcomes. Understanding those associations and quantifying their impact is an important step in the quality improvement process.

To study outcomes from antireflux procedures we studied over 86,000 patients and found that while the rates of splenectomy have decreased significantly with time the rate of in-hospital mortality and esophageal injury have not. Furthermore, while the rate of adverse outcome identified was low (~2% chance of splenectomy, <1% likelihood of death, ~1% chance of esophageal injury), these rates were between 2 and 20 times higher than results published in large case series.

This illustrates the importance of population-level results in estimating risk for the average patient. This research technique is also helpful in checking conventional wisdom about the benefits of new technology. For example, of ~10,000 patients undergoing incisional hernia repair we quantified the rate of reoperative repair and found no improvement in this measure of recurrence in the era of laparoscopy. It is also important in addressing two important forms of bias in published estimates of outcome. Cholecystectomy-related bile duct injury is the leading source of surgical malpractice claims. Determining outcome after bile duct injury is challenging because the results of surgical experts are excellent (publication bias) while reports of cases that progress to litigation (selection bias) detail dismal outcomes.

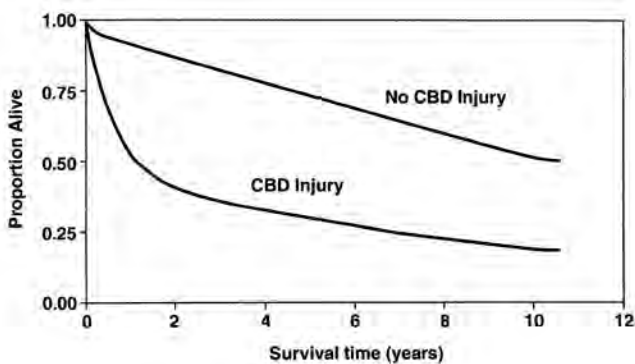


FIGURE 1: Survival after bile duct injury in Medicare beneficiaries (n=1.57 million)

We recently evaluated the risk of death after bile duct injury among all Medicare beneficiaries nationwide and found they were 2.5 times more likely to die within the first few years after an injury compared to uninjured patients (Figure 1).

Another way to assess the impact of care is to quantify patient-described outcomes as they relate to quality of life, function and well-being. Standard quality-of-life instruments measure chronic health states and do not adequately capture the dynamic process of pre-operative states, anticipatory stress, post-operative morbidity and then evolution to either recovery or chronic states. Working with industry, we are developing an Internet-based interactive survey instrument aimed at capturing, quantifying and validating changes in Quality Adjusted Days (QAD) “lost” over the relevant time course of a patient. We hope that “lost” QADs will be an important outcome measurement tool that captures the patient level burden of surgical procedures. By quantifying outcomes both on an individual and community level we can then move on to the next step in improving clinical outcomes.

What are the avoidable factors associated with these adverse outcomes?

Health services researchers believe that most adverse outcomes have a system-level component. While all individuals make mistakes, it is a flawed system that allows these mistakes to adversely impact the patient. To that end there are almost always avoidable factors that are associated with adverse outcomes. Understanding those associations and quantifying their impact is an important step in the quality improvement process.

For example, using administrative data we have quantified the degree to which both surgical inexperience and the failure to use a cholangiogram are associated with CBD injury. Surgical inexperience (the surgeons’ 1st through 19th cholecystectomy) and failure to use a cholangiogram result in a 60-70% increase in the likelihood of CBD injury. When combined, these factors have even greater impact. Surgeons are 2.2 times more likely to have a CBD injury

during their first 20 operations if they do not use a cholangiogram compared to procedures performed at later points in the experience curve. Defining the risk relationship associated with CBD injury is also important in informing patients and surgeons of the predicted probability of this adverse outcome (Figure 2). This may be a more effective way of “informing” the informed consent process.

This work was reinforced by a study of all Medicare beneficiaries undergoing cholecystectomy. In that study we found that patients who did not have a cholangiogram were approximately 70% more likely to have had a CBD injury. We also determined that this “protective” effect of cholangiography was noted whether or not the surgeon was a routine or infrequent cholangiographer. The lowest rates of injuries were found among routine cholangiographers (Figure 3).

What are the implications (using cost/decision analysis and randomized trials) of avoiding those factors?

Once we have quantified the problem and determined the avoidable factors that influence these outcomes we can try to imagine what the practice of clinical surgery would be like with these factors controlled. For example, a recently completed cost and decision analysis demonstrated that if routine cholangiograms were required, the cost per CBD injury avoided would range between \$50-86,000. The incremental cost per operation of adding the cholangiogram would be \$100. When considering the overwhelming costs (both system wide and medicolegal) of a CBD injury, this may be considered a cost effective intervention. Another example is a cost analysis showing that nationwide,

nearly \$740 million is spent each year on misdiagnosed appendicitis. Modeling potential ways to improve care is also being applied in a theoretical decision and cost analysis for routine CT scanning of patients with presumed appendicitis and teleproctoring in antireflux surgery.

These models are often helpful when the practical barriers of a randomized trial are significant. With colleagues in the general surgical division, however, we are hoping to develop and get funding for randomized trials in the management of appendicitis (routine versus selective CT scan use), for incisional hernia (laparoscopic versus open), and for the optimal management of patient with diverticulitis.

How can we make system level changes and monitor the impact of those changes?

The ultimate goal of this work is to improve surgical care for the average patient in the average hospital. The first steps are detailed above and involve getting good data, and performing effective analyses. The next step is system-level change either on the local, professional organization, or statewide level. Another opportunity for system-level change is found in working with the main financial stakeholders. For example, in coordination with administrators from the Healthcare Financing Administration (Medicare) we are helping to determine the mechanisms that could be used to increase the number of cholangiograms performed nationwide. Similarly, administrators at Group Health Cooperative are interested in optimizing the care of patients with presumed appendicitis and look to our analysis of their CT scan use as an opportunity to determine future care pathways.

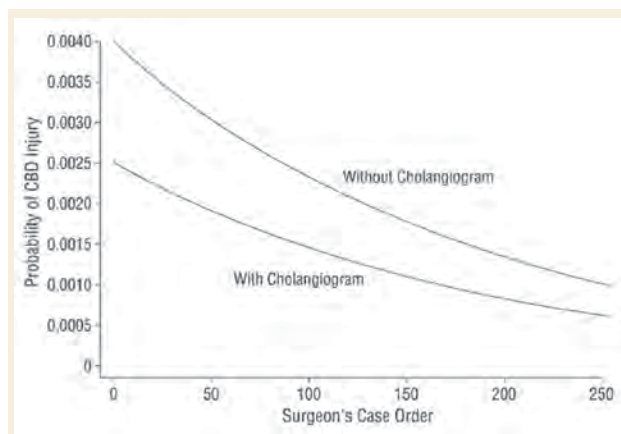


FIGURE 2: Probability of bile duct injury with and without cholangiogram, by case-order of surgeon (n=36,000)

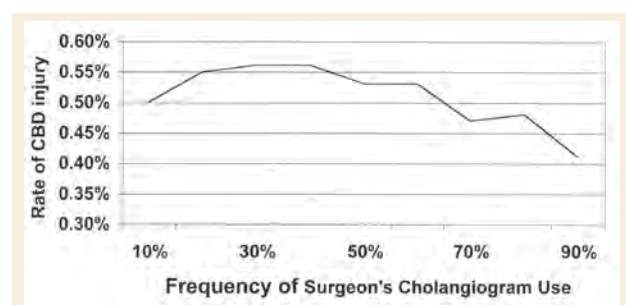


FIGURE 3: The effect of increasing the surgeon's frequency of cholangiogram use on the rate of common bile duct (CBD) injury

In collaboration with the Washington State Health Care Authority, the Center for Medicare Services, the Foundation for Healthcare Quality, Medicaid and Qualis, our group is developing a statewide system for helping hospitals identify adverse outcome outlier status and use the techniques of the QI community to address outliers. This Surgical Clinical Outcomes Assessment Project (SCOAP) is part of a 5-year project to create a surgical quality infrastructure in the state that will assure the incorporation of evidence-based approaches to surgical care in common practice. (<http://depts.washington.edu/sorce/>)

Involving the financial stakeholders may be the most effective way to improve system level care, but it may not be the best way. Over the last century, the surgical community has shown real leadership in addressing adverse outcomes and taking responsibility for them. The morbidity and mortality conference, for so long a part of the surgical culture, was ahead of its time in trying to improve the results of future interventions by avoiding past mistakes. Unfortunately, it has become apparent that conferences alone cannot deal with system-level factors involved in adverse outcome. Outcomes researchers are doing just that, and the surgical community has an opportunity to use this research in leading the way towards quality improvement.

RELATED PUBLICATIONS

1. Flum DR, Koepsell T, Heagerty P, Sinanan M, Dellinger EP: Common bile duct injury during laparoscopic cholecystectomy and the use of intraoperative cholangiography; Adverse outcome or preventable error? Arch Surg 2001; 136:1287-1292.
2. Flum DR, Morris A, Koepsell T, Dellinger EP: Has misdiagnosis of appendicitis decreased over time? A population-based analysis. JAMA 2001; 286:1748-1753.
3. Flum DR, Flowers C, Veenstra D. A cost analysis of cholangiograms in the prevention of common bile duct injury. J. American College of Surgeons 2003;196:385-396.
4. Flum DR, Cheadle A, Dellinger EP, Chan LC, Koepsell T. Intraoperative cholangiography and risk of bile duct injury during cholecystectomy. JAMA 2003; 289:1639-1644.
5. Flum DR, Cheadle A, Prela C, Dellinger EP, Chan LC. Bile duct injury during cholecystectomy and survival in Medicare beneficiaries. JAMA 2003;290:2168-2173.
6. Flum DR, Dellinger EP. The impact of gastric bypass operation on survival: a population-based analysis. J Am Coll Surg 2004 Oct;199(4):543-551.
7. Anaya D, Flum DR. Risk of emergency colectomy and colostomy in patients with diverticular disease. Arch Surg 2005 Jul;140(7):681-685.
8. Warshaw AL, Flum DR, Sarr MG, Pellegrini CA. Introduction: The rise of translational outcomes research. Surgery 2005 Nov;138(5):813-814.
9. Flum DR. The asymptomatic hernia: "if it's not broken, don't fix it". JAMA 2006 Jan 18;295(3):328-329.

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- Surgical Outcomes Research: Clinical Trials
- Surgical Education



AWARDS

National Institutes of Health

Society of Gastrointestinal Endoscopic Surgeons

• SAGES Young Researcher Award

Surgical Outcomes Research: Clinical Trials

While the field of outcomes research is relatively new, the research methods are an evolution of familiar clinical methodologies. These methods include: analysis of large databases; organized or structured reviews of the literature, known as meta-analysis; small-area analysis of healthcare utilization; prospective clinical studies emphasizing patient-oriented outcomes of care including quality of life analysis; development of decision-making analytical modes; cost-effectiveness studies; and practice guidelines.

Three important factors have stimulated the field of outcomes research: the need to contain the rapid rise in costs of healthcare, regional differences in utilization of healthcare, and increased awareness of clinical research deficiencies. The 14% of the gross domestic product that is spent on healthcare in the U.S. is significantly more than the 10% spent by the other developed nations. While this large expenditure has provided medical care of the highest quality to most Americans, an estimated 37 million Americans still do not have adequate access to medical care. It is a matter for concern that we spend such a large portion of our national resources on healthcare and still do not provide adequate care for all of our citizens. The basic costs of healthcare plus unacceptable inefficiencies in the system emphasize the need of health professionals to review current medical practices with a view to implementing the best and most cost-effective therapies.

The second major factor that stimulated the emergence of outcomes research came about as the result of work by Wennberg and Gittelsohn. Using large databases, they showed that the rates of utilization of almost all kinds of medical care are strikingly different in different

geographic areas. Moreover, the variations appeared to be almost exclusively the result of differences in *beliefs* among physicians about the best way to treat various conditions.

A significant factor that has contributed to the growth of surgical outcomes research is the evidence demonstrating that clinical research in the surgical literature has a number of deficiencies. A review of the surgical literature recently published in *Lancet* pointed out that much of the content of surgical journals is anecdotal. While 40% of surgical techniques are amenable to randomized controlled trials, only 3-6% have been subjected to this type of analysis. The deficiencies in clinical research include lack of prospective studies; the absence of comparisons of alternative treatments; inadequate, inconsistent definitions of terms and measures; the focus on the process of care rather than on measurements of function and quality-of-life; and incorrect statistical methods.

My primary interest is in surgical outcomes research utilizing clinical trials and quality of life assessments of surgical treatments. A current NIH-funded project involves a multi-institutional, phase II study of video-endoscopic assisted retroperitoneal debridement (VARD) for infected peripancreatic fluid collections following necrotizing pancreatitis. Open surgical necrosectomy for infected pancreatic fluid collections is highly effective, but is associated with significant morbidity primarily related to the large abdominal incision. Percutaneous catheter techniques are much less morbid, but require long treatment times and intensive drain manipulations to produce moderate success. Effectiveness is limited because the large amount of necrotic tissue debris cannot be easily drained via small diameter

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percutaneous catheters. All patients who fail percutaneous techniques crossover to open surgical necrosectomy as the definitive treatment.

Preliminary data suggest that VARD is a promising new method that combines the benefits of open surgical necrosectomy and percutaneous catheter drainage while avoiding problems associated with each. It is anticipated that this new, minimally invasive technique will be associated with decreased patient morbidity, length of hospital stay and associated health care costs compared to open necrosectomy. The *hypothesis* is: For in patients with infected pancreatic fluid collections following acute pancreatitis, VARD is a safe and efficacious procedure for draining infected pancreatic fluid collections, avoiding the need for crossover to open surgical necrosectomy.

This project is a multicenter, single-arm, Phase II clinical trial designed to obtain pilot data in preparation for a large, Phase III trial. Patients studied are limited to hemodynamically stable patients with documented infected pancreatic necrosis or pancreatic abscess as defined by the Atlanta Symposium. Patients are strictly classified based on the following criteria: CT classification, time from onset of pancreatitis to external drainage, and patient disease severity. Five major teaching hospitals are enrolling 40 consecutive patients over 18 months. Safety issues are being monitored by a Data Safety and Monitoring Board. All patients are being followed for six months from the onset of pancreatitis using standard methodology.

The primary aim is to assess the safety and efficacy of VARD of infected pancreatic fluid collections in preparation for a phase III trial. Outcome measures include: 1) The ability of the procedure to treat the patient without need for crossover to open surgical necrosectomy; 2) Mortality (in-hospital or 30-day mortality); 3) Number and type of intra-operative complications; 4) Number and type of secondary complications (in-hospital and 30-day).

The secondary aim is to assess the clinical and functional outcomes of patients treated with VARD in preparation for a phase III trial. Outcome measures include: 1) Length of ICU stay; 2) Length of hospital stay; 3) Total treatment time; 4) Pancreatic endocrine (hgbA1C, fasting blood sugar) and exocrine status (qualitative fecal fat stain) at 6 months; 5) Health-related quality of life scale (SF-36) at three and six months from onset of pancreatitis.

The results from this prospective pilot study will assess the safety and efficacy of VARD as a viable therapeutic modality. Patients eligible for the VARD procedure would be the same as patients eligible for an open surgical necrosectomy procedure. The long-term goal is to conduct a multi-center, Phase III, randomized, controlled study comparing VARD to the current standard of care: open surgical necrosectomy. In this latter study, short and long-term outcomes would be further analyzed, including disease-related outcomes, health-related quality of life, and cost-effectiveness.

Surgical Education

The traditional methods of teaching surgical residents have not changed much over the years, despite the many changes in a surgical residency. The days are past when a surgical residency meant that a resident actually lived at the hospital. The more leisurely days are also past when a patient was admitted two days before an inguinal hernia repair or hemorrhoidectomy and then remained in the hospital for a week of recovery. The total number of patients in a service may not have increased, but each bed is more likely to be occupied by a critically ill patient, so the daily pace of residents is faster and more intense.

As an additional result of technological progress, residents now need to cope with CT, PET, MRI and nuclear scans, sophisticated lab tests and computerized lab reports, ECMO, reverse I:E ratio ventilation, gene therapy, etc. The "old" ways of training residents are increasingly inappropriate in this newer fast-paced world. My major interest in research on surgical education is twofold: to investigate

systematic, standardized sign-out systems to ensure better transfer of patient care, and to determine how to modernize the methods for training surgical residents.

One project focuses on better sign-out systems when patients are transferred from resident to resident. A UW surgery resident has developed a Computerized Resident Signout System (UW Cores), and we are interested in seeing if patient care is improved using this tool.

The medical establishment and society are less receptive to the concept that patients should serve as a source for residents to learn operating techniques. Moreover, training

junior residents to operate in the OR may not be time and cost-effective. A project currently underway is the continued development of a laboratory-based, basic operating skills module for residents, with this training to be then evaluated in a clinical environment. Learning surgical techniques in a lab module should give the residents enough confidence in their surgical skill for them to be able to broaden their focus in the operating room on the operative details rather than on their technique.

RELATED PUBLICATIONS

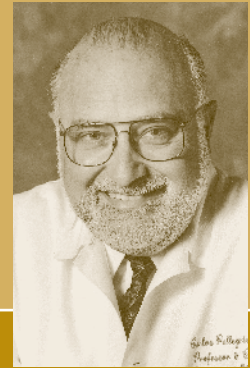
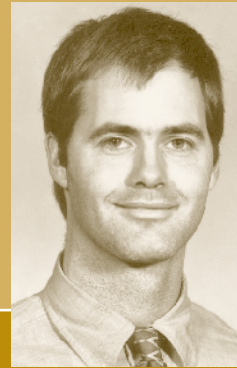
1. Horvath KD, Kao LS, Ali A, Wherry KL, Pellegrini CA, and Sinanan MN. Laparoscopic Assisted Percutaneous Drainage of Infected Pancreatic Necrosis. *Surg Endosc* 2001;15(7):677-82.
2. Horvath KD, Kao LS, Wherry KL, Pellegrini CA, and Sinanan MN. A Technique for Laparoscopic-Assisted Percutaneous Drainage of Infected Pancreatic Necrosis and Pancreatic Abscess. *Surg Endosc* 2001;15(10):1221-5.
3. Moskovitz AH, Bush WH and Horvath KD. Anaphylactoid reaction to intraoperative cholangiogram. Report of a case, review of literature and guidelines for prevention. *Surg Endosc* 2001;15(10):1227.
4. Flum D, Horvath KD, Koepsell T. Have outcomes of incisional hernia repair improved with time? A population based analysis. *Ann Surg* 2003;237(1):129-35.
5. Pavlin DJ, Horvath KD, Pavlin EG, Sima K. Preincisional treatment to prevent pain after ambulatory hernia surgery. *Anesth Analg* 2003;97:1627-32.
6. Kellogg T, Horvath KD. Minimal access approaches to complications of acute pancreatitis and benign neoplasms of the pancreas. *Surg Endosc* 2003;17:1692-1704.
7. Worrell SA, Horvath KD, Blakemore T, Flum D. Endorectal ultrasound detection of focal carcinoma within rectal adenomas. *Am J Surg* 187 (2004):625-629.
8. Van Eaton EG, Horvath KD, Lober B, Pellegrini CA. Organizing the transfer of patient care: The development of a computerized resident signout system. *Surgery* 2004;136:5-13.
9. Horvath KD, Coluccio G, Foy H, Pellegrini CA. A Program for Successful Integration of International Medical Graduates (IMGs) into US Surgical Residency Training. *Curr Surg* 2004;61(5):492-8.
10. Van Eaton EG, Horvath KD, Lober WB, Rossini AJ, Pellegrini CA. A Randomized, Controlled Trial Evaluating the Impact of a Computerized Rounding and Sign-out System on Continuity of Care and Resident Work Hours. *J Am Coll Surg* 2005;200(4):538-45.
11. Van Eaton EG, Horvath KD, Pellegrini CA. Professionalism and the Shift Mentality: How to Reconcile Patient Ownership with the 80-Hour Work Week. *Arch Surg* 140:230-236;Mar 2005.

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• The Swallowing Center at the University of Washington



FUNDING

Cook Surgical, Inc
Society of American Gastrointestinal and
Endoscopic Surgeons
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Dennis and Mary Wise Esophageal
Research Fund

Multichannel Intraluminal Esophageal Impedance

Multichannel Intraluminal Impedance (MII) is a new technology available for the detection of bolus presence within the esophageal lumen. This has potential applications for measuring esophageal motility (bolus moving from mouth to stomach) and reflux (bolus moving from the stomach retrograde up the esophagus). Based on ionic flow current, it has the capability of detecting the bolus presence characteristics (liquid, gas or mixed) as well. The catheter has multiple pairs of sensors distributed along the esophagus (figure 1); with continuous monitoring, the direction of propagation (oral or aboral) is determined. Approved by the F.D.A., it is used in combination with standard diagnostic tests (stationary manometry and 24hr pH study), giving additional information to make difficult clinical decisions.

Esophageal Motility

Traditional measurement of esophageal motility consisted of manometry only, which measured the contraction of the esophageal muscle while swallowing. The addition of impedance gives an objective measurement of whether the swallowed material (usually water) moves completely through the esophagus. With this test we also have the patient swallow a viscous material that theoretically “tests” the motility of the esophagus more than water. When we investigated patients with GERD before antireflux surgery, we found that in 278 water swallows, 5% had normal esophageal motility and incomplete bolus clearance, as well as 9% with abnormal manometry and complete bolus clearance from the esophagus. When challenging esophageal motility with viscous material, our results showed that in 252 swallows, 6% had normal manometry but incomplete bolus clearance and 5% had abnormal manometry and

complete bolus clearance. These results coincide with the ones obtained by other investigators, specifically those patients with incomplete bolus clearance and “normal” manometry tracings and vice versa. This phenomenon was unrecognized before this new technology.

Combined Multichannel Intraluminal Esophageal Impedance and Manometry does not Predict Post-operative Dysphagia after Laparoscopic Nissen Fundoplication

Laparoscopic Nissen fundoplication (LNF) is an effective treatment for GERD, although side effects such as dysphagia may occur. Manometry is used to evaluate peristaltic disorders, but alone is not effective in determining which patients may be at risk for postoperative dysphagia. MII is a relatively new technology that allows us to evaluate the transit and clearance of swallowed air, liquid and viscous material from and within the esophageal lumen. Combined MII and manometry is considered an advanced tool for esophageal function testing since it provides simultaneous evaluation of esophageal contraction (manometry) and bolus transit (MII); thus revealing the functional aspects of esophageal motility. Because of the relationship between dysphagia and esophageal clearance, we hypothesized that the addition of MII to manometry may detect those patients most at risk of developing postoperative dysphagia.

We prospectively evaluated 69 patients undergoing LNF. All patients completed a pre-operative symptom questionnaire, MII/manometry, and 24-hour pH monitoring. We defined post-operative dysphagia as occurring more than once a month with a severity ≥ 4 (0-10 scale).

TABLE 1: Manometric and impedance findings between patients with and without post-operative dysphagia

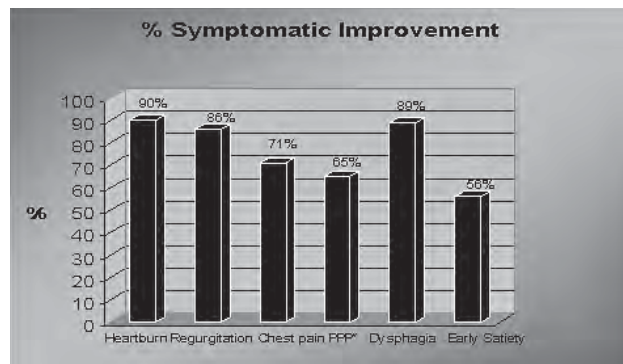
	Post-operative dysphagia (n=12)	No post-operative dysphagia (n=57)	p-value
LESP (mmHg)*	20.4 ±12.4	13.7 ±10.7	.354
% Peristalsis	80 ±32	88 ±18	.685
% LBT**	78 ±30	79 ±24	1
Liquid BTT† (secs)	6.4 ±1.4	7.0 ±1.4	.838
%VBT††	66 ±33	64 ±29	.526
Viscous BTT (secs)	7.4 ±2	7.8 ±2	.561

* Lower esophageal sphincter pressure

† Bolus transit time

** Liquid bolus transit

†† Viscous bolus transit



* PPP: postprandial pain

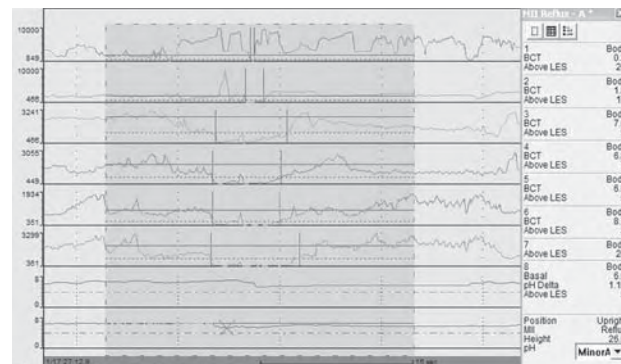
FIGURE 1: Impedance and manometry tracing showing complete bolus transit (MII) with a normal manometric tracing.

Thirty patients (43%) reported pre-operative dysphagia, but there was no significant difference in manometric (LES pressure and relaxation, peristalsis) and MII findings (esophageal transit and clearance) between patients with pre-op dysphagia (n=30, 43%) and those without (n=39, 57%). Among patients with pre-operative dysphagia only 9 (30%) had persistent post-operative dysphagia. After LNF, only 3 (8%) out of 39 patients without preoperative dysphagia developed new dysphagia. Patients with post-op dysphagia had similar manometric and MII findings to those who did not develop dysphagia. (Table 1)

We concluded that neither manometry nor MII appear to be effective in predicting dysphagia before and after Nissen fundoplication.

Evaluating Patients with GERD and Respiratory Symptoms

Patient history and standard diagnostic tools to detect reflux are not good predictors of pharyngeal reflux episodes. For this purpose, pharyngeal pH monitoring has been developed, but this study is still not a perfect one. Pharyngeal reflux episodes are usually brief, occur in the upright position and are accompanied by esophageal acidification. Our previous studies showed that pharyngeal acid reflux was present in

**FIGURE 2: Pharyngeal reflux episode detected by impedance.**

40% of patients with airway symptoms and abnormal reflux. While interesting, this result leads one to wonder if microaspiration in some patients might go undetected by pharyngeal pH testing. Pharyngeal reflux detection with MII is being carried out in our department. Our results in normal subjects are showing that the previously thought “normal value” (one pharyngeal episode) might not be the case if non-acid reflux is taken into account. In fact, when using this technology in normal, asymptomatic subjects, as many as 10 episodes of reflux reach the pharynx. Nearly all of these episodes are non-acid in nature and would be undetectable with traditional pH monitoring studies. (Figure 2)

Pathophysiology of Laryngopharyngeal Reflux (LPR)

We have hypothesized that the character (nonacid vs. acid) and proximal extension (esophagus and pharynx) of gastroesophageal reflux episodes is different in patients with reflux-induced laryngitis. We designed a prospective study using Multichannel Intraluminal Impedance to test this hypothesis. We studied 30 consecutive patients with suspected reflux-induced laryngitis and had a control group of 10 asymptomatic volunteers without GERD symptoms. Esophageal motility was also evaluated with manometry and impedance (esophageal clearance of a swallowed bolus).

Table 2 depicts the % of time pH was below 4 in the distal esophagus as well as the number and character of reflux episodes in the esophagus and pharynx. Table 3 shows the manometric and % of swallows that had a normal transit (EBT).

From this we concluded that patients with reflux related laryngitis have the same number of episodes of gastro-esophageal reflux as controls, but more are non-acid and more reach the pharynx. Impaired esophageal motility may facilitate upward extension of reflux episodes by delaying esophageal clearance.

The Role of Non-Acid Reflux in Respiratory Disease

Gastroesophageal reflux disease (GERD) has been implicated as a factor in the pathogenesis of respiratory diseases such as Asthma and Idiopathic Pulmonary Fibrosis (IPF). Studies have estimated that the prevalence of upper respiratory symptoms in GERD patients may be as high as 85%. Esophageal pH monitoring has traditionally been used to document the relationship between acid reflux and these diseases. However, in studies of patients with chronic cough and in children with persistent respiratory symptoms, their poor response to aggressive medical therapy suggests that a non-acid reflux (NAR) may contribute to respiratory symptoms. Currently, NAR and its impact on respiratory pathology have not been fully determined. As MII is currently being used to study both acid and non-acid reflux events, we are using this technique to investigate the extent to which non-acid reflux is associated with specific respiratory disease processes.

A retrospective review of all impedance pH studies done at the UWMC Swallowing Center is being conducted. Patients with diagnoses of asthma, IPF or COPD, as identified by a pre-testing questionnaire or review of medical records, are included in the study. The MII tracings of patients with respiratory symptoms will be examined to determine the degree to which they experience both acid and non-acid reflux. These results will then be compared to those of patients referred for typical symptoms of GERD such as heartburn, regurgitation, and dysphagia.

The total number of patients undergoing pH/MI studies from 2003 to October 2006 who have reported respiratory disease and/or respiratory symptoms (cough, shortness of breath) is 251. These include the following subgroups:

- Respiratory symptoms only=114
- IPF=73
- Asthma=9
- COPD=2
- Unspecified respiratory disease=53

The results of this study may lend further support to the theory that NAR is related to respiratory disease and symptoms. It has been proposed in the surgical literature that GERD patients whose respiratory symptoms do not respond to medical anti-acid therapy (PPIs and H2 receptor blockers) are not good candidates for surgical therapy.

TABLE 2

	Esophageal Reflux			Pharyngeal Reflux	
	% acid	Total reflux events*	Non-acid reflux*	Total reflux events*	Nonacid reflux*
Laryngitis	4.85	51	23	13	11
Control	2.18	50	10	5	4
p value	0.09	0.87	0.03	0.01	0.02

TABLE 3

	LES pressure	Peristalsis	Peristaltic amplitude	EBT liquid %*	EBT viscous %*
Laryngitis	16.2 mmHg	89 %	87 mmHg	74	58
Control	20.1 mmHg	100 %	71 mmHg	100	94
p value	0.30	0.15	0.36	0.02	< 0.001

* Multichannel Intraluminal Impedance

TABLE 4

	Dysphagia Severity	LESP	# Interventions	Heartburn Frequency	% pH <4
SM	4.6 ± 2.3	17 ± 8.6	18	1.5 ± 1.6	4.9 ± 7.5
EM	3.1 ± 2.6*	10.9 ± 5.7 †	4 †	1.2 ± 0.9	7.2 ± 6.3

* = $p < 0.005$ † = $p < 0.05$

TABLE 4A

Group	Dysphagia Severity	% With Peristalsis	% Liquid Clearance	% Viscous Clearance
Primary Achalasia	6.5	8%	5%	2%
Botox/Dilation	5.3*	40%*	16%*	11%*
Heller Myotomy	2.0†	63%*	28%*	19%*

* $p < 0.05$ vs. Primary Achalasia† $p < 0.005$ vs. Primary Achalasia and Botox/Dilation

However, these recommendations have been based on the postulate that in these patients, atypical symptoms are unrelated to GERD. By means of this investigation, we hope to characterize the relationship between respiratory disease states and non-acid reflux. By demonstrating an association between non-acid reflux and respiratory symptoms, we can identify a subset of the patient population unresponsive to medical therapy that may still benefit from anti-reflux surgery. Furthermore, MII monitoring may be a useful tool in screening for possible surgical candidates.

Surgical Treatment of Achalasia

LONG-TERM RESULTS OF EXTENDED HELLER MYOTOMY

The standard operative procedure for achalasia is a Heller myotomy, in which the muscle of the distal esophagus is divided. In a standard myotomy (SM) this division of the muscle extends 1-2 cm onto the stomach. We proposed the use of an extended (≥ 3 cm) myotomy (EM) and in 2003 reported better relief of dysphagia than with SM at 16 months. We designed a retrospective study to look at whether these improved outcomes were still present with an extended follow-up. Patients with achalasia who had a laparoscopic Heller myotomy between 1994 and 2003 were identified from a prospective database that includes symptom questionnaires and esophageal physiology studies.

From September 1994 to August 1998 we performed a SM with Dor fundoplication ($n = 55$), and from September 1998 through 2003 we performed an EM with Toupet fundoplication ($n = 102$). In 2001 we performed a telephone survey of all available patients. This was repeated in 2005 for those with EM. The survey included scales of symptom frequency (0 = never, 1 = 1x/month, 2 = 1x/week,

TABLE 5: Symptoms evolution after LARS

	Disappeared	Improveds	No Change	Worse
Heartburn	67%	23%	8%	2%
Regurgitation	78%	15%*	5%	2%
Dysphagia	62%	15%	10%	13

23% of patients are currently taking proton pump inhibitors daily.

3 = 1x/day, 4 = >1x/day) and severity (0–10, where 0 = no symptoms, 10 = symptoms equivalent to before surgery), as well as need for post-operative intervention for dysphagia. We were able to contact 35 patients following SM (46 mo median F/U) and 67 patients following EM (46 mo median F/U). Patient demographics were similar between groups.

Post-op results are shown in the Table. Of the SM group 9 patients (26%) required a total of 14 endoscopic interventions and 4 re-operations while 4 EM patients (6%) required one endoscopic intervention each. Of the EM group, 31 were contacted in both 2001 (16 mo median F/U) and 2005 (64 mo median F/U). There was no significant change over time in dysphagia severity (2.5 ± 1.8 vs. 2.9 ± 2.3 , $p = 0.4$). This study shows that extended gastric myotomy provides excellent durable relief of dysphagia, and is superior to a standard myotomy for the treatment of achalasia.

Return of Esophageal Function after Treatment for Achalasia

Treatment for Achalasia is aimed at the lower esophageal sphincter (LES), although little is known about the effect, if any, of these treatments on esophageal body function (peristalsis and clearance). We sought to measure the effect of various treatments using combined manometry (peristalsis) with MII (esophageal clearance).

Fifty-six patients with achalasia had been studied using manometry and MII between January 2003 and January 2006. Each was grouped according to prior treatment: 38 were untreated (Primary Achalasia), 10 had undergone Botox injection or balloon dilation, and 16 a laparoscopic Heller Myotomy. The preoperative studies for 8 of the Myotomy patients were included in the Primary Achalasia group. Each patient completed a dysphagia severity questionnaire (scale 0-10); peristalsis was analyzed by manometry and esophageal clearance of liquid and viscous material by MII.

Mean dysphagia severity scores were significantly better in patients after Heller Myotomy than in either of the other groups. Peristaltic contractions were seen in significantly

greater numbers of both Heller Myotomy and Botox/Dilation patients compared to Primary Achalasia, and liquid and viscous clearance rates were significantly better as well (Table). Similar trends were observed in these parameters for the subset of patients who underwent manometry/MII both pre- and post-Heller Myotomy, but the sample size was too small for these to be significant. In the overall Myotomy group, rates of peristalsis weakly correlated with liquid clearance ($r=0.46$, $p=0.09$) and more strongly with viscous bolus clearance ($r=0.63$, $p<0.05$). There was no correlation between peristalsis and bolus clearance in the Botox/Dilation group.

We concluded that with treatment Achalasia patients exhibit some restoration in peristalsis as well as improved bolus clearance. After Heller Myotomy, the return of peristalsis correlates with esophageal clearance, which may partly explain its superior relief of dysphagia.

Dor vs. Toupet Fundoplication: Multi-Center Randomized Trial

The development of gastroesophageal reflux is essentially guaranteed after a well done Heller myotomy. Our experience has shown that there is no way to maximally relieve the dysphagia of achalasia and at the same time prevent GERD. For this reason, most surgeons add a partial fundoplication to this procedure. The most common fundoplications are a Dor (anterior) and Toupet (posterior) fundoplication. The theoretical advantage of the Toupet is that it holds the edges of the myotomy open (possibly better relief of dysphagia) and is considered a better antireflux procedure, while the Dor fundoplication is placed over the exposed mucosa of the esophagus, thus buttressing a microperforation, should it occur.

A group of four major esophageal surgical centers have organized a multi-center randomized trial to answer whether one of these fundoplications is superior to the other in this situation. They are performed fairly equally around the world at this time and we hope to definitively determine whether there is a difference.

Laparoscopic Antireflux Surgery

LONG-TERM OUTCOMES OF LAPAROSCOPIC ANTIREFLUX SURGERY: GENERAL OUTCOMES AND PREDICTORS OF SUCCESS

Gastroesophageal reflux is a highly prevalent disease, affecting between 10 to 40% of US adult population. Laparoscopic antireflux surgery (LARS) has well-documented short-term outcomes, but long-term efficacy has not yet been established. For that reason, we reviewed the information of all the patients who had LARS at the UWMC between 1993 and 1999. We successfully contacted 288 patients (65%). The

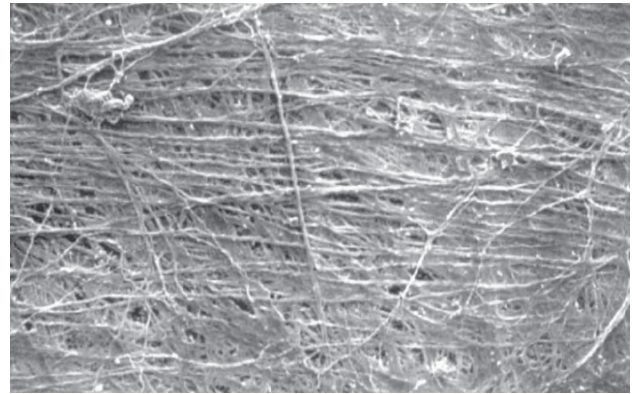


FIGURE 3: Small intestinal submucosa (SIS).

median follow up time was 72 months. (Range 48–111 mo.) No patients had a follow up of less than four years.

Of the 288 patients, 51 (18%) had preoperative diagnosis of Barrett's esophagus. 11 patients (22%) had complete regression of Barrett's after surgery. 2 patients developed high grade dysplasia after LARS. Of the 237 patients that had no Barrett's preop, only one patient developed Barrett's after LARS (0.02% per patient year)

12 patients had a redo surgery, two for acute complications, 8 for recurrent GERD, and two developed HGD after LARS and had an esophagectomy. One patient died as a result of postoperative complications.

In conclusion, this study shows that LARS is a safe operation and has few long-term side effects; LARS is an excellent durable treatment of GERD; the development of Barrett's esophagus after LARS is rare, and LARS may facilitate regression of Barrett's esophagus.

Paraesophageal Hernia

REPAIR OF PARAESOPHAGEAL HERNIAS WITH SMALL INTESTINAL SUBMUCOSA (SIS)

Laparoscopic paraesophageal hernia repair (LPEHR) is associated with a high recurrence rate. Repair with synthetic mesh lowers recurrence, but can cause dysphagia and visceral erosions. This trial was designed to study the value of a biologic prosthesis, small intestinal submucosa (SIS) (Figure 3) in LPEHR.

Patients undergoing LPEHR ($n=108$) at 4 institutions were randomized to primary repair (1°) ($n=57$) or primary repair buttressed with SIS ($n=51$) using a standardized technique. The primary outcome measure was evidence of recurrent hernia (≥ 2 cm) on UGI, read by a study radiologist blinded to the randomization status, 6 months after operation.

At 6 months, 100 (94%) completed clinical symptomatic follow-up and 94 (90%) had an UGI.

TABLE 6

	Heartburn Pre-op	Heartburn Post-op	PP pain pre-op	PP pain post-op	Dysphagia Post-op	A-P Hernia Diameter	Recurrent Hernia (≥ 2 cm)
1 ^o	5.1	0.4*	4.4	1.1*	0.7	5.8 cm	25%
SIS	5.2	1.0*	4.9	1.5*	1.5	6.4 cm†	7%†

* Pre-op vs. Post-op, $p < 0.01$

† 1^o Repair vs. SIS $p < 0.05$

Symptoms score: visual analog scale (0-10)

TABLE 7: Change in Symptom Severity after LPEHR

Symptom	Pre-op SSI*	Post-op SSI*	p-value
Heartburn (n=38)	7.3+/-2.3	0.8+/-2.1	<.0001
Regurgitation (n=29)	6.8+/-1.8	1.1+/-2.2	<.0001
Chest pain (n=24)	7.3+/-2	2+/-2.9	<.0001
Postprandial pain (n=20)	6.4+/-2	2.1+/-2.8	<.0001
Dysphagia (n=18)	6 +/-2.3	0.6+/-1.7	<.0001
Early satiety (n=18)	5.7+/-2.3	2.4+/-2.7	.002

*Symptoms score Index: visual analog scale (0-10)

PERI-OPERATIVE OUTCOMES: The SIS group had larger anterior-posterior hernia diameter, but similar esophageal length. Operative times (SIS 202 min vs. 1^o 183 min, $p=0.15$) and peri-operative complications did not differ. There were no operations for recurrent hernia nor mesh related complications.

PRIMARY OUTCOME: At 6 months, 3 patients (7%) developed a recurrent hernia > 2 cm in the SIS and 12 patients (25%) in the 1^o group.

SECONDARY OUTCOMES: Both groups experienced a significant reduction in all measured symptoms (heartburn, dysphagia, regurgitation, chest and post-prandial (PP) pain) after operation. There was no difference between groups in either pre or post-operative symptom severity.

This trial has demonstrated that adding a biologic prosthesis during LPEHR reduces the likelihood of recurrence, without mesh related complications or side effects.

Symptom Relief after Laparoscopic Repair of Paraesophageal Hernias (PEH): Insights from a Prospective, Randomized Trial

Traditionally, the presence of a PEH was an indication for surgery because of the high risk of life-threatening complications. However, in the last years several studies have shown that the real incidence of acute complications is low and only symptomatic PEHs have to be repaired. Therefore, the symptom response to surgical repair takes

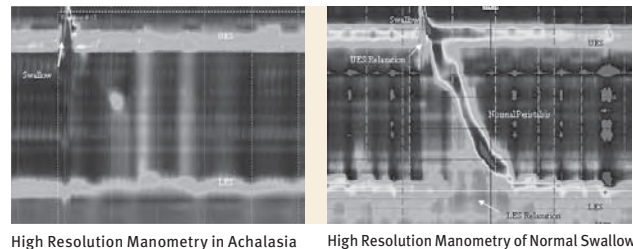


FIGURE 4: The incidence of symptom improvement after LPEHR

*PPP: postprandial pain

on greater importance today. Taking advantage of the data from a recently completed prospective and randomized trial, we studied the response to laparoscopic PEH repair for each presenting symptom as well as the relationship of symptom resolution to successful anatomic correction of the hernia.

Overall, the symptom severity index (SSI) improved significantly after LPEHR. (Table 7) The number of patients who improved varied depending on their presentation, being GERD-related symptoms the most likely to improve. (Figure 4)

Similarly, the frequency of improvement for most presenting symptoms among patients without recurrence was higher compared to the overall population. Nevertheless, the trend toward a variable response among symptoms remained the same.

With this study we have concluded that in patients with PEH, primary symptoms improve significantly after laparoscopic repair, however there are some symptoms that are more likely to improve than others. This information is helpful in counseling patients and setting expectations of symptom improvement after LPEHR.

In studies of patients with chronic cough and in children with persistent respiratory symptoms, their poor response to aggressive medical therapy suggests that a non-acid reflux may contribute to respiratory symptoms.

Esophageal Cancer

LONG-TERM OUTCOME AFTER ESOPHAGECTOMY FOR HIGH-GRADE DYSPLASIA OR CANCER FOUND DURING SURVEILLANCE FOR BARRETT'S ESOPHAGUS

Endoscopic surveillance of Barrett's esophagus is recommended to detect dysplastic or malignant changes at an early stage. We analyzed the outcomes of 39 consecutive patients who underwent esophagectomy after progression was detected while on a Barrett's surveillance program. We were able to contact 37 of 39 (95%) patients, and two patients refused to participate in this study. The mean follow-up time was 44 months (range 13-89 months).

We performed this study to identify the impact and factors affecting quality of life in patients with esophagectomy, and to determine our incidence of recurrence or progression of esophageal cancer.

No mortality was related to operation. 18 months after surgery 39/39 of patients were alive. One patient eventually died of esophageal cancer progression.

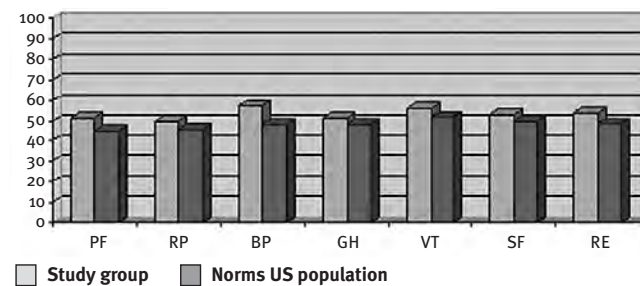
Using a standardized survey, patients were asked questions about their quality of life in seven areas: physical functioning (PF), role-physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), and role-emotional (RE). The results show that our patients have an above average quality of life with respect to national averages (Table 8)

In conclusion: this study revealed that esophagectomy is curative in the great majority and can be accomplished with minimal mortality and excellent quality of life

OUTCOMES OF LAPAROSCOPIC ASSISTED ESOPHAGECTOMY FOR ADENOCARCINOMA OF THE ESOPHAGUS

The incidence of esophageal adenocarcinoma has increased more rapidly than any other gastrointestinal malignancy in the last decade. The prognosis of patients with esophageal cancer remains poor. Only 56% of the patients who present with esophageal cancer have resectable disease, with an overall 5-year survival rate of 10%. Esophageal resection

TABLE 8:



remains the gold standard, not only in providing the optimal chance for cure, but also the best palliation for dysphagia. However, the conventional open operations are quite invasive, with a morbidity of 50% and a mortality of 5-10% in high-volume centers. Laparoscopic procedures offer an advantageous alternative to conventional open operations, such as less operative trauma than experienced with thoracotomy or manual blind and blunt transhiatal esophagectomy; less perioperative blood loss; shorter ICU stay. Furthermore, a minimally invasive procedure does appear to offer the potential for a more radical mediastinal resection, under direct vision, when compared with transhiatal esophagectomy. However, controversy still exists about what is the best approach to and extent of the dissection. At the University of Washington, we started performing laparoscopic-assisted esophagectomy in 1995 for tumors of the distal esophagus and gastroesophageal junction. We are conducting this study to determine the short-term (complications, length of stay, pathologic staging, lymph node harvest, blood loss, etc) and long-term (cancer free survival and overall survival, quality of life) outcomes with this approach.

High-Resolution Esophageal Manometry (HRM)

HRM is a recently-developed tool in the evaluation of esophageal motility which utilizes many closely-spaced pressure recording sites along a manometry catheter in order to display a relatively continuous profile of esophageal motor activity from the upper esophageal sphincter, along the length of the esophageal body, and across the lower esophageal sphincter. A recording device produces color-contour plot, with time on x-axis, esophageal length on y-axis, and pressure represented by a color scale. Data between recording sites is interpolated to demonstrate pattern and pressure gradients. The result is a more complete and detailed picture of esophageal motility, with potentially better and more accurate characterization of esophageal function than standard manometry.

The Swallowing Center of the University of Washington has contracted to purchase a cutting-edge system that combines HRM with MII, which has just become available, in the spring of 2007. Preliminary proposals for research investigation using this new equipment include:

EVALUATION OF DYSPHAGIA USING HRM-IMPEDANCE

Because HRM illustrates a continuous motility pattern along the entire esophagus and provides a detailed display of pressure across the LES and proximal stomach, it may reveal abnormalities at specific loci of bolus retention when combined with MII, and may be able to elucidate previously uncharacterized causes of dysphagia. All patients presenting with dysphagia as the primary complaint would be eligible. After completion of a detailed dysphagia questionnaire, combined HRM/MII would be performed and compared to results from control groups of patients with heartburn and healthy volunteers. We would then identify and localize any areas of bolus retention on MII and correlate with peristaltic activity in both dysphagia subjects and control groups.

FACTORS RESPONSIBLE FOR FUNDOPLICATION FAILURE

Because HRM is able to illustrate separation of high-pressure zones (HPZ) much more clearly than standard manometry, it is potentially a highly useful tool in determining the etiology of recurrent reflux after fundoplication.

All post-fundoplication patients would be evaluated using HRM+MII, pH monitoring, EGD, and UGI, and would complete a detailed symptom questionnaire. Subsets of patients having recurrent reflux symptoms, dysphagia, and gas bloat would be defined. HRM/MII results between asymptomatic patients and patients with recurrent symptoms would be compared, examining the length of

the HPZ, the HPZ “pressure profile,” and the presence of a single vs. a dual HPZ, possibly indicating recurrence of a hiatus hernia, or a “slipped” fundoplication. Combined detailed motility and bolus clearance patterns in patients complaining of dysphagia would be defined, attempting to determine where in the esophagus clearance fails, and what the motility characteristics are in that segment. Furthermore, HRM findings would be compared to information obtained from EGD, pH monitoring, and UGI, to correlate HRM data with these more traditional investigations in patients with recurrent reflux after fundoplication. Finally, we hope to determine specific factors associated with a “successful” fundoplication, such as a certain minimum length of HPZ, postoperative motility patterns, or postoperative bolus clearance patterns.

Evaluation of the Gastric Motility After Natural Orifice Transluminal Endoscopic Surgery (Notes)

Natural Orifice Transluminal Endoscopic Surgery (NOTES) represents a new paradigm shift, which may significantly change the management of gastrointestinal and intraabdominal diseases. The idea is that we would operate within the abdominal cavity without incisions, with instruments placed through a natural orifice (e.g. mouth or anus). There are potential disadvantages with NOTES, which were issued by the Natural Orifice Surgery Consortium for Assessment and Research (NOSCAR). Some are technical, like limitations in instrumentation to operate via current flexible endoscopes. There is also the potential for gastric leak and/or intraabdominal contamination and infection. We and some other centers around the world are actively involved in trying to work out these problems. Few, however, are involved in the study of long-term effects of NOTES, such as the investigation of gastric motility and function after NOTES. The primary aim of this study is to test the hypothesis that transgastric endoscopic surgery does not affect gastric motility. Using EGG and radiography with swallow of barium-impregnated rings, we want to evaluate the affects of transgastric endoscopic surgery on the gastric motility of dogs from electrophysiological and mechanical points of view. We are also actively involved in the development of instrumentation and equipment to make this feasible. While we currently do not know what role NOTES will have within the field of surgery, it clearly has the potential to revolutionize it similar to how laparoscopy did 20 years ago.

RELATED PUBLICATIONS

1. Chang L, Oelschlager BK, Barreca M, Pellegrini. Improving accuracy in the identification of the gastroesophageal junction during laparoscopic antireflux surgery. *Surg Endosc.* 17(3), 390 - 393, 2003.
2. Oelschlager BK, Chang L, Pellegrini CA: Improved Outcome after Extended Gastric Myotomy for Achalasia. *Arch Surg.* 2003;138(5):490-497.
3. Oelschlager BK, Barreca M, Chang L, Oleynikov, Pellegrini CA. Clinical and pathologic response of Barrett's esophagus to laparoscopic antireflux surgery. *Ann Surg.* 2003, 238:458-466.
4. Oelschlager BK, Barreca M, Chang L, Pellegrini CA The Use of Small Intestinal Submucosa (SIS) in the Repair of Paraesophageal Hernias: Initial Observations of a New Technique. *Am J Surg.* 2003;186:4-8.
5. Oelschlager BK, Chang L, Barreca M, Pope CE II, Pellegrini CA. Typical GERD Symptoms and Esophageal pH Monitoring are not enough to diagnose Pharyngeal Reflux. *J Surg Res.* 2005;128:55-60.
6. Cuenca-Abente F*, Quiroga E, Isch JA, Oelschlager BK. Gastroesophageal and pharyngeal reflux detection using impedance and 24-hour pH monitoring in asymptomatic subjects: defining the normal environment. *J Gastrointest Surg.* 2006 Jan; 10(1):54-62.
7. Oelschlager BK, Lal DR, Jensen E, Cahill M, Quiroga E, Pellegrini CA. Medium- and long-term outcome of laparoscopic redo fundoplication. *Surg Endosc.* 2006; 20:1817-1823.
8. Kaufman JA, Houghland JE, Quiroga E, Cahill M, Pellegrini CA, Oelschlager BK. Long-term outcomes of laparoscopic antireflux surgery for GERD-related airway disease. *Surg Endosc.* 2006; 20(12):1824-1830.
9. Cuenca-Abente F, Parra JD, Oelschlager BK. Laparoscopic sleeve gastrectomy: an alternative for recurrent paraesophageal hernias in obese patients. *JLS.* 2006 Jan-Mar; 10(1):86-89.
10. Chang LC, Oelschlager BK, Quiroga E, Parra JD, Mulligan M, Wood DE, Pellegrini CA. Long-term outcome of esophagectomy for high-grade dysplasia or cancer found during surveillance for Barrett's Esophagus. *J Gastrointest Surg.* 2006; 10: 341-346.
11. Quiroga E, Cuenca-Abente F, Flum D, Dellinger EP, Oelschlager BK. Impaired esophageal function in morbidly obese patients with gastroesophageal reflux disease: evaluation with multichannel intraluminal impedance. *Surg Endosc.* 2006 20:739-743.
12. Oelschlager BK, Pellegrini CA, Hunter J, Soper N, Brunt M, Sheppard B, Jobe B, Polissar N, Mitsumori L, Nelson J, Swanson L. Biologic prosthesis reduces recurrence after laparoscopic paraesophageal hernia repair: a multi-center, prospective, randomized trial. *Ann Surg* 2006;244:481-90
13. Wright AS, Williams CW, Pellegrini CA, Oelschlager BK. Long-term outcomes confirm the superior efficacy of extended Heller myotomy with Toupet fundoplication for achalasia. *Surg Endosc.* 2007 Mar 1.

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- Objective Assessment of Surgical Skills
- Operating Room of the Future



FUNDING

Defense Advanced Research Projects Agency (DARPA)
U.S. Army Medical Research & Materiel Command
• Telemedicine & Advanced Technology Research Center

Objective Assessment of Surgical Skills

There is a totally new paradigm in surgical education and training based upon surgical simulation. A national consortium of surgical training centers will define new metrics and outcome performance measures, establish criterion-levels of performance, validate efficacy of simulators as educational tools and then train residents to criterion and evaluate the performance in the operating room.

The conceptual change is to train residents (in the future) not for a given time, but rather to a given criterion level, a level which reduces errors to the absolute minimum and provides maximum quality, especially for patient safety. The above will be implemented by using the Minimally Invasive Surgery Trainer – Virtual Reality (MIST-VR) and the Xitact Laparoscopic Cholecystectomy simulator, in addition to other systems such as the “Blue Dragon” that are described elsewhere.



FIGURE 1: MIST-VR basic surgical skills simulator illustrating the image on the simulator screen, and the input handles for tracking motion

This new educational system will initially be implemented and validated at UWMC, then expanded to the WWAMI region, and finally to a national level.

Operating Room of the Future

Recent introduction of robotic systems into clinical surgery indicates a fundamental new direction for surgeons. Research will be conducted to integrate robotics into an entirely new concept for the operating room – one which decreases the number of personnel required, increases efficiency and quality control, and which incorporates the robotic system into the hospital information system. In addition the robotic systems will be used to train, objectively assess and certify competence of surgeons.



FIGURE 2: Xitact Laparoscopic Cholecystectomy simulator illustrating the portable system and video image.

The conceptual change is to train residents (in the future) not for a given time, but rather to a given criterion level, a level which reduces errors to the absolute minimum and provides maximum quality, especially for patient safety.



FIGURE 3: Zeus surgical robotic system

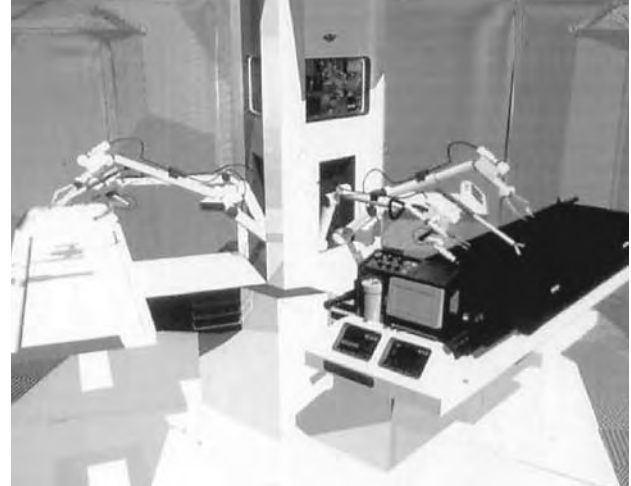


FIGURE 4: OR of the future – concept drawing from Integrated Medical Systems

RELATED PUBLICATIONS

1. Satava, RM, and Jones, SB, Preparing Surgeons for the 21st Century, Implications of Advanced Technologies, In: Surgical Clinics of North America, Minimal Access Surgery, Part 1, vol. 80, no 4, pp. 1353 – 1365, August 2000.
2. Pearson, AM, Gallagher, AG, Rosser, JC, and Satava, RM, Evaluation of Structured and Quantitative Training Methods for Teaching Intracorporeal Knot Tying. In: Surgical Endoscopy, Vol 16, pp. 130-137, November 2001.
3. Satava, RM, Accomplishment and challenges of surgical simulation, Dawnng of the next-generation surgical education (Review article). In: Surgical Endoscopy, Vol 15, No. 3, pp.232-241, March 2001.
4. Satava, RM, Bowersox, JC, Mack, and M, Krummel, TM, Symposium: Robotic Surgery State of the Art and future trends. In: Contemporary Surgery, Vol. 57, no. 10, October 2001.
5. Satava, RM, Surgical Robotics: The Early Chronicles A Personal Historical Perspective. In: Surg Laparoscopy Endoscopy & Percutaneous Techniques, Volume 12:1, pp. 6-16, February 2002.
6. Satava, R, Cuschieri A, Jakimowicz J, Deane S, Hamdorf J, Darzi A, Hanna G, Gallagher A, Buess G, Fried G, Regher R, Krummel T, Hasson H, Narwold D, Dunn M., Metrics for Objective Assessment: Preliminary Summary of the Surgical Skills Workshop, In: Surg Endos, 2003 Feb;17(2):220-6.
7. Satava RM and Fried MP. A Methodology for Objective Assessment of Errors: An Example Using an Endoscopic Sinus Surgery Simulator (ES₃). Otolaryngol Clin North Am. 2002 Dec;35(6):1289-301.
8. Seymour NE, Gallagher AG, Roman SA, O'Brien MK, Bansal VI, Andersen DK and Satava RM. Virtual Reality Training Improves Operating Room Performance: Results of a Randomized, Double-blinded Study. Ann Surg. 2002 Oct;236(4):458-63.

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- Biorobotics Laboratory Collaboration
- ISIS (Institute for Surgical and Interventional Simulation)



Building on prior work, the ongoing collaboration between the Department of Surgery and the Biorobotics Laboratory based in Electrical Engineering continued this year at an active pace. Andrew Wright joined the team and immediately initiated an active investigation program. ISIS, the subject of last year's Research Report, also initiated several major collaborative research studies in simulation-based training.

Collaborative research with the Biorobotics Laboratory focused on three areas: A major effort on further development and refinement of the UW Surgical Robot, now named RAVEN (Figure 1), with several on-campus demonstrations of the surgical telerobotic system; a major demonstration for the sponsor of the U.S. Army's Medical Research and Materiel Command / Telemedicine and Advanced Technologies Research Center (MRMC / TATRC) in Simi Valley, CA as a simulation of an actual field deployment of the robot in a military extreme environment (Figure 2); and most recently, a clinical demonstration in the Center for Videoendoscopic Surgery (CVES) laboratory using an animate porcine model for a variety of surgical maneuvers (Figure 3,4).

The second area of focus was a correlation of the performance metrics of the Red Dragon system with the objective scoring system built into the FLS (Fundamentals of Laparoscopic Surgery) training and assessment system ratified by the Society of American Gastrointestinal Endoscopic Surgeons (SAGES) and the American College of Surgeons (ACS). The Red Dragon is a second generation passive tracking device developed in the Biorobotics Lab based on a similar design of the RAVEN surgical robot (Figure 5). It uses a spherical mechanism with position sensors and force sensors to track forces and torques of two laparoscopic instruments during laparoscopic tasks.

These data are captured with video and synchronized at every 1/30 of a second. Pattern analysis of the output from a surgeon of unknown skill can be statistically mapped onto the performance of novices and experts at the same task to derive a score that represents the degree of expertise in the performance by the surgeon, a score that incorporates efficiency, grace, and the specificity of the surgeon in working to complete the task. More importantly, this is an objective score derived automatically without any expert observation or subjective scoring, removing a major hurdle to most scoring systems that are quite subjective in character and costly in terms of physician time. This work builds on the prior work in the Blue Dragon system (Figure 6), where performance of laparoscopic tasks was correlated with skill level using a hidden Markov statistical analysis of the forces and torques and tool velocities captured by the device.

The FLS system, which includes five tasks that have been independently validated to correlate with minimally invasive surgical skill, will be used to compare a nationally accepted objective scoring system of skill with the Markov analysis developed in the Biorobotics Lab, to further refine the discrimination of the Red Dragon and permit direct comparison between Red Dragon scores and other scoring systems. The FLS includes peg transfers, precision cutting, placement and securing of a ligating loop, simple suturing with an intracorporeal knot, and simple suturing with an extracorporeal knot (Figure 7). This work is ongoing and will later incorporate measures of performance in standardized FLS tasks between RAVEN and the Da Vinci clinical robot in the UWMC OR.



FIGURE 2: Biorobotics Team in Simi Valley, CA, testing a teleoperated control algorithm using a military theater wide area network for remote control of RAVEN.

The third area of focus for the biorobotics lab is the Ph.D. work by Smita De in collaboration with all members of the group dealing with damage at the tool-tissue interface in minimally invasive surgery (MIS). The premise of this work has been that in moving to a MIS environment, surgeons have relinquished not only three dimensional depth perception but also an appreciable component of the tactile feedback from handling tissues. This lack of haptic feedback with standard MIS instruments introduces the potential for inadvertent tissue injury during standard procedures, tissue injury that translates into injured bowel, a perforated gastric wall, or a torn, leaking gallbladder in clinical situations.

In the studies we have carried out, standard MIS tools mounted on a computer controlled gantry (the Motorized Endoscopic Grasper or MEG) were used to apply graded

stresses from 0 to 300 kPa and durations of 10-60 seconds to tissues (liver, small bowel, ureter, and bile duct) in an anesthetized porcine model. The tissues were then photographed and harvested two hours later for standard histology and immunocytochemical analysis, evaluating for evidence of direct tissue damage, ischemia, and apoptosis. Further experimental data and a novel finite element modeling and statistical analysis have correlated expected and demonstrated tissue stresses to the degree of damage observed. This work, to be presented at regional and national surgical meetings this year, provides surprising information on the range and severity of stress that tissue is exposed to during normal instrument handling (Figure 8), stresses that certainly influence the risk of perforation, local tissue inflammation, and postoperative healing.

Summary of ISIS Research Work

Research work in ISIS has been concentrated in the R and D committee with Aaron Jensen's work on the role of the surgical mentor in acquisition of technical surgical skills, Tom Lendvay's work on development and validation of a suprapubic catheter training system, and two other specific research projects.

In collaboration with a commercial partner, Red Llama, we have embarked on developing a series of cognitive skills platforms specifically aimed at the cognitive component of specific surgical procedures. Red Llama has contributed a development platform, SimPraxis™, and programming expertise. ISIS has developed a cognitive map (Figure 9) for the first procedure to be addressed, laparoscopic cholecystectomy, that details the steps in patient selection,

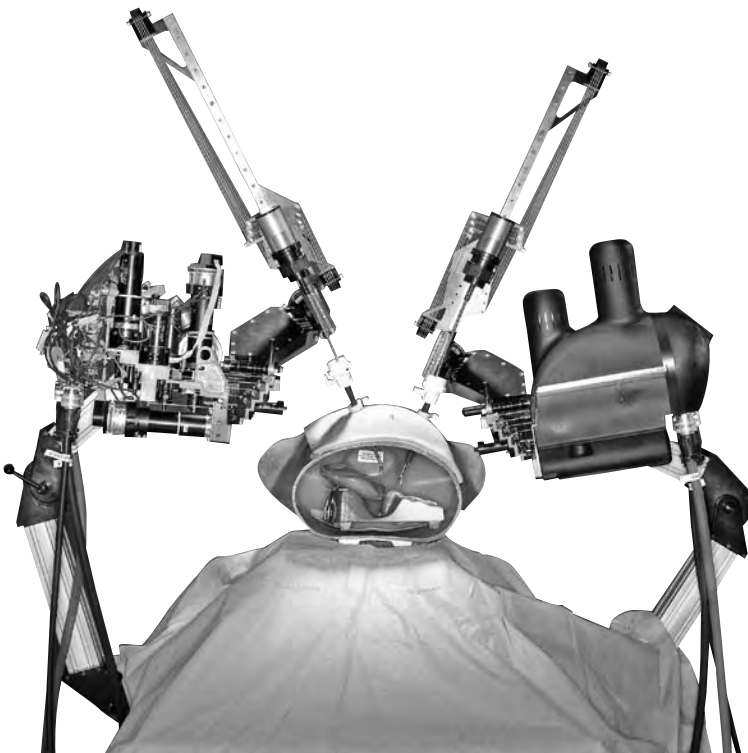


FIGURE 1: RAVEN – the UW Surgical Robot (RAVEN), based on a spherical mechanism. Two robotic arms are mounted each on a “C-arm” that will carry the robotic arms longitudinally along the table. The arms currently operate independently.



FIGURE 3: RAVEN in the CVES lab.



FIGURE 4: Dr. Andrew Wright performing intracorporeal suturing with RAVEN in an animate porcine surgical model.

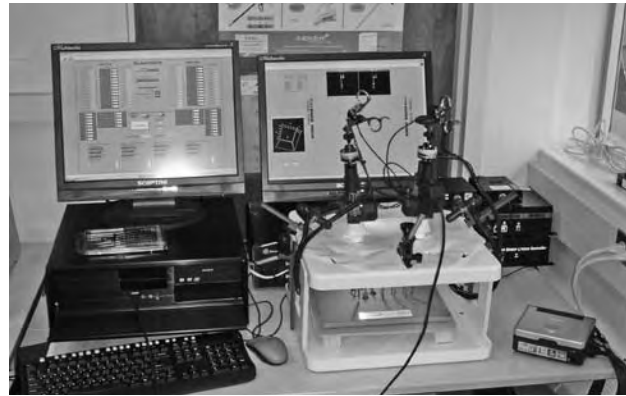


FIGURE 5: Red Dragon is a passive spherical mechanism for precise tracking of forces and torques generated through the surgical instruments along with the tool position during laparoscopic surgery



FIGURE 6: Blue Dragon, the predecessor to Red Dragon, using a four bar mechanism for capturing surgical forces and torques. Although effective, the Red Dragon design substantially decreased the footprint of the device in a MIS setup .

preparation, instruments, positioning and setup, and then detailed specific steps to the procedure. Errors at each step are included. The cognitive map is being ported into the SimPraxis engine with video commentary, clinical footage, and text prompts. This interactive platform invites the trainee to select instruments and then to choose the location of the next step in dissection or surgical treatment, mimicking the real choices that must be made in the OR (Figure 10). If the trainee chooses correctly, video segments of the procedure are shown that move to the next step. If the trainee chooses incorrectly, the video mentor provides further expert commentary. Metrics captured from these choices during training and the influence on this type of training on the trainee's comfort and perceived expertise in moving through the clinical procedure (separate from his or her skill in actually making the instruments do what he or she wants to do) are important next steps in refining this

model of simulation training, and validating the specific modules. If possible, we hope to extend these cognitive training modules to other ACS accredited simulation training centers.

The second research project being actively pursued by the ISIS R and D group is development of a UW Medicine-wide training system for placement of central venous catheters (CVCs). Placement of these catheters in the internal jugular and subclavian venous systems are one of the specific skills that intensivists and general surgeons must acquire and practice during their training. However, it is also a procedure with substantial morbidity and risk associated with it. Well defined technical innovations to the procedure: appropriate patient selection, preparation, and identification, use of sterile precautions, appropriate vital sign monitoring, ultrasound guidance for catheter

Placement of central venous catheters in the internal jugular and subclavian venous systems are one of the specific skills that must be acquired and practiced during training. We now have the capability to train, in simulation, a generation of surgeons and intensivists with a standard protocol and certify competency in the procedure.

placement, and appropriate sterile management and prompt removal have been shown to largely ameliorate these risks but have not been systematically included in resident training programs.

With the development of ultrasound capable simulators for CVC placement through our commercial partner, Simulab, we now have the capability to train, in simulation,

a generation of surgeons and intensivists with a standard protocol and certify, in simulation, competency in the procedure. Given the demonstrated risks of the procedure, such training and certification is of tremendous interest to our hospital administrations at UWMC and HMC as it should improve patient safety and reduce the cost of infectious, thrombotic, and acute surgical complications from CVC placement. At the present time, we are developing a Flash computer-based, interactive training program that will be delivered via the web. After successful completion of this program, including a number of scenarios that require complication avoidance, detection, and management, the trainee will graduate to training on the physical simulator, Central Line Man™ (Figure 11). Once the trainee can complete the entire procedure successfully, they will be certified in CVC procedures by ISIS.

All elective CVC procedures will be performed with the assistance of dedicated nursing staff and all catheters tracked with a unique numerical code. Research elements will derive from validating the training and then tracking acute and long term complications of CVC placement to demonstrate that training in simulation has real potential to improve performance and patient safety around technical procedures.



FIGURE 7: FLS desktop system for training and assessment.

ANIMAL EXPERIMENTS



FIGURE 8: Porcine liver, ureter, and small bowel after test grasping with the Motorized Endoscopic Grasper (MEG) for evaluation of the effect of graded stress on tissue integrity and healing.

Cognitive Procedural Map

LAP CHOLE WITH CHOLANGIOGRAM

DISSECTION OF TRIANGLE OF CALOT

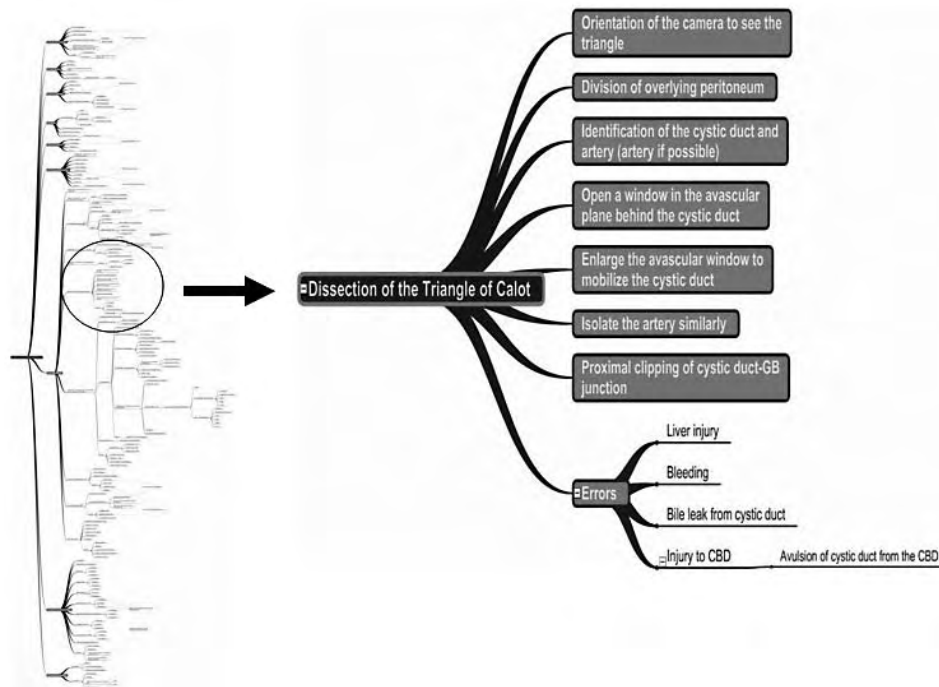


FIGURE 9: A graphical representation of the sequential thought processes that comprise a standard operative procedure, laparoscopic cholecystectomy. This representation is termed, for operational purposes, a “cognitive map,” as it illustrates the steps, variations, branchpoints, potential errors, and recovery from error for each step of a procedure.



FIGURE 10: The SimPraxis™ training platform for cognitive, interactive, computer based training in surgical skills. This panel shows one step in the laparoscopic cholecystectomy procedure, dissection of the Triangle of Calot.



FIGURE 11: The Central Line Man™ from Simulab, which will form the physical platform for simulation-based training in CVC placement.

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Raymond S. Yeung, M.D.

- The role of the TSC1/2 complex in tumor development



AWARDS

- American Association for the Surgery of Trauma
- Peter C. Canizaro Award
- Junior Faculty Research Award
- Surgical Infection Society Foundation
- Faculty Research Award

FUNDING

- National Institutes of Health

Over the last several decades, the study of hereditary tumor syndromes has laid a solid foundation for the genetic basis of cancer. While the number of patients suffering from these syndromes is small, the identification and elucidation of the underlying genetic pathways have shown to be of broad relevance to many forms of sporadic human cancers.

Investigations have found that the majority of hereditary tumors involve mutations of certain tumor suppressor genes. This latter class of genes has diverse functions including cell cycle regulation, DNA repair, apoptosis, protein degradation, cell-cell interaction, and signal transduction. However, a common feature of these genes is the “two-hit” genetic mechanism to inactivate their function during tumorigenesis. In the case of hereditary cancers, the first hit is inherited as a germline mutation of one of the alleles of the tumor suppressor gene, and the second hit is an acquired somatic mutation of the remaining allele of the same gene. This results in the loss of function of the tumor suppressor, thus creating a setting to promote tumor development.

One of the latest examples comes from the study of the tuberous sclerosis complex (TSC), an autosomal dominant disorder affecting more than 50,000 Americans. As a member of the phakomatoses, TSC is characterized by the appearance of benign tumors involving many organ systems, most notably the central nervous system, kidney, heart, lung, and skin. While classically described as ‘hamartomas,’ the pathology of the lesions is diverse, with features of abnormal cellular proliferation, growth (size), differentiation and migration.

Occasionally, TSC tumors progress to become malignant lesions (i.e., renal cell carcinoma). The genetic basis of this disease has been attributed to mutations in one of two unlinked genes, *TSC1* and *TSC2*. The protein products

of these genes are found to negatively regulate the mTOR pathway, which controls protein synthesis, among other functions. Many human cancers have been found to exhibit abnormal activation of the PI3K/Akt/mTOR pathway, and recent clinical studies showed a therapeutic advantage in patients treated with a mTOR inhibitor. The key areas of current investigation focus on the elucidation of the molecular mechanisms of mTOR-related tumorigenesis, and the involvement of this pathway in liver cancer.

Growth factor and energy metabolism in TSC tumors

Studies in *Drosophila* have revealed a novel role of hamartin and tuberlin in the PI3K/mTOR signaling pathway that is pivotal to the cellular response to growth factors (e.g., insulin) and nutrients. Genetic screens in mosaic flies for cell size control identified loss-of-function mutants of the *Drosophila* homologs of TSC1 and TSC2 that exhibit increased cell size in a cell-autonomous fashion. Conversely, over-expression of dTSC1 and dTSC2, but neither alone, effectively rescued this phenotype (i.e., reduced cell size). Genetic epistatic experiments in flies showed that the effects of dTSC1 and dTSC2 were dominant over dInR and dAkt but not dTor and dS6K. Biochemical studies confirmed a negative regulatory role of the hamartin-tuberlin complex in mTOR-dependent protein synthesis.

The current model suggests that tuberlin inhibits mTOR activity by serving as a GTPase activating protein for Rheb, a Ras-related protein, and consequently reduces p70S6K and 4E-BP1-dependent protein translation (Figure 1). Upon growth factor stimulation of PI3K, downstream activation of Akt results in phosphorylation of tuberlin and releases its inhibition on mTOR. In TSC tumors, cells have lost TSC1 or TSC2 activity, thus resulting in uninhibited cell growth associated with elevated levels of mTOR and

One possible mechanism for separating multiple activities within the cell could be on the basis of unique subcellular localization of the proteins.

p70S6K activities. Indeed, pharmacologic blockade of mTOR with rapamycin, an immunosuppressant drug, causes profound anti-tumor response in vivo. However, it is not currently known how up-regulation of mTOR results in tumor formation, nor do we understand the mechanisms of tumor response to rapamycin.

Other unanswered questions include the physiologic role of TSC1/TSC2 in cellular metabolism, the function of PI3K/mTOR pathway in tumor initiation, and the long-term efficacy of rapamycin in TSC pathology. These issues are being addressed using various cellular and in vivo models of TSC.

The β -catenin pathway and the TSC genes

At present, not all of the TSC phenotype can be explained by one pathway. Our lab has explored the role of the TSC genes in the Wnt/ β -catenin pathway. The latter has been implicated in the regulation of cell proliferation, differentiation, and migration. The Wnt family of secreted growth factors acts on multiple signaling cascades among which the β -catenin canonical pathway is best understood for its role in various human cancers (e.g., colon, skin, liver). β -catenin is a highly conserved 95-kD protein involved in cell-cell adhesion and intracellular signaling. In its latter role, β -catenin shuttles from the cytosol to the nucleus upon Wnt stimulation, where it binds the LEF/Tcf family of transcription factors to activate downstream target genes such as cyclin D1 (Figure 1).

Our observations showed that renal tumors derived from our TSC animal model expressed high levels of β -catenin and cyclin D1. In 293T renal epithelial cells, expression of TSC1 and TSC2 reduced β -catenin levels by promoting its degradation. Correspondingly, TSC1/TSC2 inhibited β -catenin dependent activity of the LEF/Tcf transcription factors. Evidence suggested that TSC1 and TSC2 act at the level of the β -catenin degradation complex by associating with its components (i.e., GSK3, Axin) in a Wnt-dependent manner. Collectively, the TSC proteins likely function in multiple pathways giving rise to the diverse manifestations

of the pathology resulting from their inactivation (Figure 1). Efforts to demonstrate in vivo participation of these pathways and their relative contribution to the disease phenotype are currently our focus of investigation.

The role of TSC1/2 in microtubule organization and function

If indeed hamartin and tuberlin act on distinct molecular targets in various pathways, how may their function be regulated? One possible mechanism for separating multiple activities within the cell could be on the basis of unique subcellular localization of the proteins. Since signaling complexes function as modules, the context in which they interact with other proteins depend on their localization. For example, insulin stimulation of PI3K leads to localized increased concentration of PIP3 at the plasma membrane. This, in turn, recruits Akt from the cytosol to the membrane where it becomes activated.

In studying the subcellular localization of hamartin and tuberlin, we found that they indeed reside in multiple compartments (i.e., cytosol, microsome, cytoskeleton). Of particular interest is the vesicular component in which tuberlin was previously shown to interact with rabaptin-5 to modulate endocytosis. Biochemical analyses showed that the microsomal fraction of TSC2 belongs to the lipid raft domains and interacts with caveolin-1, a cholesterol-binding, structural protein of caveolae. Cells devoid of tuberlin have mis-localized caveolin-1 and reduced formation of caveolae at the plasma membrane.

Recent studies point to a role of tuberlin in regulating the transport of proteins such as caveolin-1 from the Golgi apparatus to the membrane. The molecular mechanism mediating this function of tuberlin and the consequence of faulty protein trafficking in tumorigenesis remain to be elucidated.

Genetic modifiers and phenotypic heterogeneity

One of the unexplained observations of the TSC syndrome is the variability in disease severity. This so called phenotypic heterogeneity can be seen in related individuals carrying the same genetic mutations, thus implicating the presence of other modifying factors.

Using animal models of TSC, we studied the influence of genetic background on tumor size and found that a specific TSC2 mutation, when placed into two unrelated strains of rats, produced vastly different disease burden. By means of quantitative trait analysis, a genetic modifier was identified and mapped to rat chromosome 3.

It appears that this locus affects tumor size without significant influence on tumor multiplicity, suggesting a role in tumor progression rather than initiation. The identity of this gene and its function are currently being sought.

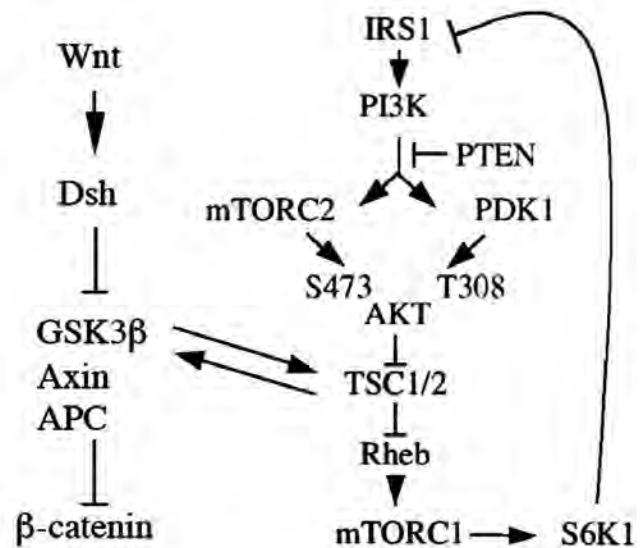


FIGURE 1: Model of TSC1/TSC2 pathway

RELATED PUBLICATIONS

1. Kenerson HL, Aicher LD, True LD, Yeung RS. Activated mTOR pathway in the pathogenesis of tuberous sclerosis complex renal tumors. *Cancer Res* 2002, 62:5645-5650.
2. Li Y, Inoki K, Yeung R, Guan KL. Regulation of TSC2 by 14-3-3 binding. *J Biol Chem*. 2002, 277:44593-44596.
3. Mak BC, Takemaru KI, Kenerson HL, Moon RT, Yeung RS. The tuberlin-hamartin complex negatively regulates b-catenin signaling activity. *J Biol Chem* 2003, 278:5947-5951.
4. Lee A, Maldonado M, Baybis M, Walsh CA, Scheithauer B, Yeung R, Parent J, Weiner HL, Crino PB. Markers of cellular proliferation are expressed in cortical tubers. *Ann Neurol*. 2003, 53:668-73.
5. Yeung RS. Multiple roles of the TSC genes. *Genes Chrom Cancer* 2003, 38:368-375.
6. Jones KA, Jiang X, Yamamoto Y, Aicher LD, Yeung RS. Tuberlin is a component of lipid rafts and mediates caveolin-1 localization. *Exp Cell Res*. 2004, 295:512-524.
7. Johnson EW, Holck PS, Levy AE, Yeh MM, Yeung RS. The role of tumor ablation in bridging patients to liver transplantation. *Arch Surg* 2004;139:825-829.
8. Wenzel HJ, Patel LS, Robbins CS, Emmi A, Yeung RS, Schwartzkroin PA. Morphology of cerebral lesions in the Eker rat model of tuberous sclerosis. *Acta Neuropath*. 2004, 108:97-108.
9. Kenerson H, Dundon TA, Yeung RS. Effects of rapamycin in the Eker rat model of tuberous sclerosis complex. *Pediatr. Res*. 2005, 57:67-75.
10. Mak BC, Kenerson HL, Aicher LD, Barnes EA, Yeung RS. Aberrant β-Catenin Signaling in Tuberous Sclerosis. *Am J Pathol* 2005, 167:107-116.
11. Mackay HJ, Billingsley K, Gallinger S, Berry S, Smith A, Yeung R, Pond GR, Croitoru M, Swanson PE, Krishnamurthi S, Siu LL. A multicenter phase II study of "adjuvant" irinotecan following resection of colorectal hepatic metastases. *Am J Clin Oncol*. 2005, 28:547-54.
12. Jiang X, Yeung RS. Regulation of the microtubule-dependent protein transport by TSC2/mTOR pathway. *Cancer Res* 2006, 66:5258-5269.
13. Carroll PA, Kenerson HL, Yeung RS, Lagunoff M. Latent Kaposi's sarcoma associated herpesvirus infection of endothelial cells activates hypoxia-induced factors. *J Virol*. 2006, 80:10802-10812.

VAPSHCS / GENERAL SURGERY

LORRIE LANGDALE, M.D.

MICHAEL SOBEL, M.D.

Lorrie Langdale, M.D.

- Hepatic Ischemia-Reperfusion: Evolution of an Injury
- JAK/STAT and SOCS in Inflammation
- SOCS Proteins, Cytokine Control and the Response to Hepatic IR Injury



FUNDING

UW Royalty Research Fund
VA Merit Review Grant

Liver failure is often preceded by a period of inadequate tissue and cellular perfusion (ischemia). Reperfusion initiates a complex chain of “second-phase” events that leads to neutrophil activation by inflammatory cytokines and a neutrophil-mediated injury (IR). Unchecked, this inflammatory response progresses to necrosis, with acute loss of liver function, an increased risk of both primary and secondary (e.g. lung) organ failure, and subsequent death from multiple organ failure. Therapeutic strategies to improve outcomes have been aimed at blocking individual components of this widely redundant inflammatory cascade prior to the onset of IR. To date, however, laboratory successes have not translated to clinically relevant therapies. Further, given that many patients present for treatment after the pro-inflammatory phase of injury is well underway, a more realistic approach would focus on understanding the mechanisms of inflammation regulation and control. Understanding the mechanisms of cellular signaling that precede, trigger and control the inflammatory response to an injury could be key to effective clinical modulation of ischemia-reperfusion injury and its complications.

Several cell signaling pathways are known to contribute to an inflammatory response to injury. Pro-inflammatory cytokines important to IR (e.g. TNF α , IL-1, Toll-like receptors) affect their target genes through MAPkinase-mediated activation of NF- κ B, ERK1/2, p38, and JNK. IL-6 and IFN γ signal their targets of the pro- and anti-inflammation cascades through the JAK/STAT pathway. Although much remains unknown as to how regulation of these pathways in an acute inflammatory response inter-relate, a family of proteins, designated Suppressors of Cytokine Signaling or SOCS proteins, have been identified as negative control mechanisms of the JAK-STAT pathway. Some of these proteins have also recently been shown to play a potential role in regulation of MAPkinase signaling.

Cell culture models have been specifically identified SOCS1 and SOCS3 as important modulators of immune and inflammatory responses. However, while SOCS1 and SOCS3 share some functionality, they do not appear to be interchangeable. In vivo, *pre-treatment* of IR with IL-6 or high dose IFN γ , potent inducers of SOCS3 and SOCS1, has been shown to be protective in models of liver IR.

We have shown that while SOCS3 is expressed over a broad range of IR injury severity, SOCS1 expression parallels the severity of the ischemic injury. We hypothesize that SOCS1 and SOCS3 are essential to the evolution and ultimate resolution of liver IR, cooperatively delimiting cytokine/chemokine-mediated primary and secondary injuries through negative regulatory cross-talk between distinct cell signaling pathways. We propose to test our hypothesis in time course studies of murine hepatic IR, correlating hepatic injury and outcomes with gene expression of inflammatory cytokines, chemokines, JAK/STAT and MAPkinase signaling factors under conditions of SOCS3 or SOCS1 conditional deletion or overexpression. Our long-term goal is to identify and potentially exploit the natural inflammatory control mechanisms as a novel avenue for clinical management of ischemia-reperfusion injuries.

Hepatic Ischemia-Reperfusion: Evolution of an Injury

The liver is particularly vulnerable to the effects of ischemia-reperfusion because of its “open architecture.” The early phase of IR injury reflects the direct effects of local toxic reactive oxygen products that injure tissue and mediate neutrophil activation and chemoattraction. Hypoxia/reoxia also initiates the recruitment of leukocytes to the specific site of injury through the actions of a spectrum of cytokines and chemotactic proteins expressed by Kupffer cells and hepatocytes. Activated neutrophils (PMNs) alter microcir-

Understanding the mechanisms of cellular signaling that precede, trigger and control the inflammatory response to an injury could be key to effective clinical modulation of ischemia-reperfusion injury and its complications.

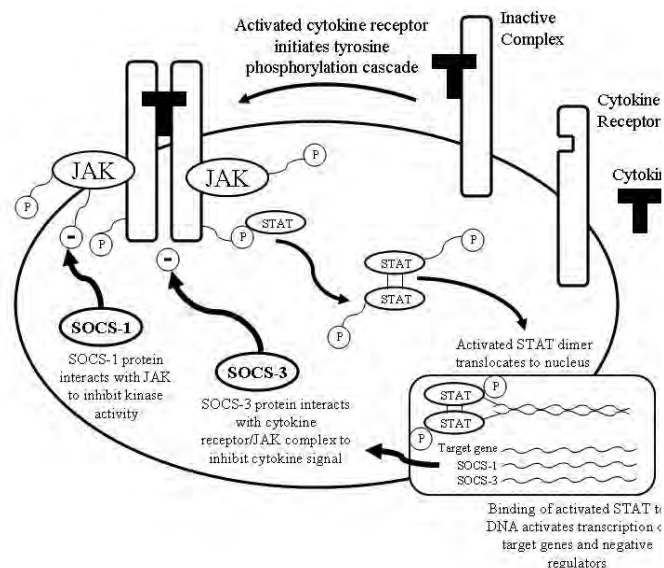
culatory perfusion through adhesion to endothelium, and adherent PMNs diapedese from the sinusoid, through the fenestrated endothelium, into the parenchyma where release of additional reactive oxygen products compounds the local injury. This PMN-mediated phase of hepatic IR occurs relatively late in reperfusion, with neutrophil infiltration becoming dominant between 8 and 24 hr of reperfusion.

The spectrum of cytokines that contribute to inflammation and its resolution utilize common cell signaling pathways to mediate their effects. A key pathway involves the Janus family of tyrosine kinases (JAK-Tyk) and the signal transducers and activators of transcription proteins (STATs). The JAK-STAT pathway requires cytokines to form a ligand-receptor complex that phosphorylates the cytoplasmic portion of the cytokine receptor. This receptor-associated Janus kinase (JAK) then forms a docking site for signal transducer and activator of transcription (STAT) and the resulting complex allows tyrosine phosphorylation of the STAT with formation of an activated dimer or tetramer. The STAT dimer/tetramer translocates to the nucleus and binds with a specific DNA sequence and/or other transcription factors to effect target gene transcription.

JAK/STAT and SOCS in Inflammation

A regulated response to injury requires both active inflammation, with the expression of pro-inflammatory cytokine and chemokine mediators and neutrophil activation and trafficking, and active inflammation control. In addition to effecting cytokine signaling, STAT-mediated cell signaling induces the expression of Suppressors of Cytokine Signaling (SOCS) proteins that serve as classic negative feedback mechanisms for cytokine expression. Numerous cytokines important to acute inflammation activate cells through JAK-STAT, including $\text{TNF}\alpha$, $\text{IFN}\gamma$, IL-1, IL-6, IL-10 and Growth Hormone (GH). These mediators are, in turn, controlled, at least in part, by SOCS proteins.

The pattern and time-course of SOCS mRNA observed following cytokine stimulation appears to be both stimulus and tissue dependent. For example, although constitutively expressed in thymus and spleen, level of SOCS-1 mRNA are very low in un-stimulated liver cells. Injection of IL-6 or $\text{IFN}\gamma$ results in dose dependent increased levels of SOCS-1, SOCS-2, SOCS-3 and CIS RNA. Both SOCS-1 and SOCS-3 mRNA are detectable in liver within 20 min after IL-6 injection in mice. SOCS-1 mRNA levels return



to baseline within 4 hr while SOCS-3 mRNA is sustained for up to 8 hr. SOCS-2 and CIS mRNA remains elevated for 24 hr.

The importance of SOCS proteins to liver injury is apparent from studies in SOCS-1 $-/-$ mice. These mice exhibit stunted growth and die before weaning with fatty degeneration of the liver and monocytic infiltration of several organs. In addition, the thymus of SOCS-1 $-/-$ mice is markedly reduced in size and there is progressive loss of maturing B-lymphocytes in bone marrow, spleen, and peripheral blood. Animals lacking SOCS-1 may be rescued by injection of antibodies to IFN γ . Mice lacking both SOCS-1 and IFN γ , however, are viable and healthy. These data suggest that it is the loss of balance between the pro-inflammatory IFN γ and its negative control mechanism that results acute fulminant liver injury. Interestingly, SOCS-1 appears to be primarily important to limiting the duration of response to cytokines, rather than the magnitude of the response. There is also evidence that SOCS-3 is up-regulated by IL-10 via a STAT-independent mechanism, implying that additional cytokine-activated transcription factors are involved with SOCS transcription. Together, these data imply a significant role for SOCS proteins in the

regulation of acute and chronic inflammation. The role of SOCS in ischemia-reperfusion has not been elucidated, but early induction of negative regulatory mechanisms would potentially explain the protection associated with high dose interferon gamma or IL-6 pre-treatment of hepatic IR.

Our current work is focusing on determining the role of JAK/STAT signaling and SOCS-mediated negative regulation on the evolution of liver injury severity and the effects of immunomodulation on injury resolution. Using a mouse model of hepatic IR, we are exploring the protective effects of SOCS-induction with erythropoietin, interferon gamma and IL-6 as well as the injurious effects of SOCS1 or SOCS3 conditional deletion from hepatocytes on liver IR severity. In addition, we are interested in the effects of SOCS induction or deletion on remote organ injury, in particular, secondary lung injury.

SUMMARY OF SIGNIFICANCE: Furthering our understanding of the cell signaling events that define and control the acute inflammatory responses to primary and secondary injury will foster the development of treatment strategies important to promoting injury progression, resolution and healing.

RELATED PUBLICATIONS

1. Langdale, LA, Flaherty, L, Liggitt, HD, Harlan, JM, Rice, CL, Winn, RK: Neutrophils contribute to liver ischemia-reperfusion injury by a CD18-independent mechanism. *J. Leuk. Biol.* 53:511-517, 1993.
 2. Winn, RK, Vedder, NB, Mihelcic, D, Flaherty, LC, Langdale, L, Harlan, JM: The role of adhesion molecules in reperfusion injury. *Agents-Actions-Suppl.* 41: 113-26, 1993.
 3. Langdale, LA, Wilson, L, Jurkovich, GJ, Liggitt, HD: Effects of immunomodulation with IFN γ on hepatic ischemia-reperfusion injury. *Shock* 11 (5): 356-361, 1999.
 4. Campbell, J., L. Prichard, F. Schaper, J. Schmitz, A. Stephenson-Famy, M. Rosenfeld, G. Argast, P. Heinrich, and N. Fausto, *Expression of suppressors of cytokine signaling during liver regeneration*. *J. Clin. Invest.*, 2001. 107: p. 1285-1292.
 5. Langdale, LA, Kajikawa, O, Frevert, C, Liggitt, HD: Sustained tolerance to LPS stimulation after liver ischemia-reperfusion injury. *Shock* Jun; 19(6): 553-8, 2003.
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DEPARTMENT CO-INVESTIGATORS

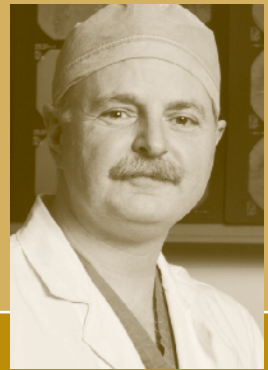
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• Heparin, Platelets, and Vascular Cells



FUNDING

National Institutes of Health
VA Merit Review Grant

Located at the Veterans Administration Puget Sound Health Care System, the Vascular Research Laboratories are led by Michael Sobel, M.D., Errol Wijelath, Ph.D., and supported by other Ph.D.'s and postdoctoral trainees. The principal focus of Dr. Sobel's research group is understanding the structure-function relations of heparin's interactions with vascular proteins and cells. Heparins are a family of structurally heterogeneous sulfated polysaccharides. Heparin is best known for its anticoagulant properties, which are exerted by heparin binding to the plasma protein antithrombin-III. But beyond their conventional anticoagulant actions, heparins have a wide range of other biological effects, antiproliferative, anti-inflammatory, as well as stimulatory actions on some vascular cells. And while the interaction between heparin and antithrombin-III is known to depend on a well defined structural domain — the heparin pentasaccharide — heparin interactions with other proteins and cells have not been as well characterized. In part, the structural complexity of carbohydrates and heparin in particular has hindered efforts to better understand its structure-function relations. Also, the biological effects of heparins have often been contradictory or confusing, due to the complexity of the biological models used.

The interactions between platelets and heparin have been especially confusing. The autoimmune-mediated phenomenon of heparin-induced thrombocytopenia is one aspect of heparin-platelet interactions. But apart from this unusual immune reaction, Dr. Sobel's laboratories have found that heparin directly influences platelet function by at least two separate mechanisms.

Heparin Interactions with von Willebrand Factor

Using biophysical methods, binding assays, and molecular modeling, they demonstrated that heparin binds to a specific domain of von Willebrand factor (vWf) (1;2). This plasma protein is essential for normal platelet hemostatic function, and mediates the adhesion of platelets at sites of vascular injury (especially under high shear, arterial conditions). When heparin binds vWf it interferes with the platelet hemostatic properties of the protein. Specific sub-species of heparin were purified that bound vWf with especially high affinity. Through scientific collaborations with Dr. Yasuo Suda, a carbohydrate polymer chemist in Japan, a structurally defined disaccharide motif was identified that was responsible for heparin's binding to vWf. A refined heparin with high affinity for vWf (and low affinity for antithrombin-III) was effective at preventing arterial occlusion in an animal model of platelet-vWf dependent arterial thrombosis (3;4). This work holds future promise for developing novel antithrombotic heparins that interfere with vWf-mediated platelet adhesion, rather than retarding plasma coagulation.

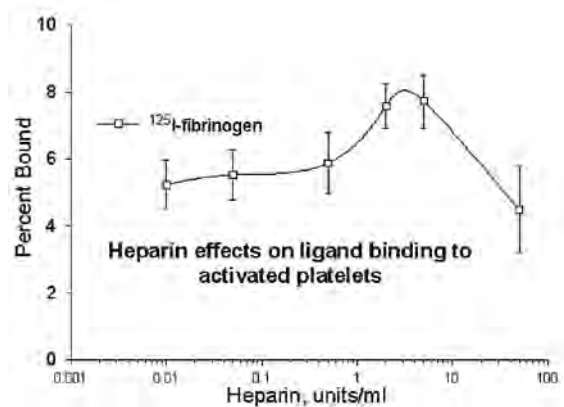


FIGURE 1: ¹²⁵I-Fibrinogen binding to thrombin-activated platelets was measured over a range of heparin concentrations. At concentrations of 2 and 5 units/ml heparin, fibrinogen binding was significantly increased.

Surprisingly, the effect of heparin on integrin function depended on the integrin subunit.

Heparin binds directly to the platelet integrin

Heparin also has a contradictory, direct stimulatory effect on platelet function. In related work, it was shown that heparin binds directly to the platelet surface, and that one of the important binding sites may be the platelet fibrinogen receptor, GpIIb/IIIa (integrin α IIb β 3). Unlike vWf, which mediates platelet adhesion at high shear rates, the fibrinogen receptor is responsible for platelet aggregation and clumping at lower shear rates. Through physiological studies of platelet aggregation, photoaffinity crosslinking, and cell-signaling work, heparin was found to bind to this platelet integrin, and enhance its binding of fibrinogen (5).

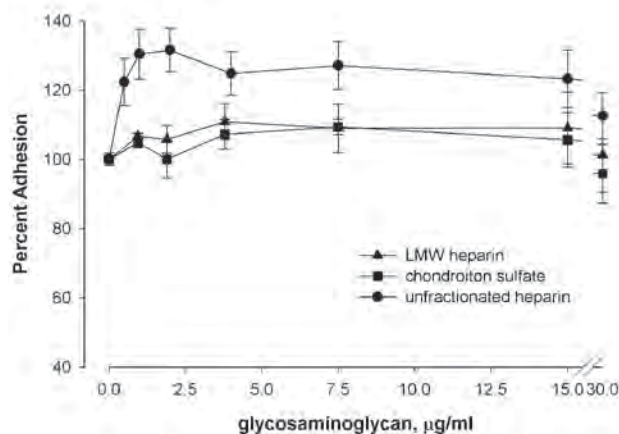


FIGURE 2: Thrombin activated platelets.

Heparin modulates β 3 integrins

How does heparin activate or enhance integrin function in the platelet? To see whether these effects were unique to the platelet integrin (α IIb β 3), the K562 cell line was transfected with different integrins, and the effects of heparin on integrin-mediated cell adhesion were studied. Surprisingly, the effect of heparin on integrin function depended on the integrin subunit. A stimulatory effect was observed in all β 3 containing integrins (α IIb β 3, α V β 3) but the type of α subunit did not seem to be as important. The effect of heparin was structure specific, as other glycosaminoglycans and low molecular weight heparins showed no enhancement of adhesion (6). Because integrins are such ubiquitous receptors in vascular cells, a detailed understanding of precisely how heparin modulates these receptors may lead to novel drugs to modulate thrombosis and vascular healing.

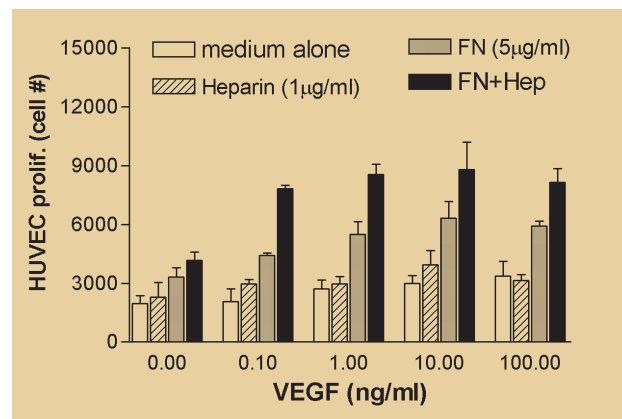


FIGURE 3: Adhesion of K562 α v β 3 cells to vitronectin. Unfractionated heparin enhances integrin-mediated adhesion, but other glycosaminoglycans do not.

Heparin modulation of endothelial cell migration and proliferation

Matrix proteins and growth factors (and their respective cellular receptors - integrins and receptor tyrosine kinases) are key actors in angiogenesis and vascular healing. Integrins and growth factor receptors work together to enhance the extracellular signals from each pathway, leading to increased endothelial cell proliferation and migration. Vascular Endothelial Growth Factor (VEGF) and fibronectin appear to have a unique complementary relationship. In a

recent publication, VEGF was shown to preferentially bind to fibronectin over other matrix proteins (7). Platelets actually release pre-formed VEGF/fibronectin complexes, and these complexes have significantly more potent mitogenic effects than VEGF or fibronectin alone on endothelial cells. Heparin further supports the synergistic biological effects of VEGF/fibronectin. Once again, heparin (and cell-surface heparan sulfate proteoglycans) may be playing a key role in modulating the extracellular assembly of specific ligands on their cellular receptors.

RELATED PUBLICATIONS

1. Sobel M, McNeill PM, Carlson P, Kermode JC, Adelman B, Conroy R et al. Heparin inhibition of von Willebrand factor in vitro and in vivo. *J Clin Invest* 1991; 87:1787-1793.
 2. Sobel M, Soler DF, Kermode JC, Harris RB. Localization and characterization of a heparin binding domain peptide of human von Willebrand factor. *J Biol Chem* 1992; 267:8857-8862.
 3. Sobel M, Bird KE, Tyler-Cross R, Marques D, Toma N, Conrad HE et al. Heparins designed to specifically inhibit platelet interactions with von Willebrand Factor. *Circulation* 1996; 93(5):992-999.
 4. Poletti LF, Bird K, Marques D, Harris RB, Sobel M. Prevention of arterial thrombosis using a novel heparin with enhanced anti-platelet and reduced anticoagulant activity. *J Vasc Surg* 26, 366-372. 1997.
 5. Sobel M, Fish W, Toma N, Luo S, Bird K, Blystone SD, Sobel M. Heparin modulates integrin function in human platelets. *J.Vasc.Surg.* 33, 587-594. 2001.
 6. Wijelath ES, Murray J, Rahman S, Patel Y, Ishida A, Strand K, Sobel M. Novel vascular endothelial growth factor binding domains of fibronectin enhance vascular endothelial growth factor biological activity. *Circ Res* 2002; 91(1):25-31.
 7. Da Silva MS, Horton JS, Jacqui M. Wijelath JM, Lisa W. Blystone LW, Wallace R. Fish WR, Errol Wijelath E., Kurt Strand K., Scott D. Blystone SD, Sobel M. Heparin modulates integrin-mediated cellular adhesion: Specificity of interactions with α and β integrin subunits. *Cell Communication and Adhesion*. 2003, Volume 10, Number 2, Mar-Apr 2003, pp. 59-67(9).
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VASCULAR SURGERY

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ALEXANDER W. CLOWES, M.D.

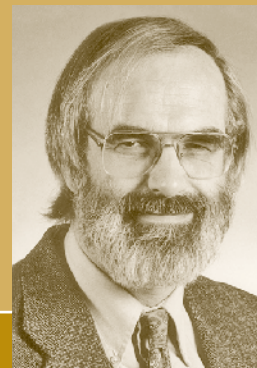
GÜNTER DAUM, PH.D

THOMAS HATSUKAMI, M.D.

TED KOHLER, M.D., M.SC.

Kirk W. Beach, Ph.D., M.D.

- Tissue Pulsatility Imaging of Brain
- Atherosclerotic Plaque Neovascularization
- Ultrasound Reading Center for Carotid Stenosis



FUNDING

National Institute of Biomedical Imaging
and Bioengineering

The purpose of this work is to use noninvasive low cost methods to characterize the vascular perfusion of tissues for diagnosis.

Since the wide clinical use of arterial and venous plethysmography to diagnose arterial and venous obstructive diseases, there has been controversy about whether the primary volume changes in tissue were due to changes in the volume of the major arteries and veins or to changes in the volume of the microcirculation. Traditional methods of plethysmography measured volume changes in an entire body part, including both major vessels and micro-vessels. The introduction of photo-plethysmography, showing both pulsatile changes in skin reflectivity with the cardiac cycle and phasic changes in reflectivity with the respiratory cycle, supported the concept that the volume changes were, at least in part, volume changes in the arterioles and venules. We have extended the study of the volume changes in arterials and venules to deeper tissues using ultrasonic strain measurement methods.

Tissue can be considered a composite structure of cells, interstitium, matrix and microvessels. We consider the cells and matrix to be of constant volume. The interstitium is an extravascular, extra-cellular space containing saline and is a space which may expand with edema. The rate of expansion and contraction of this space is slow. The intravascular volume has three regions, venular at low pressure, capillary and arterial at high pressure. Because we image tissue, microvessels are vessels too small to resolve with our imaging methods, less than 1 mm in diameter. In tissue, capillaries are near 0.01 mm diameter and spaced at intervals of about 0.1 mm, so about 1% of tissue volume is capillaries, 1% is arterioles and small arteries, and 3% is venules and small veins. About 5% of each imaging sample volume contains blood in the microcirculation. Normally

the tissue pressure is below the venous pressure, leaving the veins fully inflated. However, in regions at an elevation above the heart (above the right atrium) the veins and venules are collapsed. Whenever the local tissue pressure is below the local venous pressure, the venules are inflated; when the tissue pressure exceeds the venous pressure, the venules collapse.

We are developing ultrasonic and optical methods to measure tissue volume changes in tissue. These methods can be used to determine both whether the microvascular volume in tissue is normal, elevated (from tumor angiogenesis), or reduced (from ischemia). They can also be used to detect elevated interstitial tissue pressures by determining the local venous pressure at which the local tissue volume changes from deflated to inflated. Of particular interest are tissues at high pressures, such as arterial walls and atherosclerotic plaques on those walls.

Transmural pressure is the difference between intravascular pressure and the surrounding tissue pressure. A model made of balloons representing the microcirculation and columns of fluid representing the venous pressure, arterial pressure and interstitial pressure demonstrates that the tissue composite does not have a linear volume change as the tissue pressure rises, but there are sudden changes. As the interstitial pressure exceeds the venous pressure, the veins and venules collapse, reducing the tissue volume by 3% suddenly. Then, the volume changes little with increases in pressure until the arterial pressure is reached. At those pressures, the volume of the tissue pulsates, as arterial pressure rises above and drops below the interstitial pressure. This latter effect is the cause of large pulsations under a blood pressure cuff, because the blood pressure cuff, of course, controls the interstitial pressure.

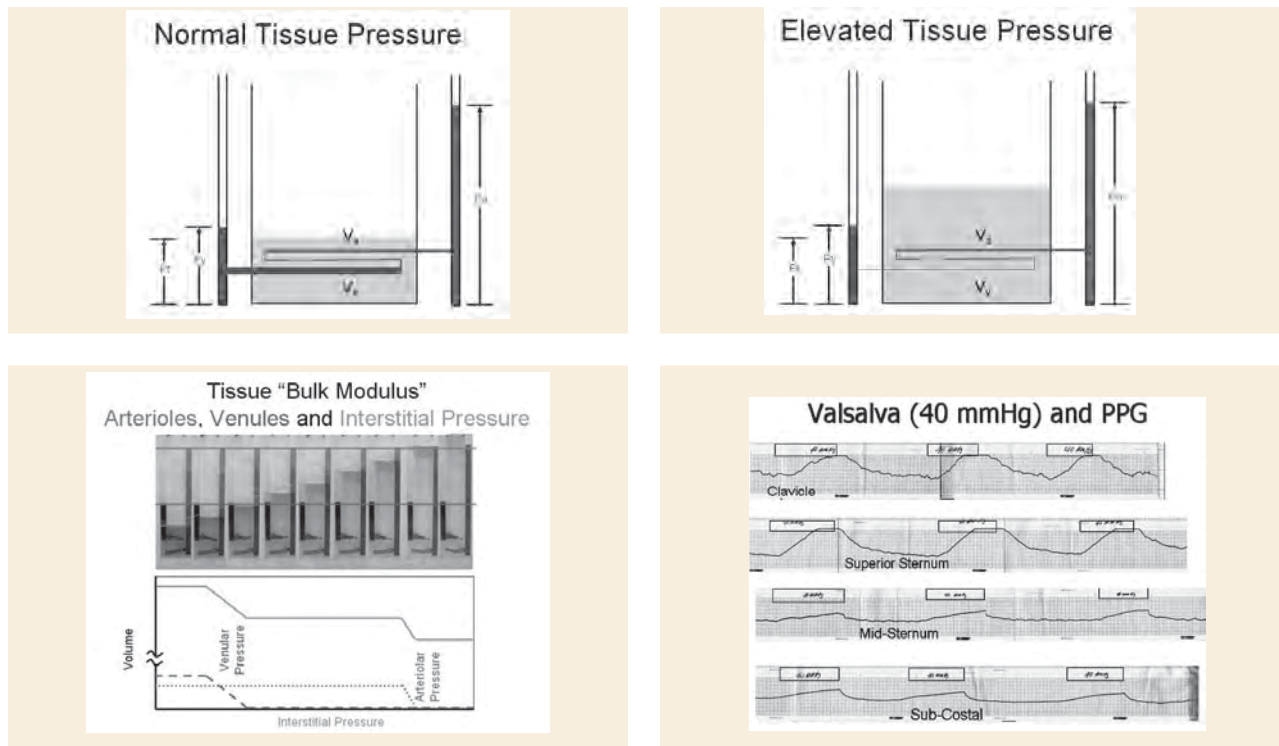


FIGURE 1: Tissue Pressure and Volume

UPPER LEFT When tissue pressure is normal, venules are inflated. **LOWER LEFT** As interstitial pressure increases above arterial pressure, the arteries and arterioles also collapse. **UPPER RIGHT** When tissue pressure is elevated, venules are collapsed. **LOWER RIGHT** Upper 2 tracings show the inflation of skin venules above the right atrium when a Valsalva maneuver raises central venous pressure; lower 2 tracings show the compliance of inflated of skin venules below the right atrium when a Valsalva maneuver raises central venous pressure.

Atherosclerotic Plaque Pulsatility

Applying these principles to the microcirculation providing nourishment to the cells within an atherosclerotic plaque, we are launching a study of the effect of Bernoulli pressure depression due to high intra-stenotic velocities on carotid plaque pulsatile strain.

A vulnerable atherosclerotic plaque is expected have a pulsatile strain waveform with a sudden inflations component in the upper right.

MIDDLE RIGHT WAVEFORMS: Upper: measured arterial diameter pulsatile waveform, Lower: measured tangential wall strain waveform.

The vasa vasorum are a network of tiny arteries and veins on the outer wall of major blood vessels which penetrate into the wall to supply respiration and nutrition to the vascular wall. In addition, these vessels penetrate through the arterial wall and into neovessels in the atherosclerotic plaques to provide nutrients and oxygen to the abnormal

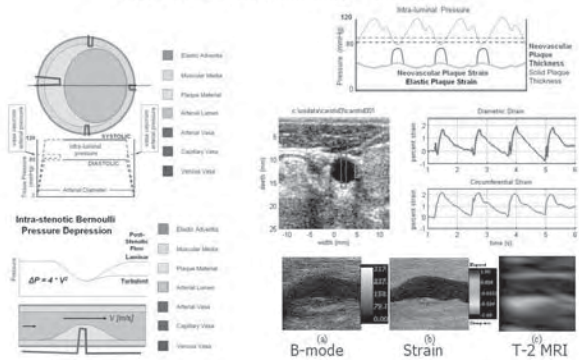
cells forming the plaque. The blood in the intraplaque neovessels is squeezed out in systole and inflates the neovessels in diastole.

We are developing an ultrasound examination method that can be performed through the skin of the neck to measure the strain (deformation) of these plaques due to the filling and emptying of the neovessels as the arterial pressure rises and falls with the cardiac cycle. By determining the arterial pressure when the neovessels inflate, the pressure in the vasa vasorum can be determined; the inflation volume is equal to the neovascular volume. Atherosclerotic carotid artery plaques which have a large neovascular volume are vulnerable to rupture, causing a stroke. The ultrasonic measurement developed in this project will differentiate plaques vulnerable to rupture from those that are stable. In this study we will measure the plaque strain in 300 patients to provide a distribution of normal and abnormal strain values in the people who have stenotic atherosclerotic carotid artery plaques.

The ultrasonic measurement developed in this project will differentiate plaques vulnerable to rupture from those that are stable.

ATHEROSCLEROTIC PLAQUE NEOVASCULAR INFLATION

Application: Vulnerable Plaque Identification



INVESTIGATORS: K. Beach, Y. Kim, J. Kucwicz, D. Leotta, S. Sikdar, RE Zierler
Grant #: 1R21EB006825-01, Score: 143, Percentile: 7.8, Project Period 12/01/06 to 11/30/08

FIGURE 2: Carotid Plaque Vascularity Theory

Brain Pulsatility

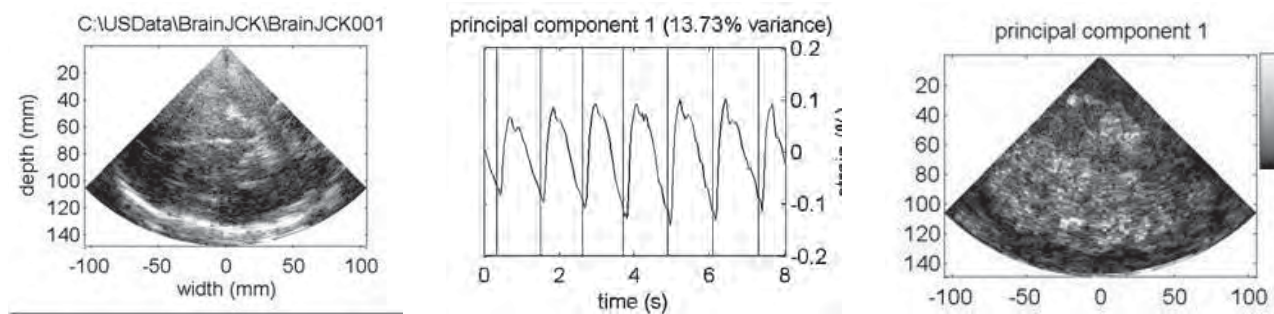
Both arterial and venous strain waveforms occur in all perfused tissues. Of particular interest is the brain, where regions of neurological activity are associated with increased local metabolism resulting in regions of increased oxygen consumption and carbon dioxide production. The associated local increases in carbon dioxide cause vasodilation and local increased perfusion. We believe that the increased perfusion can be detected as an increase in pulsatility. We

use ultrasound imaging to map the pulse amplitude in the brain through the skull (Figure 3). In the pulse amplitude image on the right, local variations in pulse amplitude in the left hemisphere (lower part of the image) can be seen.

This method can be used for functional brain imaging (Figure 4). Using a standard Evoked Response Potential protocol (functional Electro-Encephalography) used to stimulate the right visual cortex in the occipital lobe of the brain, increased pulsatility can be imaged. A flashing checkerboard is used to stimulate the visual cortex. By locating the checkerboard in the left half of the visual field, opposite a gray field on the right, the left visual cortex is activated. The “black-and-white” checkerboard alternates positive and negative each half second. By stimulating for 30 seconds and then resting with a plain gray screen for 30 seconds, pulsatility during the stimulated period is compared to the pulsatility during the resting period.

In addition to measuring pulse amplitude, pulse frequency can be useful in obstetrical imaging. Although fetal tissue can be differentiated from maternal tissue by an expert on the basis of anatomical shape, the automatic differentiation of tissue is more challenging. In addition, the identification of different pulse rates and amplitudes may be particularly useful when imaging the placenta to identify regions of infarction.

FIGURE 3: Brain Pulsatility



LEFT: 2-D B-mode brain image from the right “temporal window” through the bone above the right ear showing the falx cerebri as a horizontal line at 70 mm depth and the left skull as a curved line at 130 mm depth. CENTER: Measured brain waveform shape over 8 seconds. RIGHT: Relative pulse amplitude (gray scale 0-15 multiplied by the “average” strain on the right). John Kucwicz

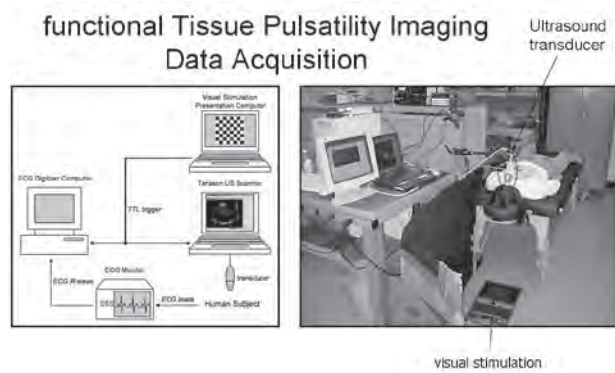
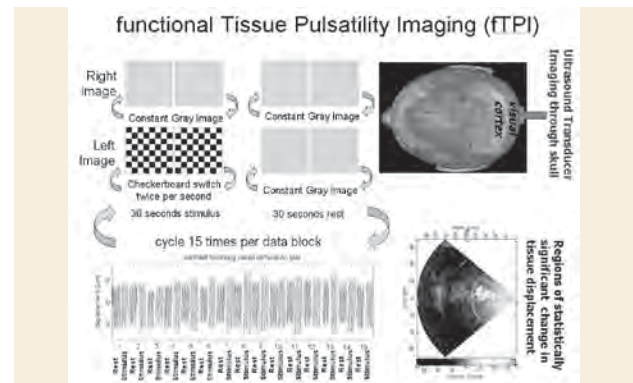


FIGURE 4: Functional Tissue Pulsatility Imaging of the Right Visual Cortex
John Kuciewicz, Barbrina Dunmire, Heracles Panagiotides



Transcranial Doppler ultrasound is limited to viewing through the thinnest portion of the skull, through the “window” above the ear, because the ultrasound reflection from blood is so weak. Ultrasound A-mode and B-mode imaging have been limited to detecting the specular echoes from the falx cerebri through the temporal window marking the midline of the brain due to the variable attenuation of the skull. Doppler methods are not sensitive to variations in attenuation. Because pulsatility imaging is a Doppler method, tissue pulsatility imaging can be done through the skull. Fortunately, the required echoes are strong as they come from the solid tissues of the brain. Pulsatility imaging of brain tissue is possible through every portion of the skull that we’ve tested (Figure 6).

In addition to applications in functional brain imaging, TPI of brain may allow the early differentiation of ischemic stroke from hemorrhagic stroke, and thus allow the early application of tissue saving thrombolytic drug therapy.

Ultrasound Reading Center For Carotid Stents

The Vascular Division, Department of Surgery, University of Washington is the Ultrasound Reading Center (URC) for the CREST (Carotid Revascularization, Endarterectomy vs. Stent Trial) and for other clinical trials. The URC processes and evaluates 1,000 to 2,000 carotid duplex-Doppler ultrasound examinations per year. The database that the URC is creating will provide new information about the hemodynamics of the cerebral circulation, the variability of ultrasound duplex Doppler examination methods and about the progression of untreated carotid atherosclerosis.

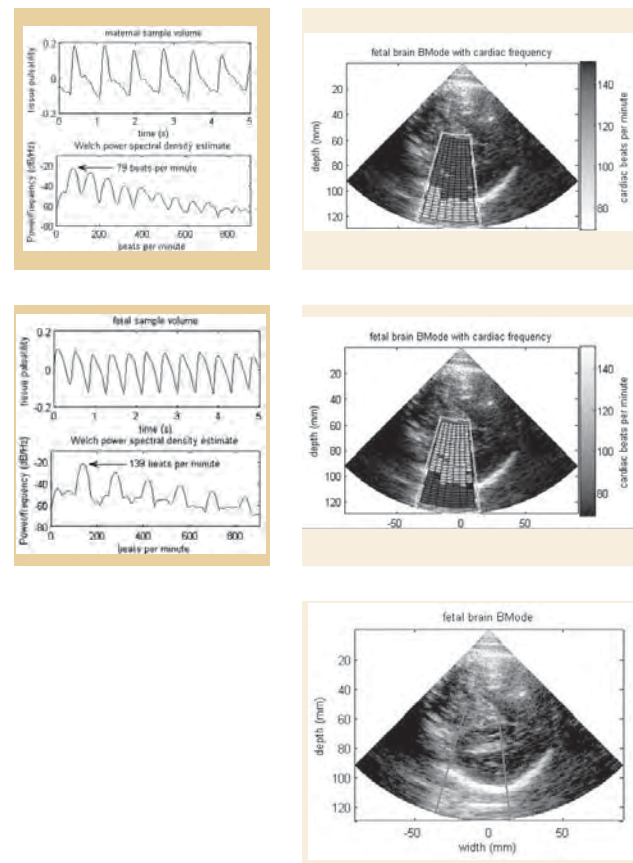


FIGURE 5: Tissue Differentiation

The pulse rate of the maternal heart beat **TOP LEFT** can be used to identify maternal tissue **TOP RIGHT** and the pulse rate of the fetal tissue **MIDDLE LEFT** can be used to identify fetal tissue. This differentiation is not made in conventional ultrasound 2-dimensional B-mode imaging **BOTTOM RIGHT**.

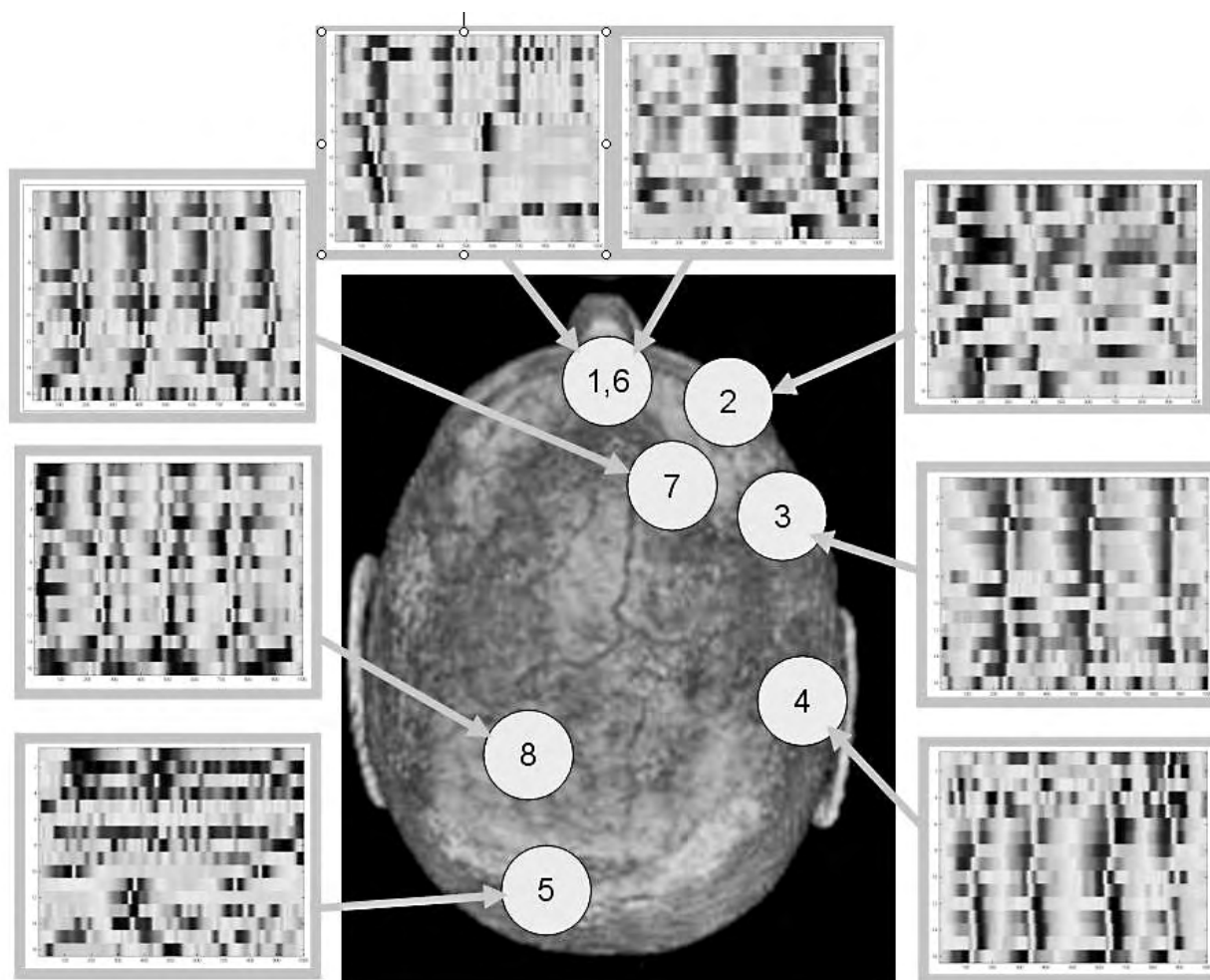


FIGURE 6: Pulsatility Imaging of the Brain Through Different Regions of the Skull

Each window shows the depth (vertical) vs. time (horizontal) record of brain motion ranging from a depth of 22 mm from the skin (top edge of each window) to 86 mm (bottom edge of each window). The gray scale cannot be quantified because that scale is set automatically to fit the range of the data for each window. John Kuciewicz, Mark Moehring

RELATED PUBLICATIONS

1. Beach KW. D. Eugene Strandness, Jr, MD, and the revolution in noninvasive vascular diagnosis: part 1: foundations. *J Ultrasound Med.* 2005 Mar;24(3):259-72.
2. Beach KW. D. Eugene Strandness, Jr, MD, and the revolution in noninvasive vascular diagnosis. Part 2: Progression of vascular disease. *J Ultrasound Med.* 2005 Apr;24(4):403-14.
3. Beach KW. D. Eugene Strandness, Jr, MD, and the revolution in noninvasive vascular diagnosis: part 3: seeking precision. *J Ultrasound Med.* 2005 May;24(5):567-81.
4. Beach Kirk, Dunmire, Barbrina, Medical Acoustics, Chapter 21, Handbook of Acoustics, Springer 2007.
5. Leotta DF, Primozich JF, Lowe CM, Karr LN, Bergelin RO, Beach KW, Zierler RE. Measurement of anastomosis geometry in lower extremity bypass grafts with 3-D ultrasound imaging. *Ultrasound Med Biol.* 2005 Oct;31(10):1305-15.
6. Leotta DF, Primozich JF, Henderson SM, Karr LN, Bergelin RO, Beach KW, Zierler RE. Display of spatially-registered Doppler spectral waveforms and three-dimensional vein graft geometry. *Ultrasound Med Biol.* 2005 Oct;31(10):1317-26.
7. Plett M, Beach KW. Ultrasonic vibration detection with wavelets: preliminary results. *Ultrasound Med Biol.* 2005 Mar;31(3):367-75. Erratum in: *Ultrasound Med Biol.* 2006 Oct;32(10):1621-2.
8. Sikdar S, Beach KW, Paun M, Vaezy S, Kim Y. Ultrasonic interrogation of tissue vibrations in arterial and organ injuries: preliminary in vivo results. *Ultrasound Med Biol.* 2006 Aug;32(8):1203-14.
9. Sikdar S, Beach K, Goldberg S, Zwink T, Baughman L, Kim Y. Ultrasonic imaging of myocardial vibrations associated with coronary artery disease. *Conf Proc IEEE Eng Med Biol Soc.* 2005;2:1087-90.
10. Sikdar S, Beach K, Goldberg S, Zwink T, Baughman L, Kim Y. Ultrasonic imaging of myocardial vibrations associated with coronary artery disease. *Conf Proc IEEE Eng Med Biol Soc.* 2005;4:4310-3.

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Alexander W. Clowes, M.D.

• Regulation of Vascular Smooth Muscle Cell Growth



AWARDS

National Heart, Blood and Lung Institute
MERIT Award

National Institutes of Health

• Vascular Surgery/Cardiology Training Grant

FUNDING

National Institutes of Health

Vascular surgical procedures are designed to rebuild diseased blood vessels and improve blood flow. While these procedures restore the circulation, they also cause injury. This injury induces a wound healing response that in some instances is associated with accumulation of scar tissue (intimal hyperplasia) and significant luminal narrowing (e.g. 20-40% of coronary arteries treated by angioplasty). Smooth muscle cells living in the arterial wall proliferate in response to injury and are largely responsible for the intimal hyperplasia (Figure 1). The primary objective of our laboratory is to understand the factors that stimulate and inhibit the growth of smooth muscle cells, and to develop new strategies for the pharmacological control of intimal hyperplasia.

REGULATION OF INTIMAL HYPERPLASIA IN DAMAGED ARTERIES: We use the rat carotid artery stripped of its endothelium by the passage of a balloon embolectomy catheter as a simplified model of vascular repair after endarterectomy or angioplasty. As in human arteries, the response to injury in rat carotid

arteries involves a series of events leading to intimal hyperplasia. Medial smooth muscle cells start proliferating at 24-48 hours. They begin to migrate into the intima at four days, and they continue to proliferate and to synthesize matrix for several weeks before resuming the resting state. The net result is a substantial increase in wall mass.

The critical issue is to define the factors that start and stop this process. We have been studying heparin as a paradigm for drugs that inhibit smooth muscle cell proliferation and migration. Since heparin-like heparan sulfates secreted by endothelial cells and resting smooth muscle cells can inhibit growth, they may play a role in maintaining the growth-arrested state in normal arteries. The current experiments are designed to test the hypothesis that heparin inhibits smooth muscle cell growth by interfering with the activation of the EGF and FGF receptors.

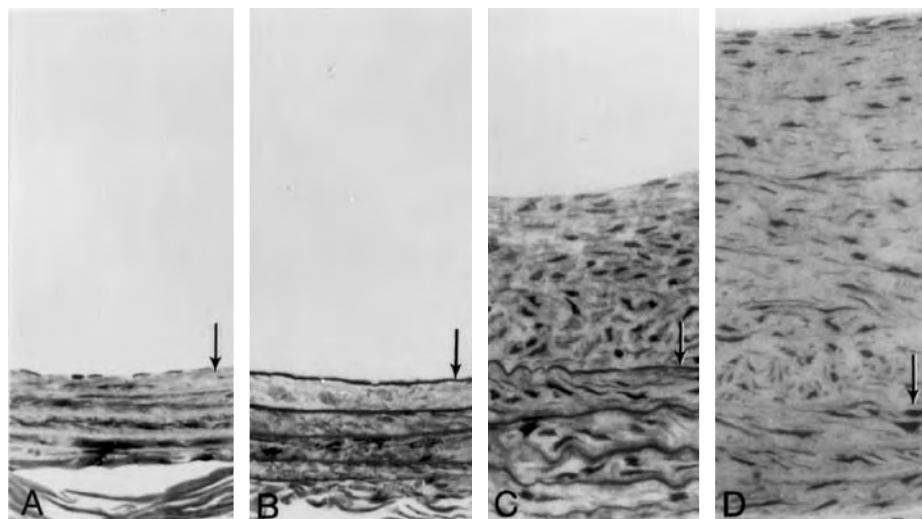


FIGURE 1: This series of photographs shows how a normal rat carotid artery (panel A-histologic cross-section) responds to injury. Angioplasty of the artery removes the surface endothelium (panel B). By two weeks (panel C), smooth muscle cells have migrated from the media into the intima (region above the elastic layer marked by the arrow) and have begun to proliferate (intimal hyperplasia). The thickening of the wall reaches a maximum by three months (panel D).

In the grafts, smooth muscle cells proliferate where endothelial cells are present, whereas in injured arteries they proliferate only where the endothelium is missing.

Recent studies in the laboratory have defined a novel pathway of smooth muscle cell activation which depends on these receptors. Thrombin can induce cell growth by interacting with its G-protein coupled receptor. In rat smooth muscle cells, the activated thrombin receptor in turn causes the release of heparin-binding EGF-like protein (HB-EGF) from the cell membrane, and the released HB-EGF then binds to the EGF receptor to induce a cell response. Blockade of the EGF receptor with specific antibodies inhibits cell growth and suppresses intimal hyperplasia in balloon-injured rat carotid arteries. In human smooth muscle cells, thrombin treatment induces the release of endogenous FGF and activation of the FGF receptor, instead of the EGF receptor. FGF mediates the cellular stimulus induced by not only thrombin but also PDGF and Factor Xa. We are currently pursuing experiments designed to understand “crosstalk” between growth factor and cytokine pathways.

NITRIC OXIDE AND SMOOTH MUSCLE PROLIFERATION: Nitric oxide (NO) is the principal arterial vasorelaxant. It is also an inhibitor of smooth muscle cell growth and injury-induced intimal hyperplasia. The mechanism of action has not been delineated although, in part, it depends on intracellular cyclic GMP and the activation of a cGMP-dependent protein kinase (PKG). We are currently studying a downstream target of NO and PKG, vasodilator stimulated phosphoprotein (VASP). Overexpression of VASP mutated to prevent phosphorylation by PKG makes cells unresponsive to NO, while overexpression of VASP mutated to prevent phosphorylation by PKC makes the cells sensitive to NO but unresponsive to serum. Thus, VASP may prove to be pivotal in the response of smooth muscle cells to growth stimulants and inhibitors, and pharmacological manipulation of this pathway might be a fruitful approach to controlling the arterial response to injury.

REGULATION OF SMOOTH MUSCLE GROWTH IN GRAFTS BY BLOOD FLOW AND PDGF: We have found that smooth muscle cell proliferation and neointimal hyperplasia in primate PTFE grafts are exquisitely regulated by changes in blood flow. Normal blood flow promotes neointimal hyperplasia, while high blood flow suppresses it or induces it to shrink (atrophy). In the

grafts, smooth muscle cells proliferate where endothelial cells are present, whereas in injured arteries they proliferate only where the endothelium is missing. Thus, depending on the physiological state, endothelial cells can have a positive or a negative effect on smooth muscle cell growth. Using molecular arrays, we are attempting to define the molecules altered by changes in blood flow that might regulate smooth muscle cell proliferation. We have recently identified bone morphogenetic protein-4 (BMP-4), a member of the TGF- β family, by array analysis. BMP-4 is expressed by endothelium, is upregulated by increased shear stress, and inhibits growth and at times kills smooth muscle cells.

Recent experiments using a mouse monoclonal antibody that recognizes and blocks the beta form of the PDGF receptor (PDGFR- β) have demonstrated conclusively that intimal hyperplasia in grafts as well as in injured arteries depends on PDGF. In collaboration with Celltech, Ltd., and ZymoGenetics, Inc., this antibody has been genetically engineered to resemble a human immunoglobulin; this “humanized” antibody has been tested in a human trial for the prevention of restenosis after coronary stent angioplasty and *failed*. We are astonished by this result and, in consequence, have gone back to the laboratory to investigate it further. Blockade of both PDGF receptors may be necessary. When we block both PDGFR- β and PDGFR- α , we not only suppress intimal thickening but we induce ca. 50% intimal atrophy (Figure 2) by two weeks. This novel finding indicates to us that restenosis might be a pharmacologically reversible process.

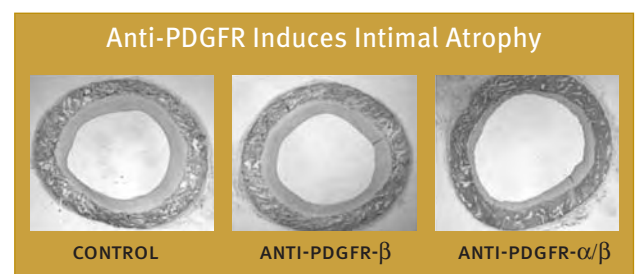


FIGURE 2: Histological cross-sections of normal flow PTFE grafts at 2 weeks following initiation of treatment with vehicle control, blocking antibodies to PDGFR- β , or blocking antibodies to both PDGFR- α and PDGFR- β . (H&E staining, 16X).

RELATED PUBLICATIONS

1. Berceli SA, Davies MG, Kenagy RD, Clowes AW: Flow-induced neointimal regression in baboon polytetra fluoroethylene grafts is associated with decreased cell proliferation and increased apoptosis. *J Vasc Surg* 36:1248-1255, 2002.
2. Serruys PW, Heyndrickx GR, Patel J, Cummins PA, Kleijne JA, Clowes AW: Effect of an anti-PDGF-beta-receptor-blocking antibody on restenosis in patients undergoing elective stent placement. *Int J Cardiovasc Intervent* 5:214-222, 2003.
3. Englesbe MJ, Hawkins SM, Hsieh PC, Daum G, Kenagy RD, Clowes AW: Concomitant blockade of platelet-derived growth factor receptors alpha and beta induces intimal atrophy in baboon PTFE grafts. *J Vasc Surg* 39:440-446, 2004.
4. Chitaley K, Chen L, Galler A, Walter U, Daum G, Clowes AW: Vasodilator-stimulated phosphoprotein is a substrate for protein kinase C. *FEBS Lett* 556:211-215, 2004.
5. Rauch BH, Millette E, Kenagy RD, Daum G, Clowes AW: Thrombin- and factor Xa-induced DNA synthesis is mediated by transactivation of fibroblast growth factor receptor-1 in human vascular smooth muscle cells. *Circ Res* 94:340-345, 2004.
6. Chen L, Daum G, Chitaley K, Coats SA, Bowen-Pope DF, Eigenthaler M, Thumati NR, Walter U, Clowes AW: Vasodilator-stimulated phosphoprotein regulates proliferation and growth inhibition by nitric oxide in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 24:1403-1408, 2004.
7. Millette E, Rauch B, Defawe O, Kenagy RD, Daum G, Clowes AW: PDGF-BB-induced human smooth muscle cell proliferation depends on basic FGF release and FGFR-1 activation. *Circ Res* 96:172-179, 2005.
8. Rauch BH, Millette E, Kenagy RD, Daum G, Fischer JW, Clowes AW: Syndecan-4 is required for thrombin-induced migration and proliferation in human vascular smooth muscle cells. *J Biol Chem* 280:17,507-17,511, 2005.
9. Conte MS, Bandyk DF, Clowes AW, Moneta GL, Seely L, Lorenz TJ, Namini H, Hamdan AD, Roddy SP, Belkin M, Berceli SA, DeMasi RJ, Samson RH, Berman SS: Results of PREVENT III: A multicenter, randomized trial of edifoligide for the prevention of vein graft failure in lower extremity bypass surgery. *J Vasc Surg* 43: 742-751, 2006.
10. Hsieh PC, Kenagy RD, Mulvihill ER, Jeanette JP, Wang X, Chang CM, Yao Z, Ruzzo WL, Justice S, Hudkins KL, Alpers CE, Berceli S, Clowes AW: Bone morphogenetic protein 4: potential regulator of shear stress-induced graft neointimal atrophy. *J Vasc Surg* 43:150-158, 2006.

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Günter Daum, Ph.D.

- A role for sphingosine-1-phosphate receptor-2 in smooth muscle cell differentiation and restenosis



FUNDING

National Institutes of Health

Restenosis is the cause for the unacceptably high failure rate (20-30%) of surgical interventions, such as vein grafts, stents, and angioplasty, to restore blood flow in occluded vessels. Restenosis is characterized by loss of luminal area due to negative remodeling (decreased vessel cross-sectional area) and intimal hyperplasia (accumulation of intimal smooth muscle cells (SMCs) and extracellular matrix). The introduction of stents prevents negative remodeling but not intimal hyperplasia. Stents allow local delivery of growth inhibitory drugs, and the use of rapamycin (sirolimus) is the most promising approach to date to inhibit stent restenosis. However, not all vascular occlusions are suitable for stenting. In addition, a systemic approach to prevent restenosis is still desirable since such treatment would be less invasive and possibly less expensive.

Despite tremendous research efforts, it is still unclear which factors drive the formation of restenotic lesions in humans. Since intimal cells exhibit a smooth muscle phenotype, i.e. they express SMC-restricted genes, the current paradigm is that medial SMCs become activated, migrate towards the lumen where they proliferate and produce matrix. To what extent adventitial cells or blood borne stem cells contribute to intimal formation is unclear. We also do not know whether a developing intima after vein grafting or arterial stenting is regulated by the same factors. Common to all growing intimae is that cells proliferate; the critical question is why? One should not forget that excessive intimal growth after arterial injury does NOT occur in 70% of patients. Thus, one may ask the question, what “goes right” in these patients? SMCs in normal arteries are extremely quiescent. For these cells to be able to proliferate, they must de-differentiate. Logically, after the healing process,

SMCs must re-differentiate to become quiescent again. It is our key hypothesis that the window of de-differentiation determines whether intimal lesions develop or not.

A role for sphingosine-1-phosphate receptor-2 in SMC differentiation and restenosis: Serum-response-factor (SRF) plays a key role in SMC differentiation. SRF is a transcription factor that in concert with SMC-specific co-factors of the myocardin-like family of proteins regulates the expression of SMC-specific genes. Potent activators of SRF are bioactive lipids, such as lysophosphatidic acid, sphingosylphosphorylcholine and sphingosine-1-phosphate (S1P). Our work focuses on S1P, which is recognized by SMCs through three receptors, S1P1, S1P2, and S1P3. All S1P receptors are G protein-coupled receptors that are linked to different G alpha subunits and thus, activate different signal transduction pathways.

Genetic ablation of S1P receptors in mice revealed that S1P1 is required for arterial development whereas mice without S1P2 or S1P3 develop normally. S1P2 was initially of interest to us since it is the only S1P receptor that activates the small GTPase Rho, which is required for SRF-dependent expression of SMC differentiation genes. To investigate whether S1P2 expression affects the response to arterial injury, we compared lesion formation after ligation of the left common carotid in wild-type and S1P2 knock-out mice. The difference between the two mice was dramatic. Wild-type mice did not form significant lesions, whereas S1P2-deficient arteries developed large lesions between 2 and 4 weeks after injury (Fig. 1). In both arteries, injury induced proliferation of medial cells. This event was transient in the wild-type artery, whereas it was continuous in the S1P2-deficient artery (Fig. 2-3).

This observation suggests that the onset of SMC activation is similar in both arteries, and that S1P2 in the wild-type vessel is responsible for the transient nature of SMC activation. Consistent with our hypothesis, we found that S1P induces SMC differentiation genes in wild-type but not in S1P2-deficient SMCs. We are currently investigating the molecular mechanisms of this process and our goal is to define a role for S1P2-induced stimulation of SRF in our mouse injury model.

CONCLUSION: Our work suggests that S1P2 regulates an SRF-dependent differentiation program in SMCs that terminates SMC proliferation and migration after injury, and thus, prevents intimal growth. We consider stimulation of SMC differentiation an intriguing possibility to limit intimal formation because such approach should have few side effects as it lacks general cytotoxicity.

Common to all growing intimae is that cells proliferate; the critical question is why?

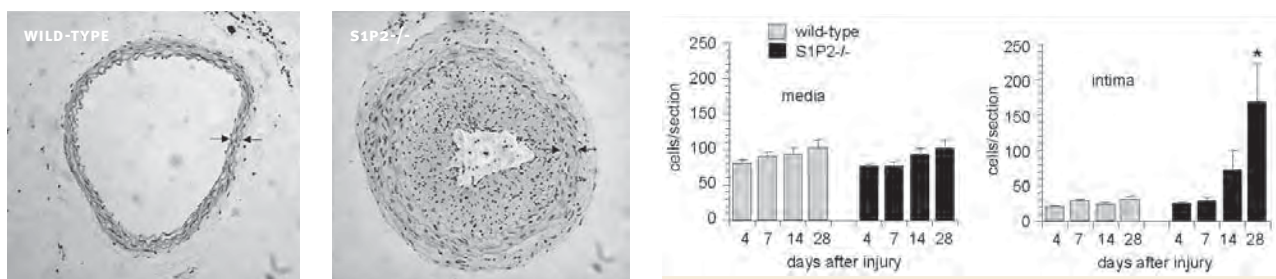


FIGURE LEGEND: Littermates of wild-type and S1P2-null mice underwent carotid ligation injury (4-6 animals/data point). Mice were injected intraperitoneally with bromodeoxyuridine (BrdU, 30 μ g/g body weight) at 1, 9 and 17 hours before sacrifice. Mice were perfusion-fixed with 4% paraformaldehyde in PBS and carotid arteries were harvested. Sections were immunohistochemically stained. Hematoxylin-positive (total) and BrdU-positive (replicating) cells were counted on 8 arterial sections (100 μ m apart). (A) A typical cross section at 28 days after injury is presented. Arrows mark external and internal lamina, which define the media boundaries. Data for medial and intimal cell number (B) and BrdU index (labeled nuclei/total nuclei \times 100) (C) are shown (mean \pm SEM, n=4-6). *P<0.05. P was calculated by unpaired t-test and indicates significance of difference between wild-type and S1P2 null mice at a given time point after injury.

RELATED PUBLICATIONS

1. Englesbe MJ, Hawkins SM, Hsieh PC, Daum G, Kenagy RD, Clowes AW. Concomitant blockade of platelet-derived growth factor receptors alpha and beta induces intimal atrophy in baboon PTFE grafts. *J Vasc Surg.* 2004; 39:440-6.
 2. Englesbe MJ, Deou J, Bourns B, Clowes AW, Daum G. IL-1b inhibits PDGF-BB-induced migration by cooperating with PDGF-BB to induce COX2 expression in baboon aortic smooth muscle cells. *J Vasc Surg.* 2004; 39:1091-96.
 3. Englesbe MJ, Davies MG, Hawkins SM, Hsieh PC, Daum G, Kenagy RD, Clowes, AW. Arterial injury repair in nonhuman primates-the role of PDGF receptor-beta. *J Surg Res.* 2004; 119:80-84.
 4. Chen L, Daum G, Chitaley K, Coats SA, Bowen-Pope DF, Eigenthaler M, Thumati NR, Walter U, Clowes, AW. Vasodilator-stimulated phosphoprotein regulates proliferation and growth inhibition by nitric oxide in vascular smooth muscle cells. *Arterioscler Thromb Vasc Bio.* 2004; 24:1-6.
 5. Millette E, Rauch BH, Defawe O, Kenagy RD, Daum G, Clowes AW. Platelet-derived growth factor-BB-induced human smooth muscle cell proliferation depends on basic FGF release and FGFR-1 activation. *Circ Res.* 2005; 96:172-179.
 6. Rauch, BH, Millette E, Kenagy RD, Daum G, Fischer JW, Clowes AW. Syndecan-4 is required for thrombin-induced migration and proliferation in human vascular smooth muscle cells. *J Biol Chem.* 2005; 280:17507-17511.
 7. Millette E, Rauch BH, Kenagy RD, Daum G, Clowes AW. Platelet-derived growth factor-BB transactivates the fibroblast growth factor receptor to induce proliferation in human smooth muscle cells. *Trends Cardiovasc Med* 2006; 16:25-28.
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DEPARTMENT CO-INVESTIGATORS

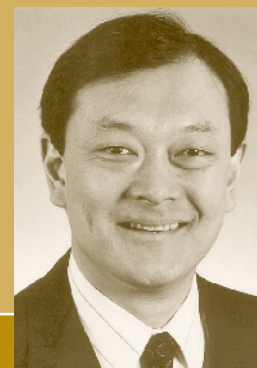
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Thomas Hatsukami, M.D.

· Magnetic Resonance Imaging of the High-Risk Atherosclerotic Plaque



FUNDING

AstraZeneca

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Over 16.7 million people die of cardiovascular disease each year – one person every 2 seconds. Our primary goal is to develop and validate high-resolution imaging methods that will improve our ability to identify individuals at highest risk. Furthermore, by allowing us to non-invasively visualize the diseased vessel wall, these imaging tools will enable us to assess the effectiveness of novel therapies for CVD.

Introduction

Cardiovascular disease (CVD) is the number one cause of death worldwide and is a leading cause of long-term disability. It is estimated that the annual cost for the care of victims of CVD is over \$390 billion per year in the U.S. alone. Most CVD events, such as heart attack and stroke, are atherosclerosis-related. Traditionally, the degree of vessel lumen narrowing has been used to identify the high-risk atherosclerotic plaque. However, there is increasing evidence that the structure, composition, and inflammatory activity of the atherosclerotic lesion are more important markers of the vulnerable plaque. Progress in understanding how vulnerable plaques develop has been hindered by our inability to serially examine these critical characteristics of the diseased vessel wall in a non-invasive fashion.

The mission of our research group is to advance high-resolution magnetic resonance imaging (MRI) technology for accurate, non-invasive examination of atherosclerosis. Our laboratory is organized along five core functions: 1) Imaging Physics: develop novel image acquisition techniques; 2) Histology: provide the histological gold-standard for validation of MRI findings; 3) Imaging Software: build custom-designed tools that permit more efficient, reproducible, quantitative image analysis; 4) Clinical Studies: apply



FIGURE 1: MRI of right and left internal and external carotid arteries demonstrating good suppression of flow artifact and clear delineation of the lumen and outer boundary of normal (right) and diseased (left) carotid arteries. Note evidence of compensatory (expansive) enlargement on the left side. The cross-sectional area of the lumen is similar on both sides, yet there is significantly greater plaque burden on the left. ICA = internal carotid artery; ECA = external carotid artery



FIGURE 2: Special care is taken to excise the carotid plaque intact, with minimal handling and disruption of the overall morphology and architecture of the lesion. Photo of the gross specimen in the left panel; Trichrome stain of serial cross-sections of the specimen from common carotid on left toward common carotid bifurcation on the right. Note the rupture of the fibrous cap in the sections near the carotid bifurcation.

MR imaging techniques to understand mechanisms leading to development of the vulnerable plaque; and 5) Reading Center: provide training, quality control, and image analysis for multi-center clinical trials using MRI.

Validation

Significant improvements in MR image quality have been made possible by a combination of hardware development and novel image acquisition sequences (Figure 1). The accuracy of this high-resolution MRI technique has been extensively validated by comparing pre-operative carotid MRI findings to matched histological sections of the

excised plaque (Figure 2). We have shown that MRI can categorize carotid plaque types according to established American Heart Association histological classification criteria (Table I), with a weighted Kappa of 0.79, indicated very good agreement between MRI and histology (*Circulation* 2002; 106:1368). Furthermore, we have shown that MRI can accurately identify the presence and precisely quantify the size of critical features of the vulnerable plaque, as defined by an expert panel (*Circulation* 2003; 108:1664). These features include the degree lumen narrowing and overall plaque burden (*Circulation* 1998; 98:2666 and *Magnetic Resonance in Medicine* 2000; 44:968), fibrous cap thinning and rupture (Figure 3; *Circulation* 2000; 102:959), the lipid-rich necrotic core and intraplaque hemorrhage (Figure 4; *Arteriosclerosis, Thrombosis and Vascular Biology* 2005; 25:234), and the degree of neovascularity and inflammatory cellular infiltration of the plaque (Figure 5; *Circulation* 2003; 107:851 and *Radiology* 2006; 241:459).

Automated Quantitative Image Analysis

Analysis of the MR images is a time-consuming process, with approximately 70 high-resolution images generated for each artery. In order to perform large-scale clinical studies, automated, quantitative image analysis tools are needed, which would improve reproducibility and efficiency. Our lab has developed a probability based segmentation method that utilizes morphological information, such as local wall thickness, coupled with active contours, to limit the impact from noise and artifacts associated with in vivo imaging (Figure 6). In experiments involving 142 sets of multi-contrast images from 26 subjects undergoing carotid endarterectomy, segmented areas of the lipid-rich necrotic core, calcification, loose matrix and fibrous tissue on MRI agreed with areas on the corresponding histological section with correlations (R^2) of 0.78, 0.83, 0.41 and 0.82, respectively. In comparison, areas outlined by expert MRI readers blinded to histology yielded correlations of 0.71, 0.76, 0.33 and 0.78, respectively (*Magnetic Resonance in Medicine* 2006; 55:659).

Clinical Studies

With funding from the National Institutes of Health, we have enrolled over 300 individuals over the past seven years in a prospective study, where participants undergo high-resolution MRI examination of their carotid arteries every 18 months. This study has demonstrated that arteries with intraplaque hemorrhage are associated with more rapid progression in overall plaque and lipid-rich necrotic core size (*Circulation*

2005; 111:2768). The percent change in wall volume over 18 months was 6.8% amongst those with intraplaque hemorrhage, compared to -0.15% for those without hemorrhage ($p = 0.009$). The lipid-rich necrotic core increased by 28.4% in plaques with hemorrhage, compared to -5.2% in those without hemorrhage ($p = 0.001$). Furthermore, those with intraplaque at baseline were much more likely to develop new plaque hemorrhages during follow-up, compared to controls (43% versus 0%, $P = 0.006$).

Lesion Type	Definition
I–II	Isolated foam cells or small foam cell layers
III	Pre-atheroma: small extracellular lipid pools
IV–V	Atheroma/Fibroatheroma: confluent lipid core with surrounding fibrous tissue
VI	Complicated lesion: surface defect, hemorrhage or thrombus
VII (Vb)	Predominantly calcified plaque
VIII (Vc)	Predominantly fibrotic plaque

TABLE I: Modified American Heart Association (AHA) classification scheme for describing atherosclerosis lesion types.

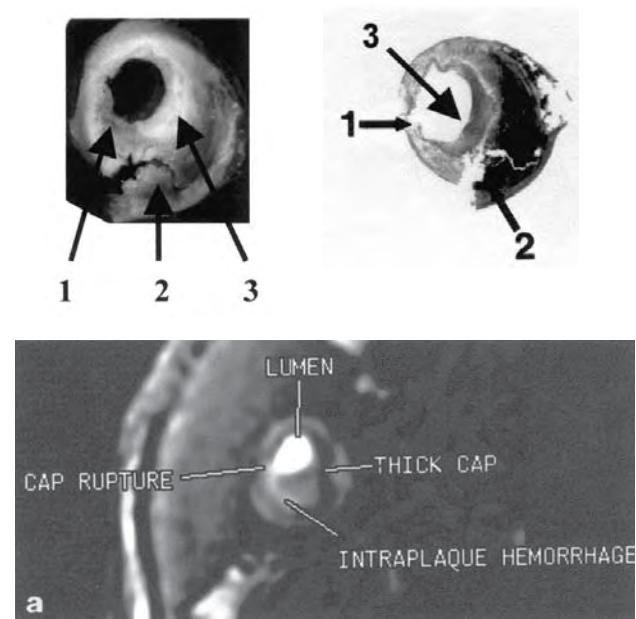


FIGURE 3: Example of a common carotid plaque with fibrous cap rupture and intraplaque hemorrhage. Photo of gross section of common carotid artery (left panel), trichrome stained histological section (middle panel), and corresponding TOF MR image (right panel). Arrow 1 indicates an area of cap rupture, arrow 2 = intraplaque hemorrhage, and arrow 3 = area of thick, collagen-rich fibrous cap. The thick cap appears as a dark band adjacent to the lumen on MRI. The dark band is absent, and there is adjacent hyperintense signal in the region of cap rupture.

Our lab has developed a probability based segmentation method that utilizes morphological information, such as local wall thickness, coupled with active contours, to limit the impact from noise and artifacts associated with in vivo imaging.

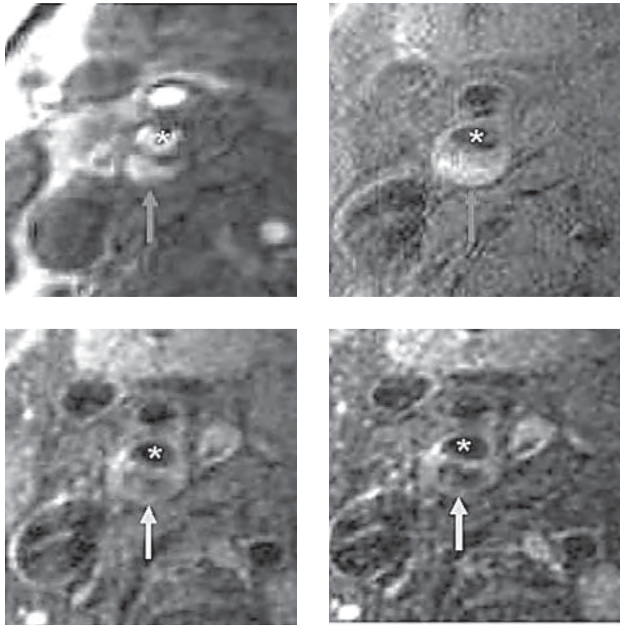


FIGURE 4: Example of an AHA Type VI (complicated) lesion with acute hemorrhage into the lipid-rich necrotic core. The asterisks indicate the lumen of the internal carotid artery. Early intraplaque hemorrhage, seen on the corresponding histological cross-section on the right, is identified by a hyperintense (bright) signal on time-of-flight (TOF) and T1-weighted (T1W) MR images, and relatively hypointense (dark) on the proton density- (PDW) and T2-weighted (T2W) images.

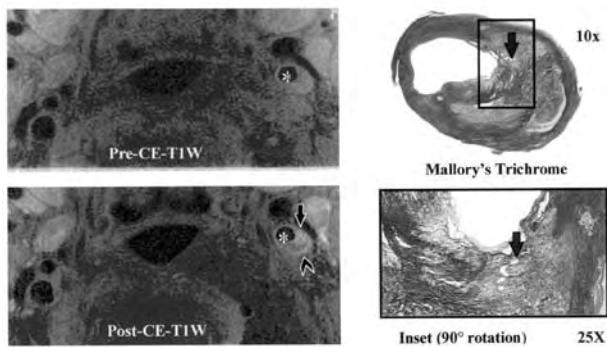


FIGURE 5: Pre-gadolinium contrast enhanced T1-weighted image of common carotid artery in left upper panel, post-contrast enhanced T1W image in left lower panel, and corresponding 10X and 25X trichrome stained histological sections. Note the enhancement seen in the shoulder region (arrow) in the post-contrast enhanced image. This enhancing region demonstrates abundant development of neovasculature and inflammatory cell infiltration on the corresponding histological section.

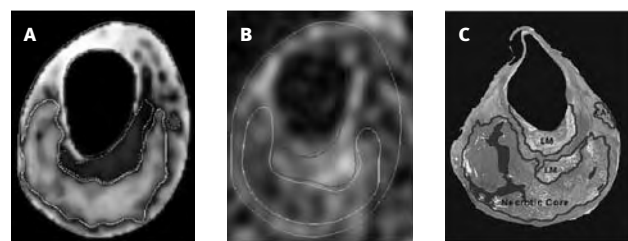


FIGURE 6: Segmentation results showing (a) automated quantitative image analysis tool; (b) manual outline by expert reviewer; and (c) corresponding histology section demonstrating a large necrotic core, loose matrix (LM) and a small area of calcification (CA). The dark regions within the necrotic core on the histology specimen are artifacts due to sectioning.

We have also shown that specific plaque characteristics, as identified by MRI, are associated with the development of subsequent transient ischemic attack (TIA) or stroke. A significant association was found between presence of a thin or ruptured fibrous cap (hazard ratio, 17.0; $p < 0.001$), intraplaque hemorrhage (hazard ratio, 5.2; $p = 0.005$), larger mean intraplaque hemorrhage area (hazard ratio, 2.6; $p = 0.006$), larger maximum %lipid-rich/necrotic core (hazard ratio for 10% increase, 1.6; $p = 0.004$), and larger maximum wall thickness (hazard ratio for a 1 mm increase, 1.6; $p = 0.008$) (*Stroke* 2006; 37:818).

Finally, we have examined the natural history of plaque volume progression and vessel wall remodeling in our study, and have found that the diseased wall area increases by 2.3%/year ($P = 0.004$). We noted that earlier stage lesions are associated with a significant increase in wall area without a corresponding decrease in lumen area (*Atherosclerosis* 2007, in press). Therefore, this prospective study confirms the hypothesis that compensatory enlargement of the outer boundary of the vessel wall (positive remodeling) accom-

modates plaque growth without encroachment of the lumen, and that imaging techniques that focus on lumen narrowing will underestimate overall plaque burden. Furthermore, we have shown that use of LDL-cholesterol lowering drugs ("statins") are associated with a significantly lower rate of progression, compared to individuals not on statins (mean wall area progression rate 1.2% vs. 4.4% per year; $P = 0.02$).

Conclusions

Magnetic resonance imaging is a promising tool for studying the pathophysiology of human atherosclerosis progression and regression in vivo. In addition to precisely assessing plaque burden, MRI is capable of accurately classifying disease according to established AHA criteria, and identifying critical plaque features such as the fibrous cap and neovasculature. A better understanding of disease mechanisms and factors leading to more rapid progression will permit identification of high-risk individuals for more aggressive treatment, and potentially lead to the development of novel methods for therapeutic intervention.

RELATED PUBLICATIONS

1. Nelson JA, Yuan C, Hatsukami TS. MR cardiovascular imaging. *Nature Medicine* 1995; 1:996-997.
2. Yuan C, Beach KW, Smith HL, Hatsukami TS. Measurement of atherosclerotic plaque size in vivo using high-resolution magnetic resonance imaging. *Circulation* 1998; 98(24):2666-2671.
3. Hatsukami TS, Ross R, Yuan C. Visualization of Fibrous Cap Thickness and Rupture in Human Atherosclerotic Carotid Plaque In-vivo with High Resolution Magnetic Resonance Imaging. *Circulation* 2000; 102:959-964.
4. Yuan C, Mitsumori LM, Ferguson MS, Polissar NL, Echelard D, Ortiz G, Small R, Davis JW, Hatsukami TS. The in vivo accuracy of multispectral MR imaging for identifying lipid-rich necrotic cores and intraplaque hemorrhage in advanced human carotid plaques. *Circulation*, 2001;104:2051-6.
5. Yuan CY, Zhang S, Polissar N, Echelard D, Ortiz J, Davis JW, Ellington E, Hatsukami TS. Identification of fibrous cap rupture with magnetic resonance imaging is highly associated with recent TIA or stroke. *Circulation*, 2002; 105(2):181-185.
6. Yuan C, Miller ZE, Cai JM, Hatsukami T. Carotid Atherosclerotic Wall Imaging by MRI. In: Fayad ZA ed. *Neuroimaging Clinics of North America* 12(3): 391-401, vi, 2002. Philadelphia: W.B. Saunders Company, 2002.
7. Saam T, Ferguson MS, Yarnykh VL, Takaya N, Xu D, Polissar NL, Hatsukami TS, Yuan C. Quantitative evaluation of carotid plaque composition by in vivo MRI. *Arterioscler Thromb Vasc Biol.* 2005;25:234-9.
8. Takaya N, Yuan C, Chu BC, Saam T, Polissar NL, Jarvik GP, Isaac C, McDonough J, Natiello C, Small R, Ferguson M, Hatsukami TS. Presence of intraplaque hemorrhage stimulates progression of carotid atherosclerotic plaques: A high-resolution MRI study. *Circulation* 111:2768-2775, 2005.
9. Takaya N, Yuan C, Chu B, Saam T, Underhill H, Cai J, Tran N, Polissar NL, Isaac C, Ferguson M, Garden GA, Cramer SC, Maravilla KR, Hashimoto B, Hatsukami TS. Association between carotid plaque characteristics and subsequent ischemic cerebrovascular events: A prospective assessment with magnetic resonance imaging – initial results. *Stroke* 2006; 37:818-23.
10. Kerwin WS, O'Brien K D, Ferguson MS, Polissar N, Hatsukami TS, Yuan C. Inflammation in Carotid Atherosclerotic Plaque: A Dynamic Contrast-enhanced MR Imaging Study. *Radiology.* 2006;241:459-468.

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Ted R. Kohler, M.D., M.Sc.

- Healing of Prosthetic Vascular Grafts
- Preventing Dialysis Access Failure
- Factors Involved in Restenosis
- Endovascular Repair of Abdominal Aortic Aneurysms

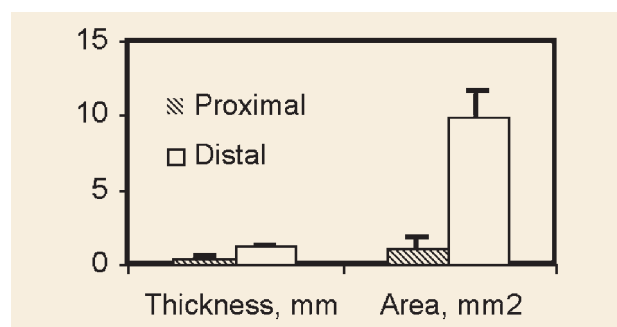


FUNDING

National Institutes of Health

Healing of Prosthetic Vascular Grafts

Vascular surgery has made tremendous advances in the last few decades. Bypass grafts, angioplasty, stents, and stent grafts are now standard treatment for arterial insufficiency and aneurysm disease in peripheral arteries. However, long-term success of these procedures, particularly small diameter prosthetic devices, is limited due to thrombosis and restenosis. Part of the problem is the lack of a natural, non-thrombogenic, biologic lining. Prior work in the Clowes lab has shown that increased porosity of standard polytetrafluoroethylene (PTFE) bypass grafts when placed in non-human primates can allow complete endothelialization of the graft surface by capillary ingrowth along the length of the graft. We tested these grafts in humans, placing composite bypasses made of standard and high-porosity PTFE in the femoropopliteal position (Figure 1). Unfortunately, there was no evidence of capillary ingrowth as measured by the surrogate marker labeled platelet activity. Subsequent studies have shown that capillary ingrowth may be dependent on species, age, and the nature of the surrounding tissues. In collaboration with Dr. Sobel, we are studying ways to enhance graft healing by addition of natural matrix components and growth factors (such as vascular endothelial growth factor) using a canine model of PTFE grafts placed in the carotid position. These grafts behave like those in our clinical trial; they fail to endothelialize. It is hoped that pretreatment will provide a more robust response and endothelialization.



GRAPH 1: Sheep Eight Week Data

Preventing Dialysis Access Failure

Effective renal dialysis requires several hundred milliliters per minute of blood flow. To accomplish this, a fistula is created between an artery and vein, typically in the arm (Figure 2). This provides a high-flow conduit just under the skin surface where it can be accessed by needle puncture. Unfortunately, these fistulae have a high failure rate, even higher than other vascular grafts. Re-operation for failed access is a major cause of morbidity, prolonged hospital stay, and increased cost in the treatment of renal failure. Most access failures are caused by intimal hyperplasia at the venous end of the graft. This is very surprising since in animal models we have found that increased blood flow reduces wall thickening after placement of prosthetic arterial grafts.

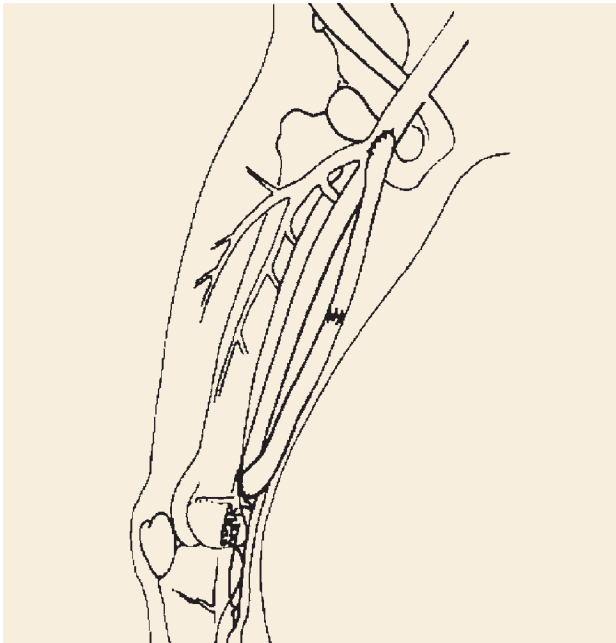


FIGURE 1: PTFE in the femoropopliteal position

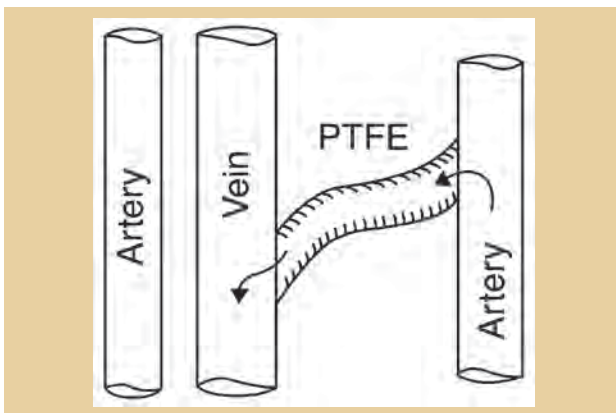


FIGURE 2: Fistula created between artery and vein to provide high-flow conduit

We are studying this problem in an animal model. PTFE grafts like those used in humans are placed in the neck of sheep, and measurements are made of the narrowing at the junction of the graft and native vessels. We have found that standard grafts fail within two to three months due to narrowing, which is much more pronounced at the venous end (Graph 1). Active thrombosis along the graft surface, particularly at the venous end, appears to be a

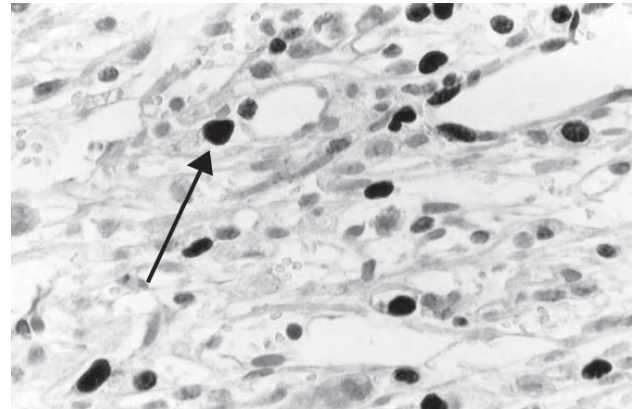


FIGURE 3: PCNA-positive nucleus

major contributing factor. Thickening is greatly reduced if the grafts are sewn into an artery instead of a vein, even if blood flow is increased by creation of an artery-to-vein fistula beyond the graft. We have also found that special coating of the graft surface with phospholipids can stop this thickening process.

The three principle components of graft healing and lumen narrowing are endothelial ingrowth, smooth muscle cell proliferation, and thrombosis. These are evaluated using scanning electron microscopy, morphometry, and immuno-histochemistry. We can also use simulated dialysis to assess the potential role in graft failure of the various components of the dialysis procedure.

Like the clinical specimens, the sheep lesions have focal regions of prominent cellular proliferation, often adjacent to thrombus and in granulation tissue surrounding the graft. This can be seen in Figure 3, showing a proliferating-cell-nuclear-protein (PCNA)-positive nucleus marked by an arrow.

Organizing thrombus contributes significantly to luminal narrowing. The continued presence of thrombus and high rates of cellular proliferation suggest ongoing injury as an important cause of lesion formation. Rapid development of lesions morphologically similar to lesions makes this model uniquely suited for study of the cellular mechanisms of dialysis failure.

Restenosis following revascularization or dialysis access is a major cause of morbidity, prolonged hospital stay, and increased cost.

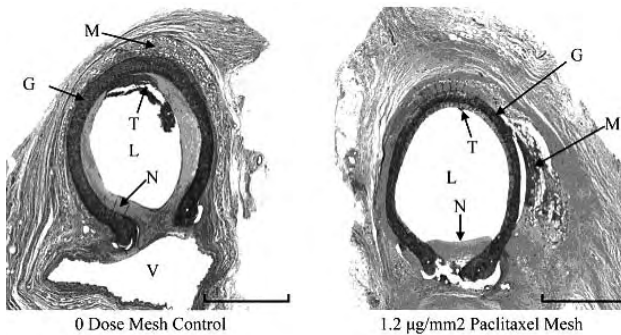


FIGURE 4: Example of a section through the toe of the graft at low power showing where capillary density in the neointima is greatest (bar represents 4 mm). The inset is a high power (bar represents 400 μ m) from this region demonstrating multiple capillaries (arrow).

TABLE 1

Location	Tissue Factor Activity (+/SD)	Tissue Factor Protein	Fibrin
Normal Artery	22.0 \pm 18.0	–	–
Graft near Artery	113.5 \pm 10.9 *	++	+
Graft near Vein	194.5 \pm 15.2 *	+++	+
Normal Vein	32.0 \pm 1.5	–	–

(N=4. *= $p < .05$, one-tailed Mann-Whitney comparison)

Paclitaxel is an effective drug that prevents smooth muscle cell growth and migration and inhibits inflammation. It is one of the drugs that is effective in preventing restenosis in drug-eluting coronary artery stents. We have now demonstrated that a bioabsorbable mesh containing paclitaxel can prevent intimal hyperplasia and narrowing of the venous end of PTFE access grafts in sheep (Figure 4 shows the venous end of these grafts at eight weeks in control and drug-treated animals). Dr. Kohler is currently consulting with AngioTech Pharmaceuticals, Inc. to start a clinical trial with this promising new approach.

Factors Involved in Restenosis

Restenosis following revascularization or dialysis access is a major cause of morbidity, prolonged hospital stay, and increased cost. Intimal hyperplasia causes failure of almost one-third of all vascular reconstructions. This process results from wall thickening due to smooth muscle cell proliferation narrowing the lumen. Inflammation caused by local trauma at the site of reconstruction (stent, atherectomy, angioplasty, or bypass) causes proliferation and migration of myofibroblasts and constriction of the vessel lumen by remodeling of the surrounding tissue. Endothelial damage causes local thrombosis that enhances this process and contributes scaffolding for further neointimal formation.

Much research has been devoted to understanding the cellular pathology of this process and to developing ways to combat it with drugs (like paclitaxel and heparin), new devices, and genetic modification of the cells involved. In collaboration with Drs. Sobel and Clowes, we are prospectively studying patients who are undergoing vein graft bypasses. We will compare inflammatory and thrombotic responses and the propensity of explanted smooth muscle cells to proliferate in patients who do and do not develop restenosis. We hope to find ways to predict which patient will develop this problem and to gain a better understanding of the process so we can target patients at high risk for failure with better preventive therapies.

Endovascular Repair of Abdominal Aortic Aneurysms

Endovascular therapy uses a catheter-based delivery system rather than conventional open techniques. Patient morbidity and hospital stay are dramatically decreased. Endovascular grafts are held open and in proper position by attached metallic stents and are placed by a simple arterial cutdown or, in some cases, percutaneously. These devices have been very successful in mid term clinical trials yet it remains to be seen if they will perform as well over the long term (decades) as conventional grafts. The primary concern is whether or not the devices will remain well attached to the native artery at either end despite the native vessel's

tendency to dilate over time. Dr. Kohler and Dr. David Glickerman, from interventional radiology, began the endovascular therapy program at the Seattle VA in 1999. We are a site for the VA Cooperative Trial of Open versus Endovascular Repair of Abdominal Aortic Aneurysms (Dr. Kohler is on the Executive Board). This trial will recruit 900 patients randomized to one or the other approach. Five-year follow-up will include analysis of cost as well as morbidity, mortality, and effectiveness in preventing death from aneurysm rupture.

RELATED PUBLICATIONS

1. Mattsson E, Kohler TR, Vergel S, Liao JK, Clowes AW. Increased Blood Flow Induces Regression of Intimal Hyperplasia. *Arterio Thromb Vasc Biol* 17:2245-2249, 1997.
2. Kohler TR, Kirkman TR. Central venous catheter failure is induced by injury and can be prevented by stabilizing the catheter tip. *J Vasc Surg* 28:59-66, 1998).
3. Kohler TR, Kirkman TR. Dialysis access failure: A sheep model of rapid stenosis. *J Vasc Surg* 30:744-51, 1999.
4. Gibson KD, Caps MT, Kohler TR, Hatsukami TS, Gillen DL, Aldassy M, Sherrard DJ, Stehman-Brenn CO. Assessment of a Policy to Reduce Placement of Prosthetic Hemodialysis Access. *Kid Int* 59:2335-45, 2001.
5. Fontaine AB, Nicholls S, Borsa JJ, Hoffer E, Bloch RD, Kohler TR. Seat Belt Aorta: Endovascular Management with a Stent-Graft. *J Endovasc Ther* 8:83-86, 2001.
6. Leotta DF, Paun M, Beach KW, Kohler TR, Zierler RE, Strandness DE Jr. Measurement of Abdominal Aortic Aneurysms using Three-Dimensional Ultrasound Imaging: Preliminary Report. *J Vasc Surg* 33:700-7, 2001.
7. Gibson KD, Stehman-Brenn CO, Kohler TR. Use of the vascular diagnostic laboratory in improving the success of angioaccess procedures. *Sem Vasc Surg* 14:222-26, 2001.
8. Gibson KD, Gillen DL, Caps MT, Kohler TR, Sherrard DJ, Stehman-Breen CO. Vascular access and incidence of revisions: A comparison of prosthetic grafts, simple autogenous and venous transposition fistulas from the United States Renal Data System Dialysis Morbidity and Mortality Study (USRDS DMMS). *J Vasc Surg* 34:694-700, 2001.
9. Yutan E, Glickerman DJ, Caps MT, Hatsukami T, Harley JD, Kohler TR, Davies MG. Percutaneous transluminal revascularization for renal artery stenosis: VA Puget Sound Health Care experience. *J Vasc Surg* 34:685-93, 2001.
10. Leotta DF, Paun M, Beach KW, Kohler TR, Zierler RE, Strandness DE Jr. Measurement of Abdominal Aortic Aneurysms using Three-Dimensional Ultrasound Imaging: Preliminary Report. *J Vasc Surg* 33:700-7, 2001.
11. Kohler TR, Toleikis PM, Gravett DM, Avelar RL. Inhibition of Neointimal Hyperplasia in a Sheep Model of Dialysis Access Failure with the Bioabsorbable Vascular Wrap™ Paclitaxel Eluting Mesh. *J Vasc Surg* (in press).
12. Kohler TR, Toleikis PM, Gravett DM, Avelar RL. Inhibition of Neointimal Hyperplasia in a Sheep Model of Dialysis Access Failure with the Bioabsorbable Vascular Wrap™ Paclitaxel Eluting Mesh. *J.Vasc.Surg.* 2007;45:1029-38.
13. Nelson KM, Reiber GE, Boyko, EJ, Kohler TR, Peripheral vascular disease in a multiethnic national sample: Data from the National Health and Nutrition Examination Survey, 1999-2002. Ethnicity in Disease. (in press)

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