SURGERY RESEARCH

DEPARTMENT OF SURGERY 2006 REPORT

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

2006 REPORT

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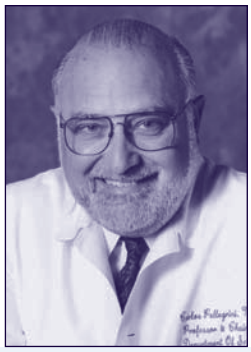

Discovering and Distributing Scientific Knowledge

Without exception, surgical procedures significantly advanced during the major wars and events of the 20th century. It was during the World Wars, for instance, that remarkable progress occurred in the disciplines of trauma, blood transfusion, and reconstructive surgery. Then the Space Age ushered in amazing discoveries and instruments such as ultrasound and the laser, thus paving the way for solid organ and soft tissue transplantation. Next we witnessed an explosion of diagnostic and disease treatment protocols from information unlocked by the genome research project. Throughout it all, one unchanging goal of the academic surgical community has been to ensure that this wealth of knowledge is easily accessible to *all* medical practitioners.

As a community resource and a regional referral center, our Department takes this responsibility seriously. Our faculty are experts in their respective specialties and are well known for their willingness and ability to collaborate with colleagues throughout the WWAMI region. It is, in fact, most likely that you know someone who has benefited from the UW telemedicine and teleconferencing routinely utilized by our community surgeons.

Moreover, our partnerships with other departments throughout the University system continue to increase. **The Institute for Surgical and Interventional Simulation** (ISIS), an educational and research center specializing in simulation technology, is an interdisciplinary effort of the Human Interface Technology Lab, School of Engineering and our Surgical Simulation Working Group. The **Center for Videoendoscopic Surgery** provides training and conducts research in minimally invasive surgical techniques; we are partnered with the UW BioRobotics Lab, other UW departments, and corporate sponsors. The **Swallowing Center** is a multidisciplinary specialty clinic headed by the Department of Surgery that evaluates people who suffer from gastroesophageal reflux disease or other swallowing disorders. The **Surgical Outcomes Research Center** (SORCE) is a new interdisciplinary center devoted to translational research on outcomes knowledge and changes of practice. The key to our success as a department lies in our ability to build new programs with colleagues throughout the entire UW system.

The driving force behind these innovative programs is our vigorous scientific research and investigation. Many of our faculty with national and international reputations for excellence in research were recognized for their investigative work this past year. A selection of these honors and awards are in the sidebar that accompanies this letter.

Our job is to provide you, your family, and your friends with the very best in surgical care. We accomplish this in two ways. First, we continue to discover new tools and new ways to meet the challenges of disease. Then, we ensure that this knowledge is made available to 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).

Carlos A. Pellegrini
The Henry N. Harkins Professor and Chairman

SELECTED RESEARCH HONORS & AWARDS FROM 2005

STEPHANIE ACIERNO, M.D., (THEN) R4, placed second in the clinical research category at the National ACS Committee on Trauma resident paper competition. Dr. Acierno was one of 13 residents competing, each having already won a regional competition, to represent the best trauma-related research in North America. Her paper, titled "An Estimation of the Number of Deaths and Severe Injuries that Might Be Prevented by Addressing Incompatibility between Passenger Vehicles and Light Truck Vehicles," sparked a great deal of interesting discussion.

EILEEN BULGER, M.D., ASSOCIATE PROFESSOR, received the *Peter Canizarro Award* from the American Association for the Surgery of Trauma for her paper entitled "The Use of Neuromuscular Blocking Agents to Facilitate Prehospital Intubation does not Impair Outcome following Traumatic Brain Injury."

ALEXANDER W. CLOWES, M.D., PROFESSOR AND CHIEF, DIVISION OF VASCULAR SURGERY, received the notable *Flance-Karl Award* from the American Surgical Association. The award, established in 1996 in recognition of Drs. I. Jerome Flance & Michael M. Karl, is presented to the surgeon who has made a seminal contribution in basic laboratory research with application to clinical surgery. It includes a stipend of $35,000. Dr. Clowes was also elected Chair of the Surgical Research Committee of the American College of Surgeons.

GORDON A. COHEN, M.D., PH.D., ASSOCIATE PROFESSOR, was awarded $156,000 from the McMillen Foundation to fund a pediatric ventricular assist device (VAD) program. The goal is to place implantable VADs in pediatric patients as a long-term bridge to transplant or recovery. Although this is not new to the adult population, there is sparse worldwide experience with this in the pediatric population and only a handful of patients have had devices placed in the USA. The funding facilitates a leading edge approach in pediatric cardiac care.

JOSEPH S. GRUSS, M.B., B.CHIR., PROFESSOR, presented the *D. Ralph Millard Lecture* at the International Symposium on Pediatrics sponsored by the Miami Children's Hospital. Dr. Gruss addressed skull abnormalities in children.

RICHARD A. HOPPER, M.D., ASSISTANT PROFESSOR, was the invited INCO Visiting Professor at the 2004 Lindsey-Thomson Pediatric Craniofacial Symposium at the Toronto Hospital for Sick Children — the largest craniofacial conference in Canada.

LORRIE A. LANGDALE, M.D., ASSOCIATE PROFESSOR, was awarded a three-year, $500,000 VA Merit Review Grant for her proposal, "Mechanisms of Injury Control after Liver Ischemia-Reperfusion." Building on her work from a recent research sabbatical, she will explore novel signaling pathways that modulate the hepatic response to injury. She was also awarded a one-year, $35,000 UW Royalty Research Grant to study the effects of SOCS3 gene deletion on the evolution of liver ischemia-reperfusion injury.

CARLOS A. PELLEGRINI, M.D., THE HENRY N. HARKINS PROFESSOR AND CHAIRMAN, was elected President of the American Surgical Association, the oldest and most prestigious organization of surgeons in the United States.

MICHAEL SOBEL, M.D., PROFESSOR, was awarded an NIH grant titled "Modulating Endothelialization of Cardiovascular Grafts." The research group, which includes **Drs. Ted Kohler & Errol Wijelath,** is developing novel molecules that enhance the natural endothelialization of prosthetic bypass grafts, both through capillary ingrowth and the differentiation of adult stem cells.

ERIK VAN EATON, M.D., (THEN) RESEARCH FELLOW, presented results of the UW Cores (Computerized Rounding and Sign-Out) system he designed at the annual meeting of the Association of American Medical Colleges. The journal *Surgery* featured Dr. Van Eaton's work in their July 2004 issue.

DR. RAYMOND YEUNG, PROFESSOR, was featured on the cover of *Pediatric Research* for his investigative work titled "Effects of Rapamycin in the Eker Rat Model of Tuberous Sclerosis Complex." Dr. Yeung's work is supported by two NIH grants.

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

3F Therapeutics, Inc.
COAP
Edwards Cardiovascular
The Medicines Company
Medtronic, Inc.
National Institutes of Health
• National Research Service Award in Heart and Vascular Diseases
Proctor and Gambel (Alexion)
St. Jude

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 biological response of the body to artificial perfusion and gas exchange through the non-endothelialized cardiopulmonary bypass (CPB) circuit.

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 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.

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.

These different approaches result in markedly different effects on inflammation and thrombin generation during artificial perfusion.

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 systemic circulation).

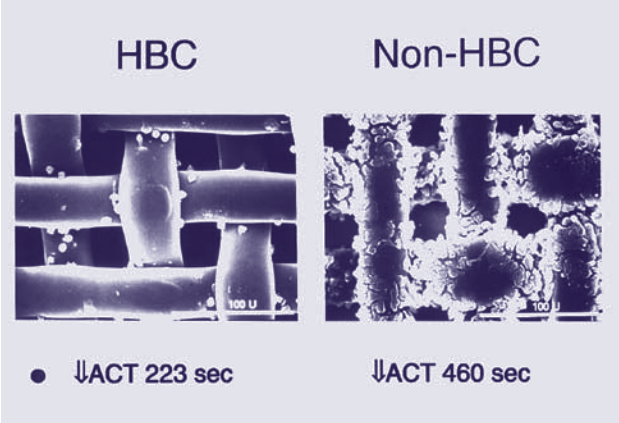

FIGURE 1: Scanning electron micrographs at 200 fold magnification of arterial filter. Lowest activating times (ACT) in seconds are noted. HBC= heparin-bonded circuits. Non-HBC- control non-heparin-bonded circuits.

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 cardiotomy suction vs. use of cell saver technology) on markers of hemostasis, inflammation, neurological and neuro-psychological deficits. Although both result in blood conservation, one (cardiotomy 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 (ß-thromboglobulin) and neurological injury (neuron specific enolase, S-100b) are all nearly completely blunted when HBC are used and cardiotomy suction is eliminated during CPB. Our results suggest that cardiotomy suction should be eliminated whenever possible. Our results challenge

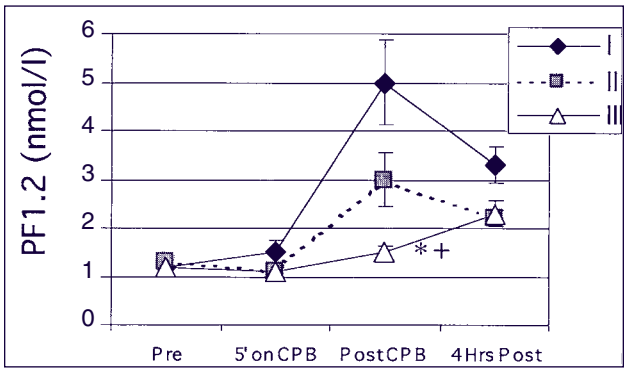

FIGURE 2: PF1.2 for thrombin generation.

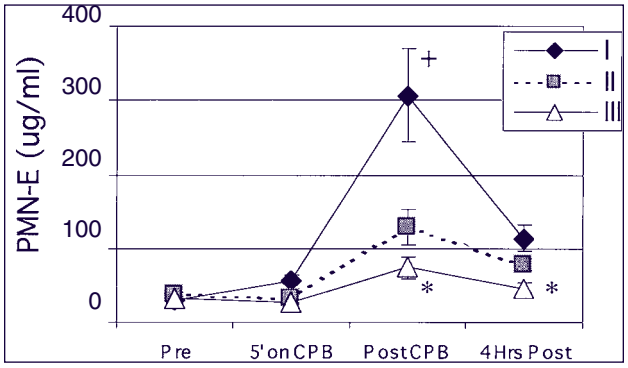

FIGURE 3: PMN-E for elastase.

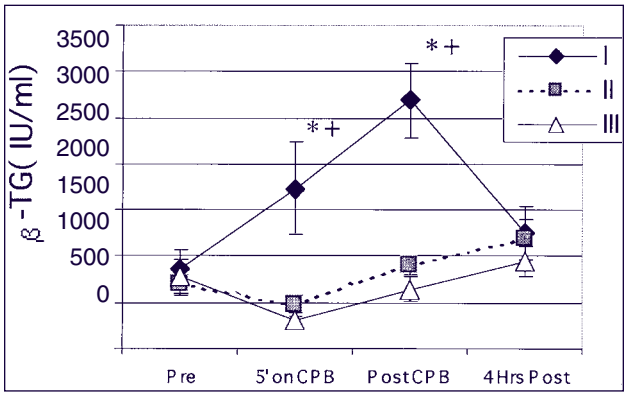

FIGURE 4: β-Thromboglobulin for platelet activation.

long held precepts that adverse 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.

Finally, 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 used a combination of altered equipment, techniques and pharmacological therapy) and hope to develop reliable specific biological essays to predict an individual patient's response to artificial perfusion and direct clinical therapy. 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.

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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, CX_3C, and C. The CC chemokines or the b 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-1α, MIP-1b, to name just a few. The second group, the CXC chemokines, are also referred to as the a 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 CX_3C families. These chemokines are chemotactic for T lymphocytes and monocytes and include lymphotactin (C) and fractalkine, also known as neurotactin (CX_3C).

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. TNFα, IL-1α, 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 MIP1α 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 expression for MIP-1α 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-1α 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 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 1α, 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-1α 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.

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.

Whole organ transplants are desirable but such studies are confounded by technical complications, and the costs can be prohibitive.

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 anastamosis 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.

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Edward D. Verrier, M.D.

- Cellular and Molecular Mechanisms of Myocardial Ischemia-Reperfusion Injury

FUNDING

Bayer Corporation
Proctor & Gamble / Alexion Pharmaceuticals
National Institutes of Health
National Science Foundation
Thoracic Surgery Foundation
ZymoGenetics, Inc.

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.

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 infection. 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 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.

Paradoxically, restoration of blood flow to oxygen-deprived tissue, the mainstay of therapy for ischemia, often causes further myocardial damage.

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-κB, 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.

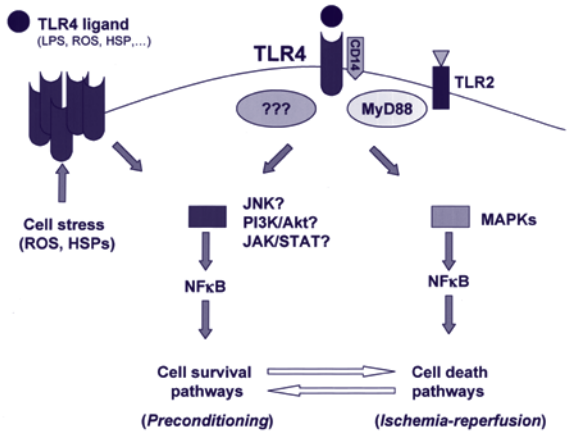

Figure 1: TLR4, and possibly other TLRs, are activated by oxidative stress during myocardial I/R injury, either by binding a putative endogenous ligand (e.g., HSPs) that circulates in response to myocardial I/R injury or because of physical alterations by oxygen radical species that cause TLR4 activation 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., NFκB). NFκB 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.

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- 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

AWARDS

American Association for the Surgery of Trauma
- Wyeth-Ayerst Research Scholarship Award
- John H. Davis Research Scholarship Award
- Peter C. Cannizaro Award 2004

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Medic One Foundation
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Based on a strong interest in trauma and critical care, my research has focused on addressing 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.

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: Al Halstrom; Co-PIs: Graham Nichol, Eileen Bulger). The Seattle and King County Medic One programs are one 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 proposed trial of hypertonic resuscitation will enroll nearly 6000 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. Investigators from three of the clinical centers including Seattle,

We identified that patients undergoing prehospital intubation facilitated by neuromuscular blocking agents actually had a better outcome than those intubated without these medications.

San Diego, and Toronto have also submitted an R01 application to conduct detailed studies of the immuno-inflammatory 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 are those 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 are now collaborating with the national experts in this area to draft a position paper regarding the prehospital management of the airway for patients with severe traumatic brain injury. In addition, we are collecting additional data to assess the impact of prehospital hyperventilation on outcome in this patient population.

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 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 5 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 I 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.

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Joseph Cuschieri, M.D.

- Toll-Mediated Signaling
- Trauma Induced Mononuclear Cell Reprogramming
- Proposed Mechanism of Lipid Raft Clustering and Reprogramming

AWARDS

American College of Surgeons
- Committee on Trauma Region X Competition, Best Basic Science Paper

Michael S. Benninger, M.D. Outstanding Resident Award

Helen & John Schilling Resident Research Symposium, First Place

Seattle Surgical Society Award

Shock Society Travel Award

FUNDING

National Institutes of Health
- National Institute of General Medicine Sciences

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 during this state, an exaggerated inflammatory response ensues, leading to the development of MODS.

The mechanism responsible for this dysregulated innate 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 and 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 lipopoylsaccharide (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 lipid rafts. In particular, attenuation and augmentation of this receptor complex formation results in dysregulated inflammatory mediator activation. 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.

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-associate 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.

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.

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. 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 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. However, we have recently been able to shed some light on this mechanism by demonstrating that activation of the PKC isoform, PKC-z, is involved. Although the full effects of PKC-z 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-z 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. Although sustained activation of CaMK has not previously been studied in mononuclear cells obtained from injured and septic patients, enhanced activation of CaMK has been demonstrated in a number of other cell types, including cardiac myocytes and smooth muscle cells during sepsis.

In addition to the activation of the regulatory kinase, CaMK II, recent evidence has suggested that sphingomyelinase activation and ceramide production may play critical roles. In fact, intracellular ceramide levels along with serum TNF-a have been demonstrated to be elevated in patients suffering from severe sepsis. This strong correlation between cell-associated ceramide and serum TNF-a supports the hypothesis that ceramide, along with sphingomyelinase, plays a role in sepsis. Although sphingomyelinase activation and ceramide production may prove to be important following acute injury, this has not been previously explored.

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-kb and the MAPK. Recently, we have demonstrated that endotoxin tolerance does in fact effect recruitment of the TLR4 complex. 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.

Finally, 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, 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.

Proposed Mechanism of Lipid Raft Clustering and Reprogramming

Based upon the material presented above and our preliminary 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 recruit-

ment 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-z. Activation of PKC-z then potentially leads to the engagement of b2 integrins on lipid rafts, leading to the formation of macrodomains, as well as cytoskeletal changes resulting in lipid raft recruitment of TLR4 components and scaffolding proteins. These cytoskeletal changes are perhaps induced through engagement of b2 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.

Reprogramming following injury is not only associated with constitutive changes in CD16 expression, but also changes within the raft. 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.

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

FUNDING

National Institutes of Health

- National Institute of General Medicine Sciences
- National Institute of Diabetes, Digestive and Kidney Diseases

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.

Cytokine Response to Thermal Injury

Our latest therapeutic approach to the acute care management of patients with thermal injury has been to reintroduce plasmapheresis into the care plan of patients with large burns that are failing resuscitation. With advances in wound closure we are able to treat patients effectively if we can help them to survive the initial resuscitative phase — or 48 hours after injury. Over the past year we have had favorable experience using plasmapheresis on selective patients with large

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.

burns. Since these patients represent anecdotal evidence that plasmapheresis may have a role in the management of patients with large burns, we are pursuing an in depth clinical and basic science study of the effect of plasmapheresis. We are looking at cytokine levels in the plasma of the patients before and after their plasmapheresis has been completed to determine which mediators are elevated during the inflammatory response to injury. We are correlating these results with the clinical course of patients that undergo plasmapheresis compared with control subjects matched in age and burn size.

Neuroinflammatory Response to Wound Repair

Our lab has been dedicated to defining the neuroinflammatory response to wound repair. The sensory nerves in skin regulate not only pain transmission, but also a local inflammatory response within the wound bed. We have identified the normal temporal and spatial distribution of pain fibers in human burn wounds.

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.

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 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.

Our lab is focused on determining the 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 focussed 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 and the EGFr as means of mediating substance P activity. These studies have been funded by the NIH.

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- National Study on Costs and Effectiveness of Trauma Care
- Washington State Trauma Registry and Central Region CQI
- Post-Traumatic Stress Disorder in Trauma Patients

FUNDING
Centers for Disease Control and Prevention
- 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 entitled, "The National Study on Cost 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 on going 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 8000 trauma patients for the study.

We contracted with Westat, one of the leading survey research firms in the world, to conduct phone follow-up 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 2000 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 the 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 for what types of patients and what 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%), 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.

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 take 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 this 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?"

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 in trauma patients, and is responsible for initiating cooperative studies between surgery, pediatrics, and psychiatry on the assessment and treatment of posttraumatic stress disorder (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 PTDS, 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).

The life-saving beneficial effects of trauma center care is most evident in the younger, more severely injured patients. Vexing questions remain on why this dramatic beneficial effect is not seen in the elderly and will be the focus of further studies.

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- 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

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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.

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.

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 antioxidant 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 multinucleated 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 ostepontin 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 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 Ð38. 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.

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 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 of 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 hierarchal 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.

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Charles Mock, M.D., Ph.D.

- Strengthening Trauma Systems in Developing Countries
- The Essential Trauma Care Project
- Capacity Building for Injury Control
- Crash Injury Research and Engineering Network (CIREN)

AWARDS

University of Washington Outstanding Public Service Award

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- Brown University Medical School Commencement

FUNDING

Atlantic Philanthropies

Centers for Disease Control

- National Center for Injury Prevention and Control

National Highway Traffic Safety Administration (NHTSA)

National Institutes of Health

- Fogarty International Center

The global burden of injuries is enormous, but has often been overlooked in attempts to improve health. There is a tendency among governments and societies in every country to consider injuries as bad luck and as unavoidable. However, much can be done to decrease the burden of injury by addressing the spectrum of injury control, including surveillance and research, injury prevention, and trauma care. Organized, scientifically based efforts can be applied at all points along this spectrum. Much remains to be done in high-income countries. However, attention is especially needed in less developed countries, where injury rates are higher, where minimal injury control activities have thus far been undertaken, and where the majority of the world's people live. My work, collaboratively with many people at work in their own home countries, has sought to address the spectrum of injury control activities globally.

In all societies, the leading cause of death was once infectious diseases; however, in developed countries, this pattern has changed over the past two centuries, with decreases in infectious diseases and increases in life expectancy. Unfortunately, some of these gains were offset by increases in other diseases, including chronic diseases and injury. Today, injury is the leading cause of years of life lost in almost every developed country.

Similar trends are underway in today's less developed countries. In middle-income countries, as in East Asia and Latin America, injury has become a leading cause of years of life lost. In low-income countries, such as in South Asia and Africa, infectious diseases continue to predominate because of their continued high toll in younger children and because of HIV/AIDS. However, even in these locations, injury is usually one of the leading causes of death among older children and working age adults.

In many developed countries, injury mortality rates have fallen in recent decades, as a result of both improved prevention efforts and improved trauma treatment capabilities. Such well-organized approaches to prevention and treatment have not been carried out in less developed countries. Moreover, basic information about the incidence, mechanisms, and causes of injury in such locations is lacking.

I and Co-workers from several countries have helped to address these concerns by working to improve the spectrum of trauma system activities (Figure 1):

1. Surveillance and research on the basic epidemiology of injury.
2. Injury Prevention.
3. Prehospital Care.
4. Hospital Based Trauma Care.

We have worked on these activities in several developing countries, including Ghana, Mexico, and Vietnam. During the conduct of this work, the

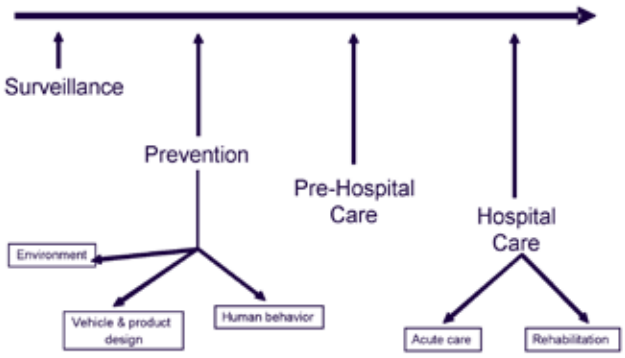

FIGURE 1. Spectrum of Injury Control

UW Department of Surgery has served as my home base. The advice and expertise of colleagues in the Department and at the Harborview Injury Prevention and Research Center has also represented a valuable resource for my work.

Strengthening Trauma Systems in Developing Countries

Surveillance and research on the basic epidemiology of injuries in developing countries

In developed countries, the usual sources of data on the incidence and consequences of injury include vital statistics registries, police accident reports and health care records. In many less developed countries, these sources are inadequate. Many or most deaths are not reported to the government. Many injured persons may never receive formal medical care, making health care records an incomplete source of data as well.

To better ascertain the incidence and consequences of injury in Ghana, co-investigators at the University of Science and Technology in Kumasi and I undertook a community-based survey of injuries in this country. Using a defined random sampling strategy, known as two-stage cluster sampling with probability proportional to size, a denominator of 21,105 persons living in 432 separate urban (city of Kumasi) and rural (Brong-Ahafo region) sites were selected.

Through direct household visits and interviews, we sought information on any injury that had resulted in one or more days of lost activity during the prior year (including fatalities). A total of 1,597 injuries were reported and analyzed. Information was obtained on the mechanism, specific body part injured, type of medical care obtained, cost of treatment, and outcome of injury, including length of disability. Information was also obtained on the economic consequences of the injury to the family of the victim.

In the urban area, the major causes of injury included falls, accidental lacerations, and transport related injuries. However, transport related injuries were more severe than the other causes, as indicated by a longer mean period of disability (64 days per injury), compared to all other injuries (37 days per injury). In the urban area, 38% of injured persons received treatment at a hospital (either emergency room visits or inpatient admissions); 30% received treatment at a government or private clinic, and 32% received no formal medical treatment.

In the rural area, the major causes of injury included agricultural injuries (30% of all injuries), falls, and transport related injuries. Compared with the urban area, significantly fewer injured persons received any type of formal medical care. Only 20% of injured persons received hospital based treatment; 31% received treatment at one of the network of non-physician staffed primary health care clinics; and nearly half (49%) of all injured persons in the rural area received no formal medical care.

Detailed information on the characteristics and outcomes of the transport related injuries has been shared with the road safety officials in Ghana. Information on health service utilization patterns has been shared with the Ministry of Health of Ghana. Data from this survey have been the basis for multiple publications on mechanisms and causes of injury, pediatric injuries, injury related disability, economic consequences of injury, trauma treatment, and epidemiologic methodology.

The data from this survey have also been useful for efforts to improve the existing information sources for trauma in Ghana. For example, a comparison of the incidence rates of pedestrian injuries in the city of Kumasi as derived from the survey with the incidence rates as derived from police reports showed that only about 10% of actual injuries were being recorded in police records. These data have prompted efforts to improve the existing information sources.

In addition to the above survey, we have undertaken research regarding trauma mortality patterns in developing countries. The goals of this study were to provide information that would help with decisions regarding trauma system development in developing countries. In developing a trauma system, decisions must be made as to the extent to which limited resources should be allocated to injury prevention programs, prehospital care, emergency room care, or other aspects of hospital based care. Hence, there is a need to know where in a nation's trauma system the greatest mortality lies.

Assessment of where the greatest improvements are to be made could be assisted by comparing such mortality patterns to those of an industrialized nation with a well-developed trauma system. In our study, we compared the trauma mortality patterns in three cities in countries at different economic levels: Seattle, WA, USA (industrialized country), Monterrey, Mexico (middle income country), and Kumasi, Ghana (low income country).

The main finding of this study was that with decreased socio-economic status, the overall rate of death among seriously injured patients increased,

In Ghana as in many low-income countries, there is no formal emergency medical system (EMS). Ill or injured persons are usually brought to the hospital by relatives, using whatever type of transportation is available.

from 35% in Seattle, to 55% in Monterrey, Mexico to 63% in Kumasi, Ghana. This was, of course, not unexpected. What was somewhat surprising however was the way in which this happened. The majority of the changes in mortality between the various cities were accounted for by changes in deaths in the field (e.g., the prehospital setting). The proportion of deaths occurring in the field increased with decreasing socio-economic status, from 59% in Seattle, to 72% in Monterrey, to 81% in Kumasi. The study pointed out the importance of injury prevention efforts and prehospital care in the setting of developing countries.

Injury prevention

We have carried out research on factors contributing to injuries in Ghana in collaboration with others involved in road safety in that country, including the National Road Safety Committee, the Building and Road Research Institute, the Motor Traffic Unit of the Ghana Police, and the Ghana Police Hospital.

One study involved a random roadside breathalyzer study to assess the prevalence of drunk driving. This used methodology similar to that developed by the National Highway Traffic Safety Administration (NHTSA) in the United States. A total of 722 drivers were randomly tested on the major roads leading into the capital city of Accra, Ghana. A total of 149 (21%) of these were positive for any level of blood alcohol. Furthermore, 53 (7.3%) had blood alcohol concentration of 80 mg/dl or higher, indicating alcohol impaired driving. This prevalence of alcohol impaired driving is higher than that reported by similar methodology in developed countries (0.4 - 3.4%). It is notable that 3.7% of bus drivers and 8.0% of truck drivers had blood alcohol concentrations of 80 mg/dl or higher.

These data indicate that alcohol impaired driving is likely to be a major contributor to motor vehicle crashes in this country. Data from this study have been used by the National Road Safety Committee in its educational campaigns and have been presented to the Ghanaian Parliament in efforts to stimulate updating of Ghana's drunk driving laws.

In Mexico, injury prevention work has involved a collaborative effort of the Harborview Injury Prevention and Research Center (HIPRC) and several local institutions in the city of Monterrey, Nuevo Leon. These include the Hospital San Jose and the TEC de Monterrey School of Medicine. As part of these efforts, we have developed a program providing injury prevention counseling for parents. This focuses on improving parents' knowledge and practices of childhood safety in the Mexican environment. It has involved adaptation of existing educational materials developed by the American Academy of Pediatrics. Thus far we have carried out pilot work in this and have put on educational seminars that have had the participation of nearly 3000 parents in the Monterrey area.

Prehospital care

My efforts in the development of prehospital care capabilities in developing countries have involved Ghana, Mexico, and Vietnam.

In Ghana as in many low-income countries, there is no formal emergency medical system (EMS). Ill or injured persons are usually brought to the hospital by relatives, using whatever type of transportation is available. In a review of the mode of transport for injured persons treated at the main hospital in Kumasi (the Komfo Anokye Teaching Hospital), the great majority (70%) were brought in by some form of commercial transportation (taxis or buses); 22% were brought in by a private vehicle; 5% were brought in by the police; and only 3% were brought in by an ambulance. All of the latter were transferred from a smaller rural hospital and a hospital ambulance was used for the transfer.

Currently, efforts to institute a formal prehospital system include plans to place ambulances along the major inter-urban roads and to build up the capability of groups such as the Red Cross and the Fire Service. Plans for EMS development are hampered by the paucity of telephones and other telecommunications in the country. Hence, current efforts also include

building upon the foundation of what prehospital care does exist; namely the commercial drivers who bring in the majority of injured persons.

I am involved with pilot training programs that are being conducted through the Kwame Nkrumah University of Science and Technology and the Ghana Private Road Transport Union, to which most commercial drivers belong. These training programs are evaluating the educational background of commercial drivers and their experience with transporting injured persons, as well as providing them with basic first aid instruction. Emphasis has been on hands-on experiences through practical drills, rather than didactic lectures and written materials (Figure 2). Approximately 400 drivers have been given first aid instruction as part of this program.

As part of the research and development aspects of this program, we have interviewed 71 drivers one year after having taken the course. Before the course, few drivers provided any type of first aid to injured

FIGURE 2. Scene from first-aid training course for commercial drivers in Ghana. Extrication is practiced using previously crashed vehicle. Rubber gloves used for universal precautions.

persons they transported. After the course, 61% of drivers indicated they had provided first aid during the interval year. Improvements included: airway management (2% before vs. 21% after*), bleeding control (4% vs. 25%*), splint application (1% vs. 10%*), and triage (7% vs. 21%*) (*$p < 0.05$). The course has cost $4 per driver trained.

In Mexico, as in many other middle-income countries, there are usually basic ambulance services, at least in the urban areas. My Mexican colleagues and I have been involved in ongoing efforts to improve the ambulance systems in the Monterrey metropolitan area over the past eight years. Efforts to upgrade this EMS there have included introduction of the Prehospital Trauma Life Support course (PHTLS). Introduced in 1994 for paramedics in the Green Cross ambulance service, this course has been conducted annually since that time.

In our evaluation of this program, we documented an improvement in both the process and outcome of prehospital trauma care after the PHTLS course. Airway maneuvers for patients in respiratory distress increased from 18% before the course to 43% after ($p < 0.05$). IV fluids for patients with BP < 100 increased from 44% to 81% ($p < 0.05$). En route mortality declined from 8.2% to 4.7% ($p < 0.05$). Regular PHTLS courses have cost $2600 per year (0.5% of the EMS budget). Hence, the improvements in both Ghana (a low income country) and Mexico (a middle income country) have been low cost and sustainable within the context of the local economies.

We are currently working on a project funded by the Medic One Foundation in Seattle to further the EMS development work in Mexico. This project builds upon the foundation that was started with the PHTLS project by specifically addressing increased training for advanced airway maneuvers, including endotracheal intubation.

We have also recently embarked on a program to conduct similar EMS development in Vietnam. In particular, we received a grant from USAID to establish a link between the world renowned Medic One program in Seattle and the Hanoi Emergency Transport Center. This center contains some highly motivated individuals. However, it is still at a rudimentary level and is estimated to meet only 20% of the need for EMS in the city. The program provided upgraded training for prehospital trauma care for ambulance personnel in Hanoi. This was done through exchanges of personnel between the two cities. Of special note is the fact that this was one of the first times that USAID has funded an EMS or trauma related project in a developing country. This recently completed (2002) pilot project has now been expanded to encompass 3 other Vietnamese cities (Haiphong, Danang, Khan Hoa). In July–August, 2003, 12 doctors from these cities visited Seattle for 2 weeks each. They participated in a project in a specially designed course to introduce them to the EMS and trauma care systems of Seattle and Washington State. This included lectures, practical sessions, and rotations in the ED at Harborview and on the ambulances of Medic One. Following this, several people from UW and Medic One visited Vietnam and par-

ticipated in training programs for over 100 doctors in the ambulance systems and EDs of the above noted cities in Vietnam. They helped to train a group of Vietnamese trainers who have provided similar "roll out" training to over 500 other doctors and nurses throughout that country.

As part of this ongoing work, in February, 2004, a delegation of health care leaders from Vietnam visited Seattle. These included representatives of the national Ministry of Health, as well as the heads of the Provincial Health Departments from Hanoi, Da Nang, and Khanh Hoa. They visited Medic One, Harborview Medical Center, the State Department of Health in Olympia, and a rural trauma system on Whidbey Island. The purpose of their visit was to see how Washington state organizes and provides trauma care, both prehospital and hospital-based.

Hospital based care

Experience with the Advanced Trauma Life Support Course (ATLS) of the American College of Surgeons has shown that using a structured educational approach, with well-planned teaching materials and evaluation of the course's effectiveness, can improve the process and outcome of trauma care in the U.S. and in developing nations with higher economic status, such as Trinidad.

However, in nations at the lower end of the economic spectrum, such as Ghana, facilities needed to implement the ATLS guidelines (including CAT scans and consultations with neuro- and general surgeons) are extremely limited. In rural areas, hospitals are staffed almost exclusively by general practitioners. Opportunities to refer patients are limited by poor roads and financial restrictions. Hence, training in this setting needs to be expanded beyond the early resuscitation and diagnostic work-up of the "Golden Hour" to include definitive treatment which general practitioners might be expected to perform in isolated rural hospitals. The experience of the ATLS program in the U.S. indicates that a similar approach, oriented for the particular circumstances of developing nations, could improve trauma care in these locations.

During the past eight years, in collaboration with the Department of Surgery at the University of Science and Technology in Kumasi, I have conducted several postgraduate lecture series on trauma management. These have formed the basis for the development of a more organized, standardized continuing medical education (CME) course. The material in these lectures has been updated based on surveys conducted in rural hospitals to ascertain general practitioners' needs and desires for CME. Evaluation of the effectiveness of the course has been conducted, including comparison of pre-course and post-course test results and interviews with course participants one year afterwards to assess how the course affected their practice of trauma care in rural hospitals. Results of this evaluation have identified problems that need to be corrected, as well as strengths that should be built upon in this course. It thus provides assistance to those developing similar educational programs in other African and other developing countries.

The Essential Trauma Care Project

The above sections give some indication of successful pilot projects in several countries. Many individuals from throughout the world have similar success stories to tell. The question then becomes how to take these lessons and make more progress, systematically and globally, in improving trauma care. A variety of "weak links" in the chain of trauma care need to be addressed: human resources (training, staffing); physical resources (equipment, supplies, and infrastructure); and organization & administration. In so doing we can build upon the experience gained by the WHO and others in international health. Working within the same tight financial constraints, these organizations have made considerable progress in several disease entities by developing the concept of "essential services." These are services that are highly effective, low cost, and which should realistically be available to most members of a given population.

Several programs have developed, refined, and promulgated such essential services, including the Expanded Program on Immunizations, the Essential Drug List, and the Safe Motherhood Initiative. I, and others working in trauma, feel that it is time for a similar approach to trauma care. In this regard the International Society of Surgery, through its trauma section (International Association for Trauma and Surgical Intensive Care – IATSIC) in 2001 created a "Working Group for Essential Trauma Care," with myself as chair, to specifically address this issue.

We feel that we have made some progress in this endeavor. We have formed a partnership with the World Health Organization's Injuries and Violence Prevention Department in Geneva. The two groups have worked together for the past three years. This has entailed mostly long distance communication. In addition, there was a meeting of the two groups in Geneva in June 2002 for the "Consultation Meeting

to Develop an Essential Trauma Care Programme." This involved trauma care clinicians from at least 2 countries on each continent. Through that meeting and its follow up, we have refined a list of 260 essential items of human and physical resources that we feel should be in place in the range of health facilities throughout the world. These are incorporated in a document entitled "Guidelines for Essential Trauma Care," which was published in June 2004, as a joint publication of the WHO and the International Society of Surgery. This publication is intended to serve as:

1. Part science, in that it includes a list of the items of trauma care that a panel of experts has evaluated as the most cost effective.
2. Part planning guide to assist clinicians, hospital administrators, and planners in ministries of health globally in their efforts to strengthen trauma care in their own countries.
3. Part advocacy tool, in that the Guidelines contain a delineation of the trauma care services that the WHO and the International Society of Surgery have endorsed as "Essential" and which can realistically be assured to virtually every injured person worldwide, even in the poorest countries.

A sample of some of the elements contained in the Guidelines is shown in a sample table (Figure 3).

The Guidelines have gone through a rigorous review process, with input from over 30 individuals from 20 countries. These have included people reviewing the document as individuals and also as representative of over 10 other professional organizations, such as international societies of neurosurgery and orthopedics.

The real test of the utility of these Guidelines is what they can accomplish on the ground in improving

	BASIC #	GP #	SPECIALIST #	TERTIARY #
Airway Skills				
Assessment of airway compromise	E	E	E	E
Manual manoeuvres (chin lift, jaw thrust, recovery position, etc)	E	E	E	E
Use of suction	D	E	E	E
Use of bag valve mask	D	E	E	E
Endotracheal intubation	D	D	E	E
Cricothyroidotomy	D	D	E	E
Airway Equipment				
Oral airway	D	E	E	E
Suction device (foot pump powered at least) and associated tubing and catheters	D	E	E	E
Bag valve mask	D	E	E	E
Laryngoscope	D	D	E	E
Endotracheal tubes	D	D	E	E
Magill forceps	D	D	E	E
Other advanced airway equipment		D	D	D

**Basic:** Outpatient clinic, often non-doctor staffed.
GP: General Practitioner staffed hospitals.
Specialist: Specialist staffed hospital, usually having a general surgeon and possibly other specialities.
Tertiary: Tertiary care hospitals, often university hospitals; wide range of specialists.
E: Essential; **D:** Desirable

FIGURE 3. Example of Essential Trauma Care Resource Matrix. **This example is for the Skills and Equipment for management of Airway obstruction in injured patients. Thirteen other matrices cover the spectrum of trauma care, including initial resuscitation, definitive acute care, and rehabilitation.**

Source: Mock C; Lormand JD; Goosen J; Joshipura M; Peden M. *Guidelines for Essential Trauma Care.* Geneva: World Health Organization, 2004.

care of injured patients in individual countries. The authors of the Guidelines view subsequent work as a collaborative process, involving national ministries of health, country offices of the WHO, professional societies, and other stakeholders. In this regard some progress has already been made. For example:

1. The 260 elements contained in the Guidelines have served as the basis for needs assessments of the facilities that provide trauma care in four countries: India, Ghana, Vietnam, and Mexico.
2. The Guidelines have served as a focus for trauma care stakeholders' meetings in all of the above 4 countries and Sri Lanka. At these meetings, participants included professional societies that deal with trauma care, ministries of health, WHO country offices, non-government organizations, and others. At all of the meetings, the Essential Trauma Care resource guidelines were adapted to local circumstances and policy recommendations/ implementation strategies were derived. Most participants felt that these meetings constituted the highest governmental attention to trauma care yet.

Capacity Building for Injury Control

All of the above work, in both prevention and treatment, demands the expertise of trained personnel from a variety of different fields: for example, epidemiologists who can handle injury data in the development of injury surveillance systems; psychologists, media experts, and public health personnel who can develop social marketing strategies to effectively improve safety related behavior; medical personnel who can undertake outcomes research and who can effect changes in trauma system design based on such research. Perhaps one of the most important things that workers from developed countries can do in assisting developing countries is to increase and strengthen such local expertise. Along with others at the HIPRC, I have been undertaking two programs for the development of local expertise in injury prevention and control, in Mexico and Vietnam.

In Mexico, we have developed a training course in injury prevention work for health care professionals. The course has now been given three times in Monterrey over the past four years. Around 150 persons, including doctors, nurses, public health professionals, teachers, and others have taken the course. We are in the process of undertaking further research and development of this course and hope to eventually export it to other areas of Mexico and other countries in Latin America.

In Vietnam, similar work is underway. The HIPRC has entered into a project in partnership with the Hanoi School of Public Health to design a program to improve injury prevention and control training and capabilities throughout Vietnam. This program has been generously funded by Atlantic Philanthropies. Through this program we have undertaken exchanges of faculty between our two institutions. Several Hanoi School of Public Health faculty have taken short courses on injury control at UW. Likewise, through this project, the Hanoi School of Public Health and UW have put on 3 major, nationwide injury control courses during 2003–2005 in Hanoi. Each lasted 2 weeks and was attended by professionals active with injury control in institutions and provincial health departments throughout Vietnam. The first graduate student from this program, a junior faculty member at the Hanoi School of Public Health, is now enrolled at UW in the MPH (Epidemiology) program.

The above injury control capacity building has now been extended to Ghana, through a recent grant from the Fogarty International Center of the NIH. Through this grant, we are planning for similar nationwide injury courses in Ghana, as well as graduate students and other trainees to work on injury control research.

Crash injury research and engineering network (CIREN)

In addition to my work in less developed countries, I am active in research on injury prevention in the U.S. Harborview Medical Center and its associated HIPRC are part of a network organized by NHTSA that includes six other trauma centers nationwide. At each center, persons injured in motor vehicle crashes are identified. A crash investigator examines the involved vehicles for crash deformation patterns (Figure 4). The automotive findings are correlated with the patient's injuries, and hypotheses are generated regarding the biomechanical etiology of the injuries. Data from this process are fed back to NHTSA to help with the development of safety regulations and to the automobile manufacturers to help with safety engineering design.

In collaboration with the NHTSA, our center (HIPRC) has investigated several issues pertinent to vehicle safety design and related regulations. We have investigated biomechanical thresholds for femur fracture and shown that, on average, femurs tend to fracture at lower energy loading thresholds than previously suspected from cadaver tests. This has implications for the crash test standards that are cur-

FIGURE 4. Seattle CIREN (Crash Injury Research and Engineering Network) site crash investigator examines crashed vehicle for clues as to causes of injuries to occupants.

rently used for frontal impact.

We have also investigated the effects of varying body sizes and found an increased risk of death and serious injury to larger occupants. This has implications for safety design, as most crash testing has been done using dummies of 70 kg size. There has been a push lately for more testing using small size dummies, to better account for the crash biomechanics of smaller size women and children. However, our research has shown that more attention may need to be given to larger size occupants as well.

We have investigated the effectiveness of different seatbelt systems and found that minimal protection was afforded by using a shoulder harness alone, without the associated lap belt. This is an issue, as many people assume that an automatic shoulder harness is protecting them and do not bother to buckle up their lap belt as well.

We have looked at the veracity of the safety ratings provided by the New Car Assessment Program (NCAP) of NHTSA. NCAP rates vehicles on their safety based on the forces transmitted to dummies in standardized crash tests. These forces are compared with the estimated thresholds for major head and torso injuries, as derived from cadaver tests. Assessment of these thresholds in real world crashes has been infrequent. Utilizing data from the CIREN project, we have determined that the relationship between forces in vehicle crashes and injury thresholds are more complex than initially appreciated. It appears that the likelihood of head injury has been overestimated for some vehicles, especially those that appeared most unsafe and had the highest forces to the head during standardized crash tests. However, the likelihood of head injury has been underestimated for some vehicles, especially those that appeared the most safe on crash tests. Such information is being fed back to NHTSA and its NCAP.

Another timely topic of crash worthiness research concerns the effects of vehicle mismatch. Such mismatch occurs when different types of vehicle collide. Most notably are the increased risks to occupants in passenger vehicles when they are struck by larger and higher light truck vehicles, such as sport utility vehicles and pickup trucks. Research by the Seattle CIREN team has identified patterns of injury to occupants of passenger vehicles, such as increased risk of head and chest injuries, when light truck vehicles strike the sides of passenger vehicles. This is primarily caused by intrusion of the door panel above the reinforcing bars that are placed in the doors to protect occupants against collision from other passenger vehicles. These sidebars were mandated by federal motor vehicles safety standards. They have been very effective at decreasing the rate of serious injury from passenger vehicles striking other passenger vehicles. However, the higher bumpers and increased mass of light-truck-vehicles overcomes this protection. This is especially significant given the growing number of light truck vehicles in the US vehicle fleet. Similar findings pertain to frontal collisions. These findings have been fed back to NHTSA and have been useful in that agency's efforts to update motor vehicle safety standards to improve side impact protection for passenger vehicles and to make light truck vehicle front ends less dangerous.

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**Indicates a student, resident, or fellow writing on a project for which Dr. Mock was his/her primary supervisor.*

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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 (TNFα), lipopolysaccharide binding protein (LBP) and CD14. LPS is a major component of the outer wall of gram-negative

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.

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 (TNFα), interleukin-1β (IL-1β), and IL-6. TLR4 is central to LPS signaling. It's 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+11996T). 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, TNFα, IL-1β, and IL-6.

Venous whole blood samples were drawn on three occasions from 12 healthy subjects (8 wild-type and 4

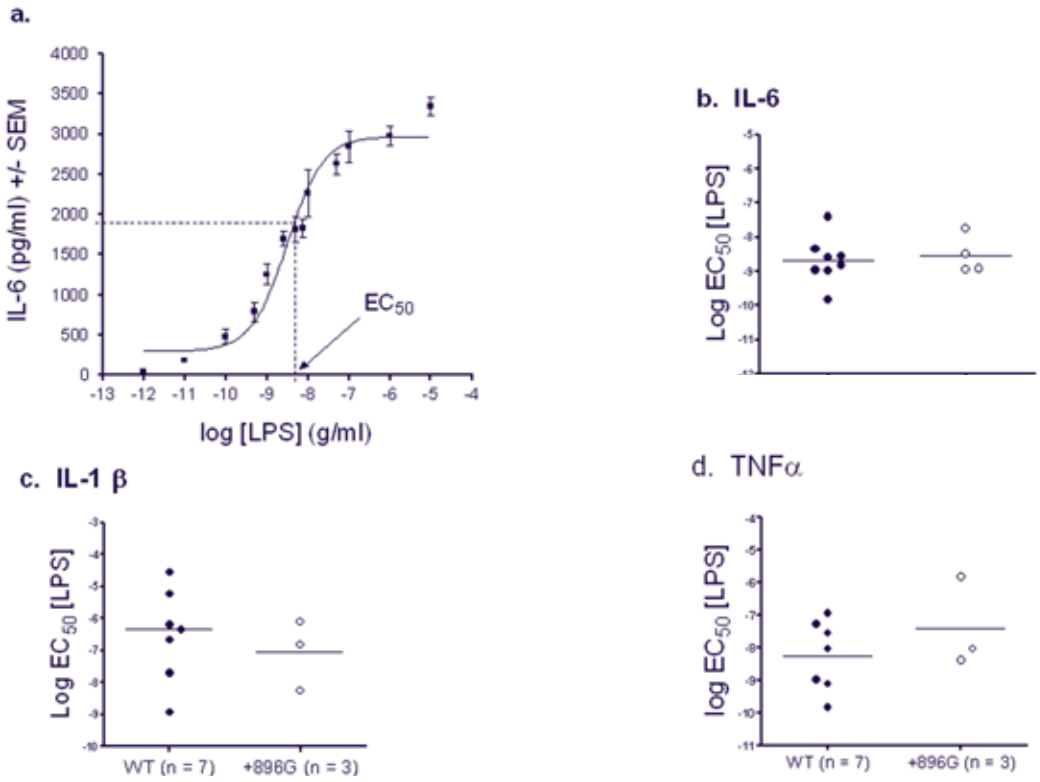

FIGURE 1

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 TNFα and IL-1β production in these same experiments (Figure 1c & 1d).

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 β2 adrenergic receptors (β2AR) 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 my 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 TNFα signaling. TNFα 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 TNFα production which is mediated, at least in part, by the nuclear factor NF-κB. There are conflicting data regarding how cAMP influences TNFα production and NF-κB 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 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.

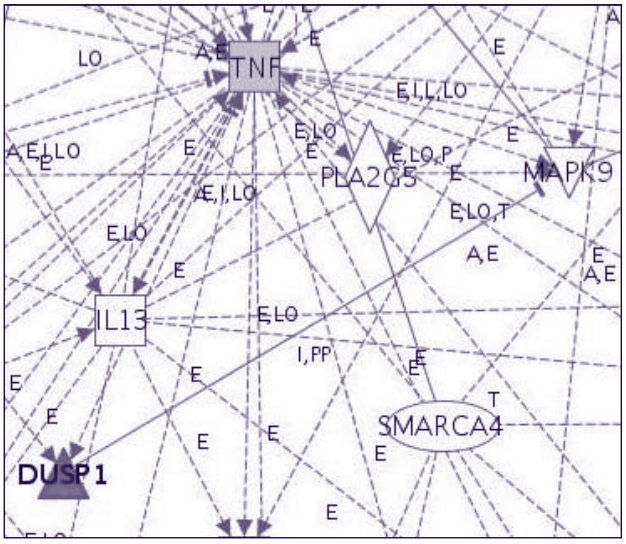

FIGURE 2

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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

Tissue loss and end-stage organ failure con tinue 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.

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.

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.

This has led to the development of methods in isolating intestinal epithelial cells with the underlying mesenchymal components as a unit (organoid units) whole 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.

Tissue engineering has recently emerged as an interdisciplinary field that works toward the development of biological substitutes that restore, maintain, or improve tissue function.

The polymer matrices used in tissue engineering serve many important functions. It provides a substrate for cell attachment and delivery, and it serves 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

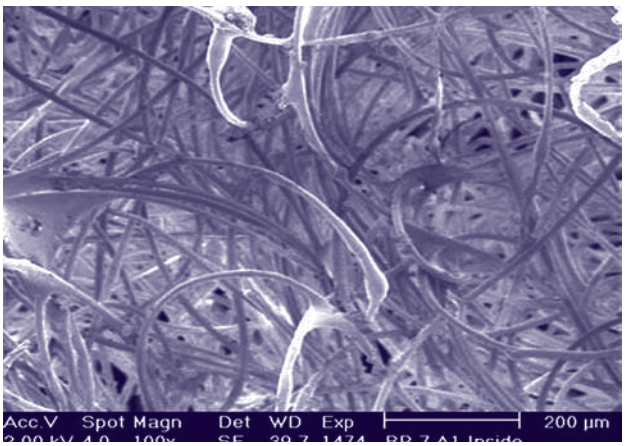

FIGURE 1. Scanning electron micrograph of different polymer matrices. a). Crystalline-based polymer matrix, b). Fiber-based polymer matrix, and c). Tubularized polymer matrix.

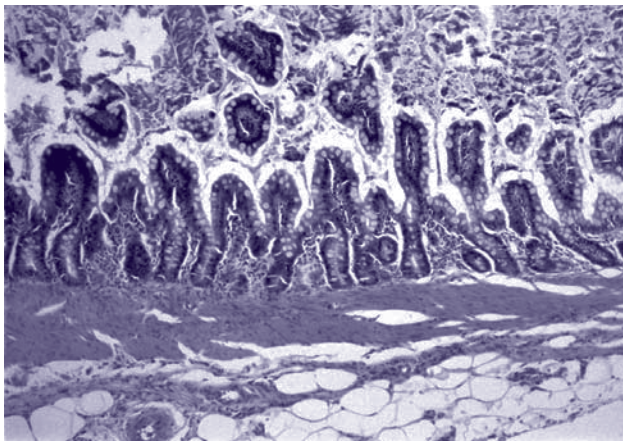

FIGURE 2. Histological section of the regenerated neomucosa.

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.

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Robert S. Sawin, M.D.

• Neuroblastoma in the Pediatric Patient

AWARDS

Robert E. Condon Surgical Resident Competition
Wisconsin Surgical Society
• Resident Competition (Scientific Division)

FUNDING

U.S. Army, Madigan Army Medical Center
• Department 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.

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.

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OTHER CO-INVESTIGATORS

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John Waldhausen, M.D.

• 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.

The treatment of Hirschsprung's disease has experienced a trend towards one stage surgical repair in the neonate rather than traditional delayed or multiple stage repairs.

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.

Other studies have answered simple questions about everyday 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 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.

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PLASTIC AND RECONSTRUCTIVE SURGERY

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Loren H. Engrav, M.D.

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

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International Association of Fire Fighters
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National Institute on Disability and Rehabilitation Research (NIDRR)
· Department of Education
Washington State Council of Firefighters

Laboratory Research

Hypertrophic Scarring

We have clarified the histological anatomy of the cones of skin (Fig.1) 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 reported on our investigation of the female, red Duroc pig and found it to be a promising animal model of hypertrophic scarring (Fig. 2). With funding from the National Institutes of Health, the National Institute on Disability and Rehabilitation Research, Department of Education (NIDRR) and the Washington State Council of Fire Fighters Burn Foundation (WSCFFBF) we are conducting further studies of these with two specific aims summarized below. Drs. Zhu, Gibran and Isik are significantly involved in these activities. The Visiting Scientists listed played major roles in these projects.

Broad Long Term Objective: To understand the cause of hypertrophic scarring after burns, with the intent of reducing or eliminating this devastating outcome and thereby greatly improving rehabilitation.

Hypertrophic scarring is perhaps the most significant negative outcome of a burn injury. Scarring affects ones 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 occur, 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.

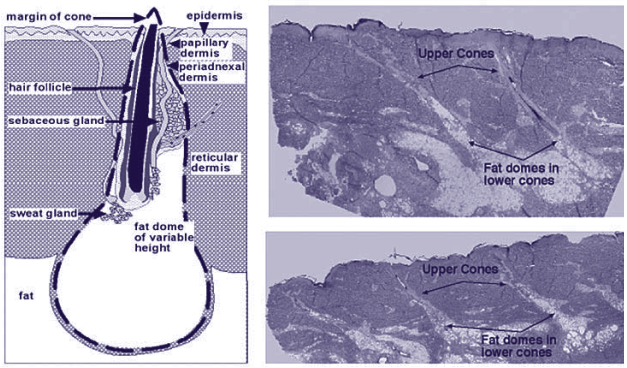

FIGURE 1: The Cones of Skin, Schematic and *in vivo*

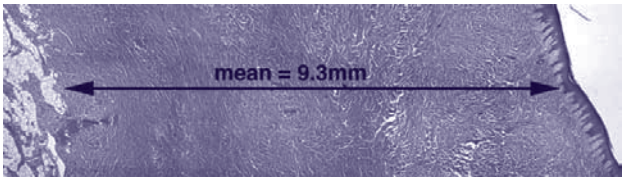

FIGURE 2: Thick Scar in Female, Red Duroc Pig

Hypertrophic scars are hard, raised, red, itchy, tender, and contracted. They are ugly and uncomfortable and may regress, but never totally go away (Fig. 3). Histologically, increased fibroblasts, collagen and other extracellular proteins characterize hypertrophic scars.

Hundreds of studies of collagen and fibroblasts in human, hypertrophic scar have been done over the past twenty years, but the pathophysiology of

hypertrophic scarring remains unknown. One fundamental reason is the lack of an animal model, which means that human tissue must be used for all laboratory studies. Human tissue cannot be obtained in a systematic and orderly fashion and at best is obtained only on a convenience basis. This severely hampers any laboratory studies.

Hypothesis: Thick scar in the female, red Duroc pig is a valid model of hypertrophic scarring and can be used to determine whether the cones of skin are the source of profibrogenic signals or fibrosis during hypertrophic scarring.

Specific Aim 1: To confirm that cutaneous scar in the female, red Duroc pig is similar to human, hypertrophic, cutaneous scar.

One reason for our lack of understanding of hypertrophic scarring is that tissue for study has historically been obtained from humans undergoing scar revision. This has been necessary since there is no useful animal model. This means that tissues are obtained on a convenience basis without order or control, which prevents systematic study of the hypertrophic scarring process. Nearly 30 years ago, Silverstein, Goodwin, Raulston, and Pruitt reported that deep donor sites in 12/12 female, red Duroc pigs healed with hypertrophic scarring. No manuscripts either confirming or disproving this model have since appeared in the literature. Because the acquisition of human, burned tissue in a systematic and controlled fashion is so difficult, and our understanding of hypertrophic scarring is limited, this animal model required to be confirmed.

This specific aim has been completed and the results are published or in press. In brief, after deep wounds on the Duroc pigs,

1. the scar is raised, hard, hyperpigmented, and contracted,
2. the scar is thick, consists of disorganized collagen and contains collagen nodules, increased numbers of mast cells, and myofibroblasts,
3. the expression of TGFß1, IGF-1, decorin, and versican is similar to human hypertrophic scar, and
4. nitric oxide levels and nerve fiber counts approximated those of human hypertrophic scar.

We concluded that the model, although not identical to human hypertrophic scar, is quite similar and worthy of study as a model of hypertrophic scarring.

Specific Aim 2 : To determine whether gene expression patterns in cones of skin suggest a role for these structures in profibrogenic signaling or fibrosis during thick scarring in the female, red Duroc.

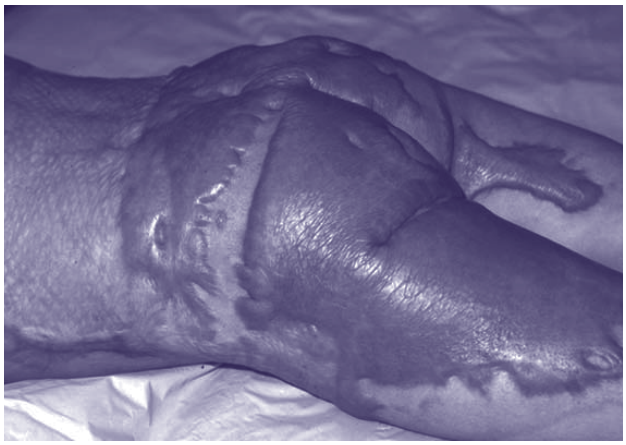

FIGURE 3: Hypertrophic Scar

A second reason for our lack of understanding of the etiology of hypertrophic scarring is that, in the past, most tissue has been minced and homogenized thereby destroying skin anatomy and homogenizing all cell populations. Thinking that skin architecture and cell location/orientation might be important to understanding the cause of hypertrophic scarring, we reviewed skin anatomy to include the cones. The cones were described in the early 1900s and re-discussed in the mid-1900s with little interest. In fact, most studies of human, hypertrophic scar either ignore the anatomy totally or mince the tissue thereby destroying the structures. We recently revisited the cones of skin and re-described the contents that include hair follicles, sebaceous glands, sweat glands, and a fat dome continuous with the hypodermis. More importantly, we demonstrated that these cones are located where hypertrophic scar occurs and are not present in those anatomic locations where hypertrophic scarring does not occur. Since these structures are anatomically related to hypertrophic scarring and since our understanding of hypertrophic scarring is so minimal, we believe the cones must be examined in relation to hypertrophic scarring.

If the cones are related to scarring, genes related to scarring should be up or down regulated within or around the cone tissues. Previous studies of hypertrophic scar have utilized tissues minced and homogenized which totally destroyed the cone structure and any observations related to that structure, a third reason for our lack of understanding of etiology of hypertrophic scarring. We will dissect the cones out of the surrounding mass of scar collagen by laser capture microdissection and study cone tissues in

isolation utilizing the porcine gene microarray to assess gene expression within this cone tissue in tissues from the female, red Duroc pig. We will give special attention to those reported to be related to wound healing, some of which were mentioned earlier, but will also study those not suspected to be significant in this regard. We will study 3 Duroc pigs and use 3 Yorkshire pigs as controls.

We now have the array data for shallow and deep wounds at 1, 2 and 3 weeks and the extracted and amplified RNA for the 3 and 5-month wounds in the Duroc pigs. We have 1, 2 and 3-week biopsies on one Yorkshire pig. We anticipate having all data in May 2006.

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. See above.

• *Project 2* is entitled 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.

• *Project 3* is entitled "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.

• *Project 4* (collaborative) is entitled "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.

• *Project 5* (collaborative) is entitled "Acute Stress Disorder Among Burn Survivors". The focus of this project will to evaluate the effectiveness of cognitive-behavioral therapy, relative to a non-directive, supportive therapy control group, and a national comparison sample in reducing the prevalence of post traumatic stress disorder diagnosis and symptom severity. The University of Washington Burn Injury Rehabilitation Model System will participate as part of the national comparison sample.

• *Project 6* is participation in the national burn rehabilitation database. The Burn Center staff listed above play a major role in gathering this data.

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 discontinue their use.

NIDRR Burn Model System Research

UW Burn Injury Rehabilitation Model System

There is very little 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 six projects managed by Drs. Engrav, Gibran, Patterson, Esselman and Wiechman. The Research Nurse Supervisor is Gretchen Carrougher, RN, MN. Drs. Kowalske, Fauerbach, Herndon and Lezotte are the other NIDRR Burn Rehabilitation Model System PIs.

• *Project 1* is entitled "A New Approach to the Etiology of Hypertrophic Scarring". The general aim for this project is to develop an increased understanding of

Projects 2-6 are well underway. 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 burn providers who prescribe pressure garment therapy after burns. Burn survivors have been targeted in order to determine the efficacy of pressure garment therapy. Burn 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 discontinue their use.

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 website.

Management of deep facial burns remains one of the greatest challenges in burn care. We have developed a protocol over the past 20 years for management of facial burns that includes excision and coverage with thick autograft. However, the results were not perfect. Deformities of the eyelids, nose and mouth as well as the prominence of skin graft junctures demonstrated the need to explore novel approaches. Integra® has been used with success in the management of burns of the trunk and extremities.

The purpose of this study was to prospectively evaluate the aesthetic outcome of the use of Integra® for deep facial burns. Twelve consecutive patients underwent excision of large, deep facial burns and placement of Integra®. Integra® provides excellent color and minimally visible skin graft junctures. The texture is good but not as supple as thick autograft. Integra® is not well suited for use in the coverage of eyelid burns due to the need to wait two weeks for adequate vascularization. In summary, thick autograft remains the gold standard for deep facial burns. However, for patients with extensive burns and limited donor sites, Integra® provides an acceptable alternative.

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• Wound Healing

FUNDING
National Institutes of Health

Much of what we see in surgical practice — especially in plastic surgery — 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.

Response to acute cutaneous injury is dependent on the temporal activation and silencing of thousands of genes.

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 334/4000 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 one hour post wounding, 471/4000 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 this 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.

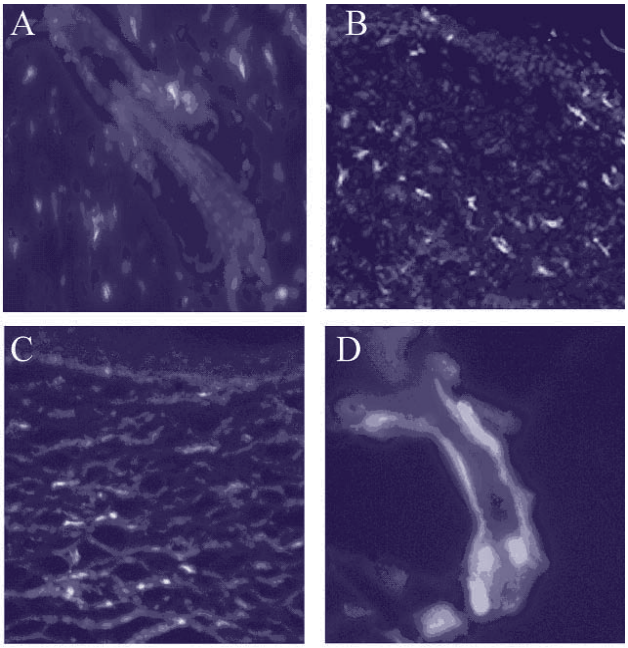

Figure 1 A: Normal skin showing numerous green cells of bone marrow origin in normal mouse skin.

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).

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, mesosderm 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 hematopoeitic 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/ß-catenin pathway is the most well characterized. Activation of the Wnt/ß -catenin signaling pathway promotes the stabilization of ß-catenin in the cytoplasm. This pool of ß-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/ß -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 ß-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/ß -catenin signaling is required for hair follicle morphogenesis and recent data also indicates that inhibition of Wnt/ß -catenin signaling may be necessary for basal epidermal cell specification. Our microarray data revealed that components of the Wnt pathway including: TCF-4, ß-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/ß -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.

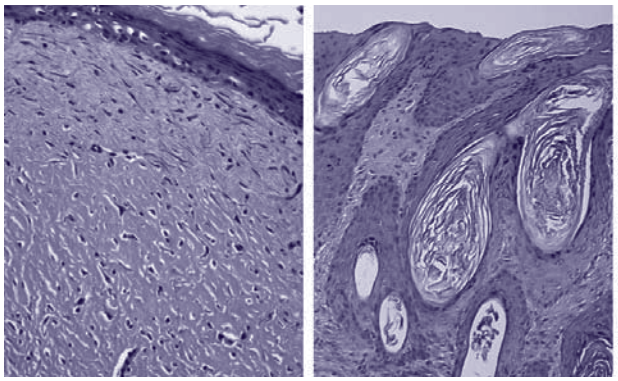

Figure 2: Figure on left shows a normal scar with simple stratified squamous epithelium over a collagen-rich dermal matrix. On right, a similar 40 day wound that overexpressed Wnt 5A retrovirus. Note the numerous epidermal cysts invaginating into the dermis, which later have structures resembling sebaceous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

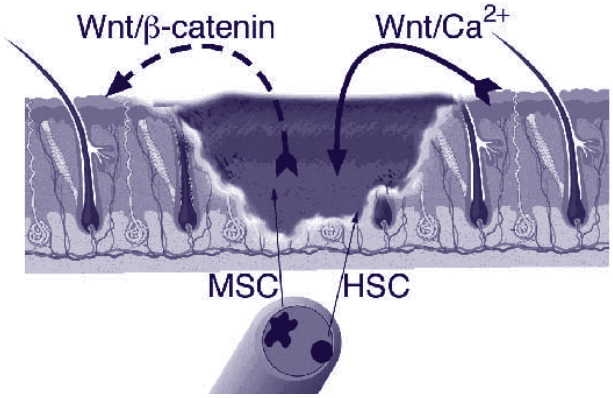

Figure 3: Graphic demonstration of our hypotheses. We think stem cells in the epidermis and stem cells from bone marrow can provide the missing cell types following loss due to injury. However, we think that the lack of morphogens in the wound may account for the lack of regeneration seen in wound repair.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

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

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KLS Martin Ltd.

Craniofacial surgery is a relatively new sub specialty 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.

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.

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 we have identified a subgroup 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 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 postoperative 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.

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

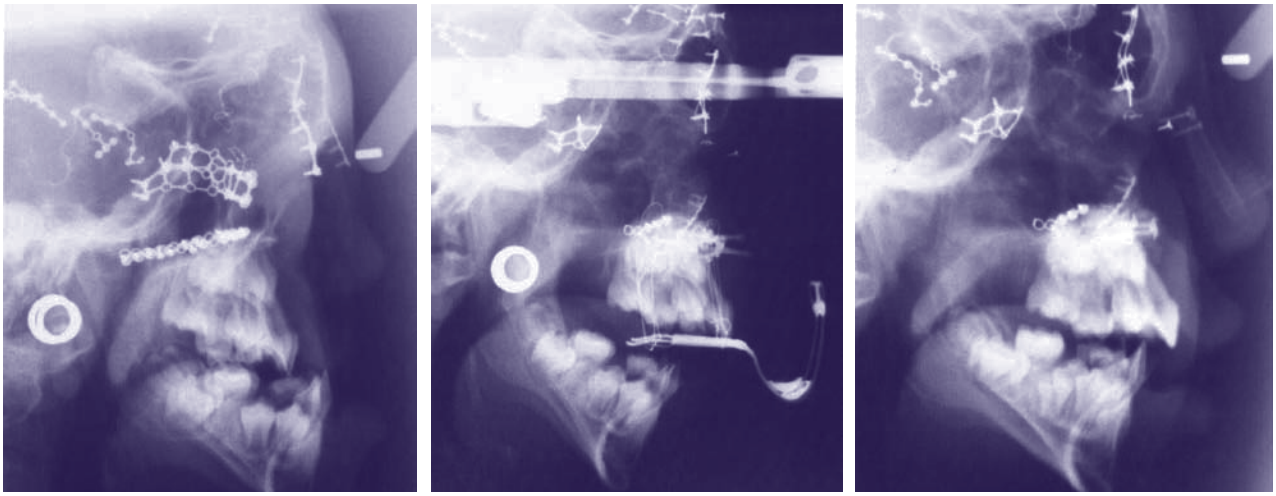

Figure 1: Lateral Cephalograms of a child undergoing midface distraction osteogenesis with an external device (Left) Before the operation, the child is having problems sleeping due to constriction of her nasopharynx, problems with dry eyes due to lack of cheek protection, and a problem chewing due to 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.

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.

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 has just been completed which examined the

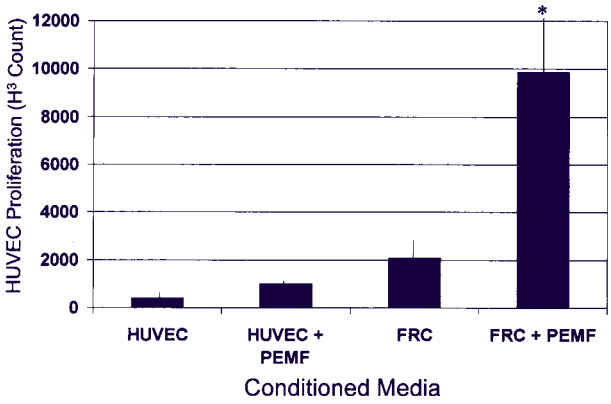

Figure 2: The effect of media from osteoblasts and endothelial cells stimulated with a pulsed electromagnetic field (PEMF) on endothelial cell proliferation. When human endothelial cells (HUVEC) were grown in media from fetal rat osteoblasts stimulated with PEMF (FRC+PEMF), their rate of proliferation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

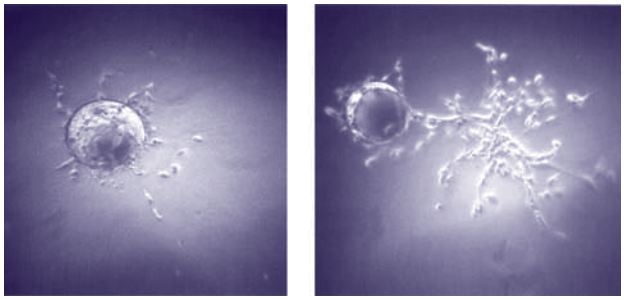

Figure 3: The effect of pulsed electromagnetic fields (PEMF) on endothelial cell tubulization. Endothelial cells attached to microspheres formed significantly more tubules when exposed to PEMF (left) than when not exposed the PEMF (right).

interaction of rat cranial osteoblasts with endothelial cells in the presence of pulsed electromagnetic fields (PEMF). We have 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).

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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- Identification of Genes Responsible for Immunologic Tolerance
- Immunologic Tolerance in a Large Animal Model
- Lymphocyte Development and Differentiation: The Role of the Notch Gene

AWARDS

American Foundation of Urologic Disease Fellowship

National Institutes of Health
- Career Investigator Development Award

FUNDING

National Institutes of Health
- National Institute of Diabetes, Digestive and Kidney Diseases

Transplantation has matured to become the treatment of choice for end-stage renal and hepatic disease. Despite many advances in immunosuppression, the majority of transplant grafts continue to be lost to immunologic causes. Of these, rejection, a lymphocyte-mediated response to foreign tissue, is a leading factor. Our research is directed toward understanding the factors responsible for this immune response and developing techniques to abrogate them. Our laboratory focuses on the control of development and differentiation as it pertains to lymphocytes and hematopoietic stem cells. We are using large-scale cDNA array techniques to ascertain the regulatory genes involved in these processes. In addition, we are interested in developing tolerance strategies in a large animal transplant model using knowledge gained from this work.

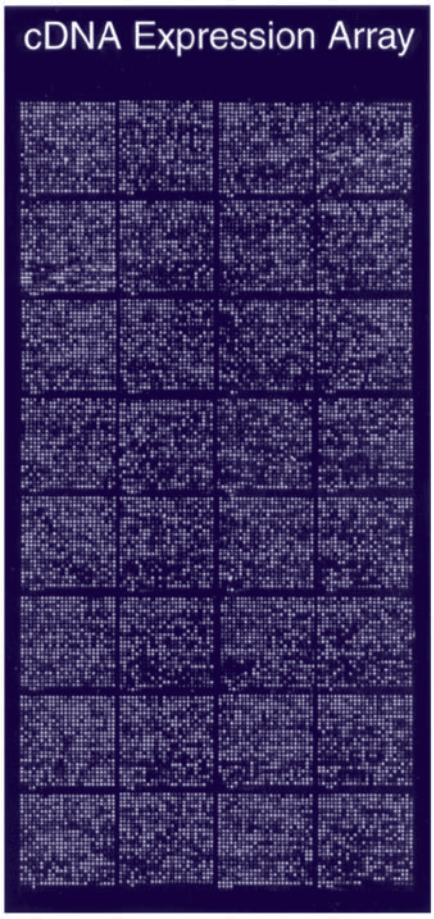

FIGURE 1

Identification of Genes Responsible for Immunologic Tolerance

Tolerance describes a state of 'donor-specific unresponsiveness.' This develops *de novo* in some patients after organ or stem cell transplant. The events responsible for this have not been elucidated and would provide important insights into therapies that would mitigate the effects of chronic non-specific immunosuppression. We are actively seeking answers to these questions by employing cDNA arrays (Fig. 1) of lymphocyte subsets both from human patients who have developed tolerance and in a transgenic mouse model of tolerance. We expect that the patterns of gene expression novel to the tolerant versus the non-tolerant state could provide a tool to determine when the tolerant state is reached. Additionally, individual genes that are differentially regulated between these two states may lead to insights into the mechanisms of tolerance induction.

Immunologic Tolerance in a Large Animal Model

This part of our work involves a large animal transplant model. In collaboration with Drs. Rainer Storb and Beverly Torok-Storb at the Fred Hutchinson Cancer Research Center, we have successfully created dog models that are hematopoietic chimeras through hematopoietic stem cell transplantation. These animals have accepted renal transplants in the absence of immunosuppression from their DLA matched littermate donor, and currently have excellent renal function more than one year after renal transplantation (Fig. 2). We

Understanding the control of lymphocyte development and maturation will lead to important insights into antigen specificity and immune dysregulation, and could be exploited to alter the immune response.

are exploiting this animal model to examine both the induction of tolerance and the robustness of hematopoietic chimerism as platform for organ transplantation in the absence of immunosuppression.

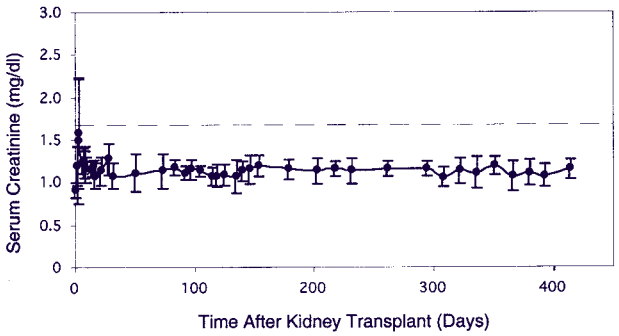

FIGURE 2

Lymphocyte Development and Differentiation: The Role of the Notch Genes

Lymphocyte development proceeds along a pathway characterized by a series of gene rearrangements that impart antigen specificity. Alterations in these pathways can contribute significantly to the development of autoimmune and immunodeficiency states. Understanding the control of lymphocyte development and maturation will lead to important insights into antigen specificity and immune dysregulation, and could be exploited to alter the immune response.

One phylogenetically conserved family of transmembrane receptors with known importance in cell-fate decisions is Notch. Originally identified in *Drosophila melanogaster*, Notch family members subsequently have been identified in other invertebrates, and four mammalian homologues are now known. Their function involves control of developmental cell-fate decisions through Notch receptor signaling which is thought to delay or block differentiation of uncommitted cells. The mammalian Notch family members are ubiquitously expressed and all are expressed in lymphoid tissue. Notch1 has been shown to influence the development of T lymphocytes, and Notch2 has recently been found to inhibit a transcription factor (E47) that is necessary for B lymphocyte. Our preliminary work shows that while the Notch family members are expressed in developing B lymphocytes, Notch2 expression is highest, suggesting unique activity in this cell population.

The focus of our work is to determine the role that Notch family members have in controlling lymphocyte development. To this end we have generated mice which overexpress the constitutively active intracellular portion of the Notch2 and Notch3 genes. We are currently analyzing the animals to characterize the phenotypic changes resulting from increased Notch activity. We plan to make use of cDNA array technology to identify downstream effectors of Notch, which remain to be fully characterized in mammals.

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- 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. 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 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.

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, particular in CD8 T cells 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 on 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 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 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 having the potential to either stimulate or inhibit immune responses. Exploiting the immune-regulatory and tolerogenic capacities of DC hold 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 TGFβ, downregulating 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 TGFβ may drive regulatory T cells 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.

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• A Model to Study the Liver's Role in Peripheral Tolerance

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.

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 Influence of the Liver on Peripheral Tolerance

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 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 mecha-

Our results suggest that different mechanisms of tolerance are influenced differently by the liver depending on the dose of the antigen.

nism 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." In a dog model, the liver has been shown to play a critical role in oral tolerance induction. The mechanisms of the liver's role in oral tolerance induction are not clear.

The Murine Transplant Model

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.

For our study, we utilized male BALB/c mice, 8-12 weeks of age. The mice were divided into six groups according to how they received liver transplants: **1)** and **2)** OVA fed donor livers (low or high doses) to non-fed recipients; **3)** and **4)** non-fed donor livers to fed recipients (low or high doses); **5)** Non-fed donor livers to non-fed recipients; and **6)** non-transplanted, non-fed groups (the controls). Two days after the last feeding or after liver transplantation, all mice were immunized with 50 µg OVA emulsified in complete Freund's adjuvant (CFA) in a total of 50 µl injected at the base of the tail. Seven days after the immunization, 50 µg OVA in 30 µl dH_2O was injected intradermally into the footpad. The footpad thickness was measured 24 or 48 hours later using digital calipers. The increase in the footpad thickness was determined by subtracting the naïve footpad thickness from the OVA-injected footpad thickness. Additionally, liver non-parenchymal cells (NPC) and spleen cells (SC) were isolated from OVA-fed BALB/c mice and adoptively transferred to naïve syngeneic mice through tail vein injection. The NPC and SC from non-fed mice were used as controls. Further OVA immunization was performed one day after adoptive transfer, and the delayed-type-hypersensitivity (DTH) response was examined seven days after the immunization.
In addition to the above *in vivo* study measuring footpad thickness, we 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.

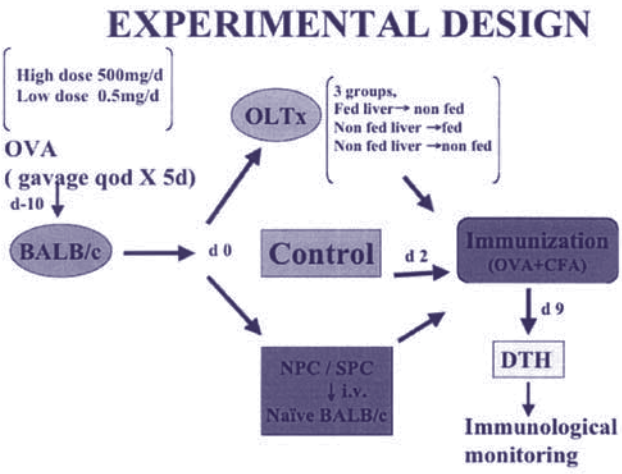

Figure 1: A model to study the liver's role in peripheral tolerance.

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.

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, the SC only 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 TH_2 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 is 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.

Future Investigations and Remaining Questions

In addition to the work we have done so far, our model would also be useful in exploring several other proposed mechanisms of liver tolerance, including the role of Kupffer cells, liver sinusoidal endothelial cells, γ/δ cells, and immature dendritic cells. The model could be manipulated to include transplanting livers between different genders, transplanting irradiated livers and other variations, and it could be used to study specific cell types previously unable to be evaluated.

We have shown that the liver is sufficient to transfer tolerance, but several other questions remain. Is the liver's role in peripheral tolerance unique to itself? Are the mechanisms of inducing tolerance operational in all peripheral nodal tissues? Does the liver's perceived influence come only from its large relative size? Are there clinical applications to the liver's role in peripheral immune tolerance, such as lowering the rejection of organ allografts, and preventing autoimmune disease, chronic viral infections of the liver, and cancer metastases to the liver? We look forward to using our model in further studies to elucidate the mechanisms of this tolerance induction. The end goal is to offer new therapeutic approaches for unsolved problems.

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• The Breast Health Global Initiative (BHGI)

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Purpose and Specific Aims

The Breast Health Global Initiative (BHGI) strives to develop 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 treatment 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-based breast care

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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.fhcrc.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 our without diagnostic mammography) can facilitate cost-effective tissue sam pling 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.

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.fhcrc.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 (International Site To-be-determined)

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." A host organization and international summit site is to-be-determined.

Panels will address early detection and access to care, diagnosis and pathology, treatment and allocation of resources, and health care systems and public policy. However, the 2007 summit focus will shift, from development and expansion of the 2006 Guidelines for International Breast Health and

Figure 1: BHGI is a structure for linkages

Cancer Treatment, to address effective implementation and integration of breast health care interventions described in the Guidelines. 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 will be explored.

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 website, 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

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. The BHGI operates 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. Currently, under development are proposals for:

- Pilot research projects
- Guideline demonstration
- Public education and provider training projects
- Special technology development projects

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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- Advancing the Clinical Science of Surgery Using Outcomes Research Tools

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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, 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.

1. 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

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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.

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.

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.

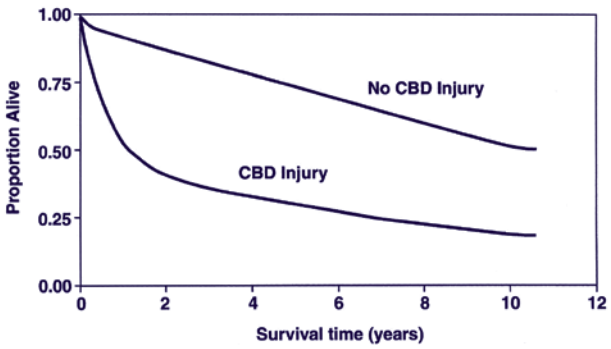

Figure 1: Survival after bile duct injury in Medicare beneficiaries (n=1.57 million)

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.

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.

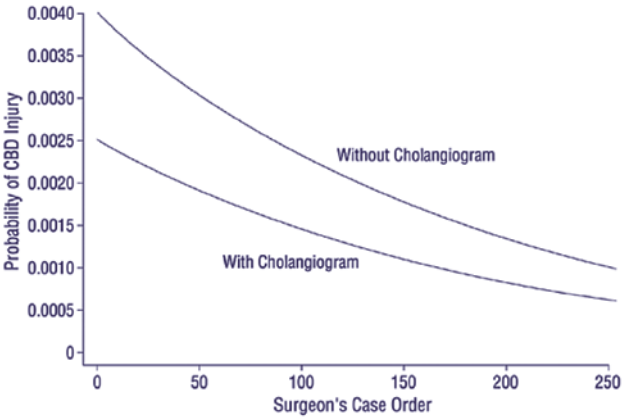

Figure 2: Probability of bile duct injury with and without cholangiogram, by case-order of surgeon (n=36,000)

2. 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).

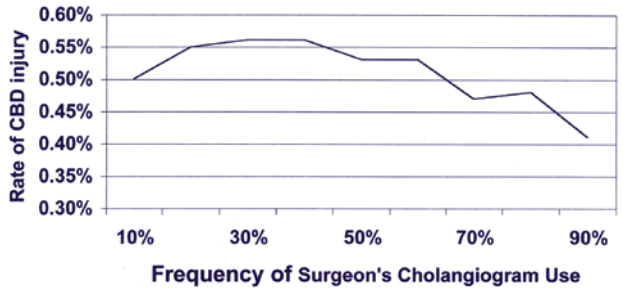

Figure 3: The effect of increasing the surgeon's frequency of cholangiogram use on the rate of common bile duct (CBD) injury

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.

4. 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.

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.

Involving the financial stakeholders may be the most effective way in improving 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.

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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.

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.

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

Preliminary data suggest that videoscopic-assisted retroperitoneal debridement (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: 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.

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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.

Motility in Morbidly Obese Patients with GERD

Morbid obesity is strongly associated with GERD, and both have an independent association with motility disorders. The importance of this is that impaired esophageal function can play a role in the development of dysphagia after fundoplications and bariatric procedures (especially restrictive procedures). However, we lack the ability to predict preoperatively who will develop dysphagia. As we mentioned above, multichannel intraluminal impedance (MII) evaluates the effective clearance of a swallowed bolus through the esophagus, thus in combination with manometry may be able to identify patients at risk for post-operative dysphagia.

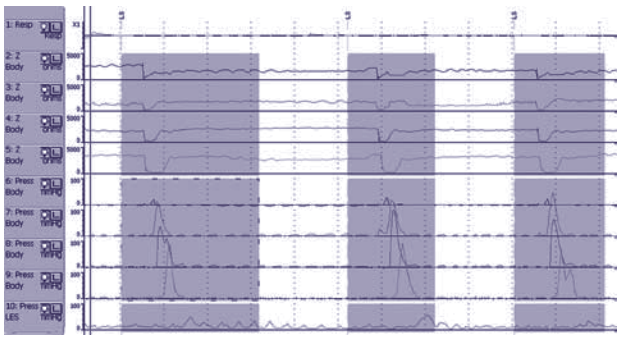


Figure 1: Impedance and manometry tracing showing complete bolus transit (MII) with a normal manometric tracing.

	DeMeester Score (mean ± SD)	Abnormal Manometry	Abnormal Impedance	Impedance Mean Bolus Clearance %
Asymptomatic (n=10)	8 ± 8	0	0	98%
GERD (n=22)	65 ± 56*	5 patients (23%)†	9 patients (41%)	88%
MO-GERD (n=22)	39 ± 30*	5 patients (23%)††	13 patients (59%)	67%**

* *vs. asymptomatic, p < 0.01; GERD vs. MO-GERD, p = 0.11*
** *Obese patients vs. Asymptomatic, p < 0.01; vs. GERD vs. MO-GERD, p = 0.01*
† *Nutcracker esophagus (n = 2), Ineffective esophageal Motility (n = 2), Hypertensive LES (n = 1)* †† *Aperistalsis(n = 2), Diffuse esophageal spasm (n = 1), Nutcracker esophagus (n = 1), Hypertensive LES (n = 1)*

We performed simultaneous MII, manometry, and pH monitoring in 10 asymptomatic subjects, 22 consecutive non-obese patients with GERD (GERD) and 22 consecutive morbidly obese patients with GERD (MO-GERD) being evaluated for antireflux and bariatric surgery at the University of Washington.

All GERD and MO-GERD patients had abnormal pH monitoring. There were similar manometric findings between the GERD and MO-GERD patients (Table). Impedance detected many more patients with abnormal motility than did manometry. MO-GERD patients have significantly impaired esophageal clearance compared to both subjects and GERD patients.

We have demonstrated that impedance often detects impairments in esophageal motility not identified by manometry, which heretofore was not known. Surprisingly, morbidly obese patients with GERD have a very high incidence of impaired esophageal motility, even more so than their non-obese counterparts. This may have significant implications in bariatric procedures, especially those that are restrictive in nature.

Gastroesophageal Reflux Monitoring with MII

Another application of the MII is the detection of GERD. Classically, detection of reflux is confirmed with a 24hr pH monitoring study. This test detects reflux by noting a drop in the pH below four. There may be episodes of reflux, in which the pH does not drop to or below 4 (non-acid reflux), and thus are undetected by pH sensors. Before MII, these episodes were unrecognizable. GERD monitoring with MII involves the utilization of both pH and impedance sensors on the same catheter. The test gives additional information such as the characteristics of the reflux (liquid, gas or mixed), as well as its height, presence within the esophageal lumen, clearance, and pH.

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 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)

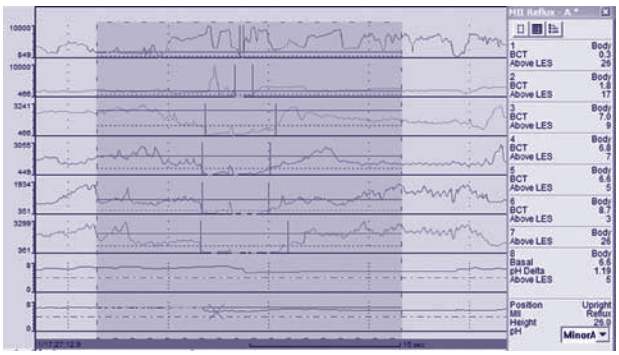


Figure 2: Pharyngeal reflux episode detected by impedance.

	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

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 hyposthesis. 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 gastroesophageal 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.

Ongoing Studies with Impedance

1) Measurement of esophageal motility measuring bolus clearance
2) Determination of the mechanism of dysphagia after fundoplication using impedance
3) Evaluating patients with GERD and respiratory symptoms
   - **a.** Normal amount of nonacid reflux in the pharynx
   - **b.** Nonacid reflux in the pharynx as a predictor of success or failure of medical therapy
4) Patients with GERD who do not respond to medical therapy
5) Influence of nonacid reflux in the pathogenesis of Barrett's esophagus

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, 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 (46mo 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

	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*

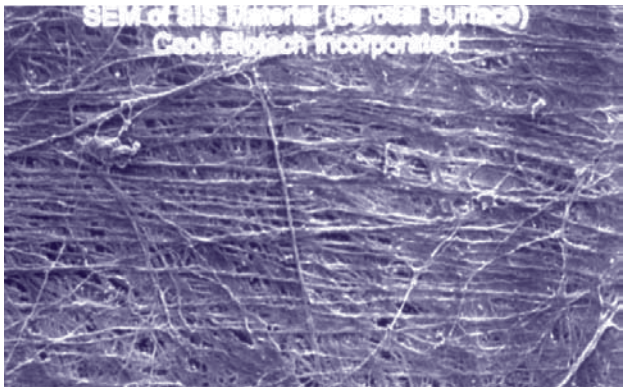


FIGURE 3: Small intestinal submucosa (SIS).

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.

	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*

Esophageal Transit After Heller Myotomy for Achalasia

Patients with achalasia typically have severe esophageal dysmotility, usually aperistalsis, which most commonly persists after Heller myotomy, despite the fact that the majority of these patients experience relief of their dysphagia. Currently there is no convenient and standardized method to objectively evaluate esophageal bolus transit in patients with achalasia after Heller myotomy. Impedance (MII), which demonstrates bolus transit success or failure, as well as transit time in the esophageal body, might be useful in the assessment of the outcome of this surgery. We are currently performing MII together with esophageal manometry in all of our patients with achalasia both before and at 6 months after surgery. The percentage of swallows with complete bolus transit as well as the total bolus transit time is being compared between the pre and postoperative studies. These data are being correlated with patients' postoperative dysphagia symptom scores and preoperative esophageal diameter in order to evaluate the results of Heller myotomy, and may enable us to make predictions about postoperative esophageal function.

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 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

Symptoms Evolution after LARS

	Disappeared	Improved	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.

Patient history and standard diagnostic tools to detect reflux are not good predictors of pharyngeal reflux episodes.

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)

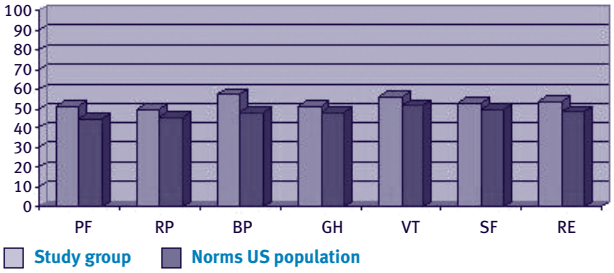
Laparoscopic techniques have been applied with increasing frequency to the repair of paraesophageal hernias, with the benefits to the patient being less pain, shorter hospital stays, and quick recovery. However, recent reports have raised concerns regarding a higher recurrence rate after laparoscopic repair when compared with open approaches. No matter which approach is used, recurrence is usually due to failure of the crural repair. For this reason, many surgeons have used the concept of a tension free mesh repair (as it is used for other types of hernias). Because the use of synthetic mesh is associated with complications such as esophageal erosion/perforation most surgeons are reluctant to use them. A new material, porcine small intestinal submucosa (SIS), has recently been introduced that serves as a temporary lattice for tissue ingrowth and a strong tissue matrix. Because it is very pliable and not synthetic, it should be less likely to cause esophageal damage, and may reduce the recurrence rate if used in paraesophageal hernia repair.

We performed an initial pilot study that confirmed its safety with very few recurrences. We have developed a multi-center clinical trial with Oregon Health Sciences, Legacy Health System, and Washington University. This trial is closed and in 2006 we should be able to answer whether bioprosthesis reduces the recurrence of paraesophageal hernias.

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 6)

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

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- Objective Assessment of Surgical Skills

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Defense Advanced Research Projects Agency (DARPA)
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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 Laproscopic Cholecystectomy simulator, in addition to other systems such as the "Blue Dragon" that are described elsewhere.

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

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

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

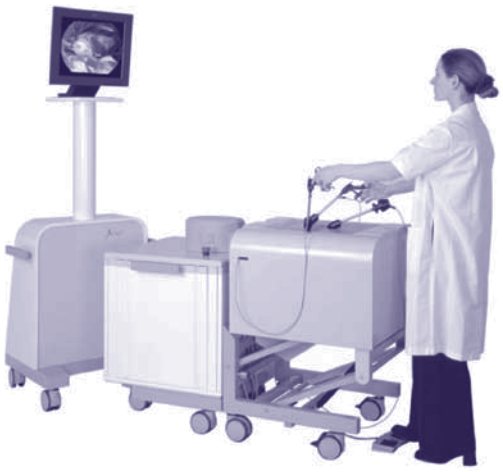

Figure 2: Xitact Laparoscopic Cholecystectomy simulator illustrating the portable system and video image.

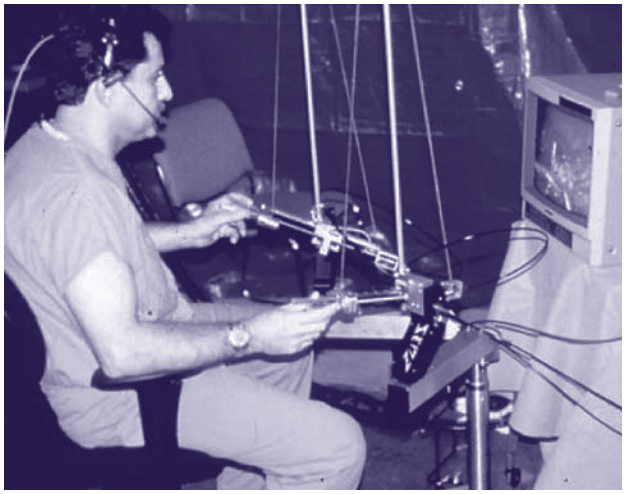

Figure 3: Zeus surgical robotic system

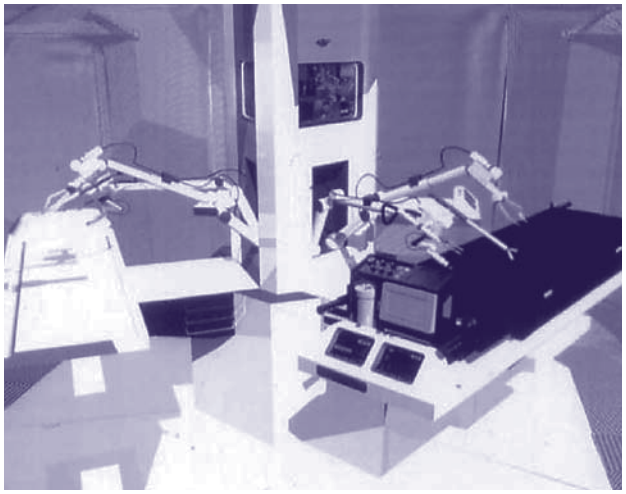

Figure 4: OR of the future – concept drawing from Integrated Medical Systems

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.

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- ISIS: Institute for Surgical and Interventional Simulation

The Institute for Surgical and Interventional Simulation (ISIS) is a pioneering collaborative effort between the Department of Surgery and other UW Medicine Departments. The goals of ISIS are to improve the safety and efficiency of surgical and other interventional procedures through training in simulated environments. The charter for ISIS calls for the development, implementation, and validation of procedural curricula based on simulated clinical environments for training of medical professionals before such procedures are incorporated into clinical practice. Although ISIS will initially focus on resident training in academic environments, eventually medical students, practicing physicians needing to acquire or polish skills, and nurses together with other allied healthcare workers will be offered training in procedural areas. Just as training in a surgical procedure may be of tremendous benefit to a resident surgeon, pre-clinical training in wound and drain management, ostomy care, and training at the interface of the patient and bedside technology holds great promise for improving safety, patient and staff satisfaction, and efficiency.

Background

ISIS is our acronym for this new concept in medical procedural training. Isis was venerated in ancient Egypt as the most powerful of goddesses: daughter of Ra, wife of Osiris, and mother of Horus. Isis was the goddess of creation, magic, and medicine, all worthy attributes for our Institute. In searching for a name for our new procedural patient safety center, this legacy seemed appropriate both for its elegant iconography and for its poignancy.

Patient safety and efficiency in procedures, the domain of ISIS, loom very large today. They are among the most significant societal issues in healthcare as evidenced by the two Institute of Medicine Reports, daily, and national weekly news reports. The prospect that avoidable harm occurs in up to 2.7% of procedures and is a contributing factor in 7.5% of deaths after procedures belies our avowed aim to alleviate suffering and cure illness through such interventions. Procedural errors have also contributed to the crisis in medical liability nationally. Increases in the cost of healthcare beyond 15% of the GNP and beyond the sustainable support of many small businesses are certainly amplified by the economic cost of waste, inefficiency, and error in procedural interventions.

Coincident with increasing recognition of safety and cost issues has been a revolution in computer and other simulation technology that makes clinically realistic, laboratory scenarios increasingly feasible for use as training platforms. The cost of many of these platforms is still high ($30K to $90K), procedural options often limited within a given platform, and the lack of a mandate for medical simulation training limiting for extensive commercial investment to expand the industry. However the capabilities of the most advanced train-

Adult students seem to acquire knowledge and skills best when those are vested in their experience and offer them immediate operational benefits, a recognition of adult learning that has driven development of simulators and curricula toward more practical goals.

ing systems, for example the anesthesia simulator or interventional cardiology simulator (CathSim), approach the realism and utility of flight simulators that have revolutionized commercial pilot training and certification.

Adult students seem to acquire knowledge and skills best when those are vested in their experience and offer them immediate operational benefits, a recognition of adult learning that has driven development of simulators and curricula toward more practical goals. Instead of saturating them with clinical experience, the new resident work hour limitations both restrict clinical exposure and offer newly available time for training outside the service imperatives of a busy clinical service. In ISIS, we plan to take advantage of this new dedication to "educational time" and offer training that is immediately applicable and practical. In this effort, we intend to construct training curricula within an investigative framework, using the dedicated time of our instructors and students to both train AND analyze the efficacy of training on clinical application of procedural skills. With our internal and commercial partners, we intend to advance and improve procedural training through better simulation and curricula.

Personnel

- Dr. Carlos Pellegrini (Chair, Department of Surgery) is Chair of the ISIS Board of Trustees.
- ISIS Board of Trustees represents leadership from UW medical and allied health care departments with an interest and commitment to procedural training using medical simulation.
- Dr. Richard Satava (Professor, Surgery and a nationally recognized expert in surgical simulation) is the Executive Director of ISIS.
- Drs. Mika Sinanan (Professor, Surgery) and Robert Sweet (Acting Assistant Professor, Urology) are Co-Directors of ISIS.
- Dr. Jacob Rosen (Research Assistant Professor, Electrical Engineering) is our Technical Consultant and strategic partner to the Biorobotics Laboratory in Electrical Engineering.
- Dr. Suzanne Weghorst (Assistant Director for Research, Human Interface Technology Lab) is our Research Consultant for VR simulation to the Human Interface Technology Lab based in Fluke Hall.

We have also called on a wealth of local expertise. Drs. Karen Horvath and Brant Oelschlager in Surgical Education and the Center for Videoendoscopic Surgery, Dr. Blake Hannaford, Director of the Biorobotics Lab in Electrical Engineering, and other colleagues in medical education at Harborview and the Veterans Administration Medical Center, in Medical Education & Biomedical Informatics, Psychology and the Department of Education will serve as UW consultants. Industrial partners in this enterprise include Simulab, based in Seattle, Surgical Science, Intuitive Surgical, and others.

ISIS Management: Dedicated training and equipment personnel will be hired to maintain the site, coordinate training and research activities, and to track and archive the training records of individual trainees.

Trainers: Curriculum development and the hands-on work of coaching, of putting simulation training into a clinical context, will fall to a cadre of surgical educators with expertise and both an academic and an educational interest in procedural simulation training. These specialist trainers will come from all participating departments and will form our core training group for specialized and cross-discipline training. Many have come forward already, expressing their interest through the "Champion's group" led by Dr. Rob Sweet. Others will need to be recruited as we expand ISIS and develop a comprehensive annual calendar of courses.

Trainees: Recruitment of trainees will proceed in several phases over the first three years of ISIS. With assistance from hospital-based QI and the respective education divisions of UW Medicine departments, we intend to identify high volume and higher risk procedures commonly performed by residents, especially procedures for which we already have basic mentoring and/or training requirements in place. This list will then be matched with available simulation platforms. For some procedures, new platforms will need to be developed or acquired. Many platforms are currently available but inaccessible and underutilized. Residents performing or participating in these procedures will be trained in simulation and the effectiveness of the training (validation) will be measured. In time, basic skills courses for medical students, nursing, and other allied health care workers will also be developed. Based on demand, training in new procedural technology and techniques will be added for practicing physicians in the community.

Simulation Platforms

Simulation training platforms span the range of complexity. In general, we have adopted the principle that the simplest valid training platform that accomplishes the goals of training will be used, to constrain cost and equipment failures. Available trainers at the UW now include:

- skills training boxes of several types (Simulab and Hassan trainers)
- latex organ models for simulation of procedures (Nissen, laparoscopic cholecystectomy)
- computer-based training for two handed coordination and special skills (Mist VR™)
- anatomically-correct VR trainings (LapSim™ training for laparoscopic cholecystectomy)
- integrated, computer-augmented trainers (anesthesia simulator, TURP simulator, ENT Sinus surgery simulator)
- surgical robotic platform (Zeus system)
- animate models for integration of skills in the experimental surgical lab

A number of additional trainers are under development or just coming to market. ISIS will seek equipment loans or donations, development funds, and training and research grant funding. These funds will be used to acquire and evaluate significant new simulation technology as it becomes available. At the same time, ISIS leadership also acknowledges the importance of developing curricula that incorporate and establish the relevance of the techniques being taught to safe and effective clinical practice.

Research

Training in simulation is a field holding great promise for improving both efficiency and safety. Military and pilot training in simulation have proven the value of simulation in creating efficiency, competency, and in developing appropriate responses to unexpected conditions. Although some studies have demonstrated improved performance in the OR after training on a few procedural simulators, many commercial simulators have been deployed without adequate validation.

We recognize that our access to trainees and trainers time is extremely limited and costly, so the effective use of this educational time will require that simulators deployed be validated in all appropriate dimensions including translation of skills to clinical practice. This will be a critical focus of research in Isis. In addition, we plan continued work on tissue and organ accuracy, the implementation of force feedback (where appropriate), and further development of interactive manipulation with multiple instruments and surgeons in open and minimally invasive procedural simulated environments.

Location

The University of Washington Medical Center allocated premium space on the first floor of the Surgery Pavilion, between the Vascular Diagnostic Service and the Urology Clinic and Prostate Center, as **ISIS-One**, the first of a number of anticipated simulation training sites distributed among the various clinical sites of the School of Medicine. "ISIS-One" will house both dedicated training simulators and a number of computer work stations with appropriate connectivity to the School of Medicine and other training laboratories. Coordination and administration of ISIS and ISIS-related research activities will be carried out from ISIS-One. Although not fully developed yet, current plans anticipate other centers in the next five years perhaps located at Harborview, Children's, and the Veterans Administration Medical Center to maximize access for residents regardless of their clinical training location.

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Raymond S. Yeung, M.D.

• Tumor Development in Tuberous Sclerosis Complex

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. TSC1 encodes an 8.6 kb transcript of which 4.5 kb of the 3' region is untranslated. It consists of 21 exons with a coding region of 3.5 kb encoding a 130kDa protein, hamartin, which contains an extended coiled-coil domain but otherwise with no significant homology to proteins of known function. The TSC2 gene contains 41 exons encoding a 5.5 kb transcript with several alternatively spliced exons. The predicted protein, called tuberin, has a sequence of 1807 amino acids showing significant homology to the Rap1GAP protein over a 200 amino acid region near the C-terminus.

Investigations have found that the majority of hereditary tumors involve mutations of certain tumor suppressor genes.

The focus of our research is to understand how tumors come about as a result of TSC1 and TSC2 inactivation. These include four areas of investigations to examine the signaling pathways, the underlying biologic mechanisms and other genes that may modify the phenotype of TSC.

Growth factor and energy metabolism in TSC tumors

Studies in Drosophila have revealed a novel role of hamartin and tuberin 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-tuberin complex in mTOR-dependent protein synthesis.

The current model suggests that tuberin 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 tuberin 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 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.

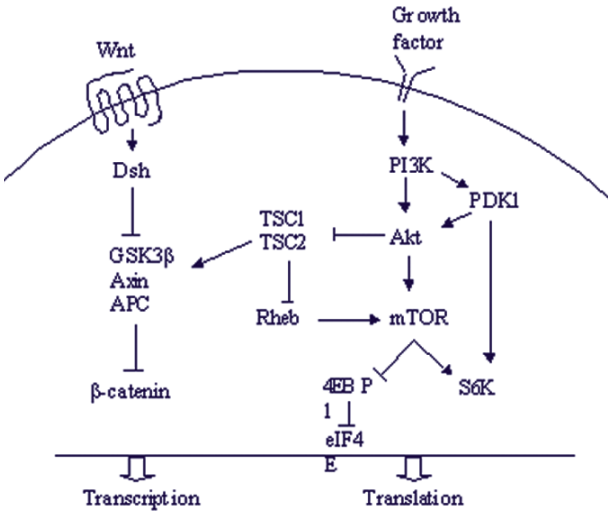

Figure 1: Model of TSC1/TSC2 pathway

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/ß–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 ß–catenin canonical pathway is best understood for its role in various human cancers (e.g., colon, skin, liver). ß-Catenin is a highly conserved 95-kD protein involved in cell-cell adhesion and intracellular signaling. In its latter role, ß-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 ß–catenin and cyclin D1. In 293T renal epithelial cells, expression of TSC1 and TSC2 reduced ß–catenin levels by promoting its degradation. Correspondingly, TSC1/TSC2 inhibited ß–catenin dependent activity of the LEF/Tcf transcription factors. Evidence suggested that TSC1 and TSC2 act at the level of the ß–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.

Subcellular localization of the TSC proteins and their role in protein transport

If indeed hamartin and tuberin 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 tuberin, we found that they indeed reside in multiple compartments (i.e., cytosol, microsome, cytoskeleton). Of particular interest is the vesicular

component in which tuberin 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 tuberin have mis-localized caveolin-1 and reduced formation of caveolae at the plasma membrane.

Recent studies point to a role of tuberin in regulating the transport of proteins such as caveolin-1 from the Golgi apparatus to the membrane. The molecular mechanism mediating this function of tuberin 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.

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VAPSHCS/GENERAL SURGERY

LORRIE LANGDALE, M.D.

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Lorrie Langdale, M.D.

- Hepatic Ischemia-Reperfusion Injury: The Search for Control
- JAK/STAT Cell Signaling Pathway and Suppressors of Cytokine Signaling (SOCS Proteins)
- SOCS Proteins, Cytokine Control and the Response to Hepatic IR Injury

FUNDING
UW Royalty Research Fund
VA Merit Review Grant

Hepatic Ischemia-Reperfusion Injury: The Search for Control

The liver is particularly vulnerable to the effects of local ischemia followed by reperfusion (IR) during resection of hepatic tumors, surgical management of direct liver trauma and organ transplantation. Ischemia initiates a complex chain of events that is augmented during reperfusion and is characterized by early expression of inflammatory cytokines and chemokines, with subsequent neutrophil activation and infiltration. The resulting injury has the potential to evolve to liver failure. Experimental therapeutic strategies to improve outcomes after IR have been aimed at disrupting individual components of this highly redundant inflammatory cascade, treating either prior to the onset of ischemia or at the time of reperfusion. To date, however, laboratory successes have not translated to clinically relevant therapies, at least in part because the pro-inflammatory phase of injury is well underway by the time patients present for treatment.

We have chosen an alternative approach to understanding the control of IR injury, focusing on signaling events that mediate the body's management of an acute inflammatory response rather than means of preventing inflammation from the outset. Given their critical role the evolution of ischemia-reperfusion injury, it is likely that events that precede, trigger and *regulate* inflammatory cytokines and chemokines play a major role in determining whether injury resolves or progresses to irretrievable damage and organ failure. Understanding these relationships is central to effective clinical modulation of ischemia-reperfusion injury once it is underway and potentially offers an exploitable new avenue for clinical control of ischemia-reperfusion injuries.

Ironically, cytokines generally accepted as pro-inflammatory (and thus potentially harmful in nature), have also been shown to confer protection under clinically relevant conditions. For example, we have previously shown that IFNγ, long accepted as a primer of macrophages and T-cell immunity, is protective in a model of liver IR when given in a dose known to restore immunocompetence. High dose IFNγ pre-treatment of normal, immunocompetent rabbits blunts progression of liver IR injury, as evidenced by decreased glutamate pyruvate transaminase (GPT) concentrations, while lower dose IFNγ pre-treatment or saline control is associated with a significantly increased cellular injury 24 hr after liver IR Histologic injury, characterized by midzonal and centrolobular necrosis, does not progress beyond the first phase of neutrophil-independent, oxygen free radical mediated injury when animals are pre-treated with high doses of IFNγ. Late neutrophil infiltration is virtually eliminated. Our data have since been corroborated by other investigators utilizing high dose IFNγ in a rat model of liver IR. They further showed amelioration of the associated secondary lung injury.

We have chosen an alternative approach to understanding the control of IR injury, focusing on signaling events that mediate the body's management of an acute inflammatory response rather than means of preventing inflammation from the outset.

Proinflammatory cytokine and chemokine expression in both liver and lung is markedly attenuated by high dose IFNγ treatment. Similarly, interleukin-6 (IL-6) is generally categorized as a pro-inflammatory cytokine but has been shown to be protective in liver IR. TNFα and IL-6 are also known to play a critical role early in liver regeneration following partial hepatectomy, serving to regulate the priming phase of regenerative repair.

JAK/STAT Cell Signaling Pathway and Suppressors of Cytokine Signaling (SOCS Proteins)

An effective response to injury requires balance between active inflammation and mediator regulation. In fact, the spectrum of cytokines that contribute to inflammation and its resolution utilize common cell signaling pathways to mediate their effects. One such key pathway involves the Janus family of tyrosine kinases (JAK-Tyk) and the signal transducers and activators of transcription proteins (STATs), which are initiated when cytokines such as IL-1, IL-6, IL-12 and IFNÐ bind to their receptor and the receptor's cytoplasmic tail is phosphorylated. 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 STATs 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 inflammatory mediator gene transcription.

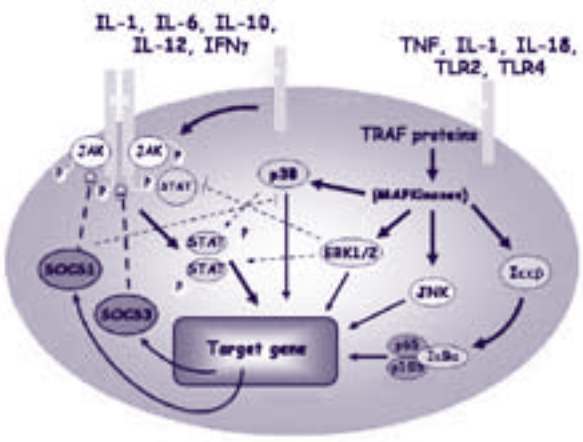

FIGURE 1: Cell Signaling Pathways of Inflammation

Among the gene targets of JAK/STAT-inducible early genes are a family of at least eight proteins, designated *Suppressors of Cytokine Signaling or SOCS proteins* (1-7, CIS) that regulate cytokine-triggered JAK/STAT signal transduction through direct negative feedback inhibition at key junctures within the pathway. In this way, the effects of major inflammatory mediators are held in check. Chemokines important to neutrophil trafficking also signal through JAK/STAT (STAT-5) and have been shown to be regulated, at least in part, by SOCS. In addition to their direct negative regulation of JAK/STAT, SOCS1 and SOCS3 have also been proposed as major inhibitors of the inflammatory processes mediated by various mitogen-activated protein kinase signaling (MAPK) mechanisms. Several cytokines important to IR, including TNFα, IL-1, IL-6 and Toll-like receptors, utilize these cell signaling pathways. Thus SOCS proteins may make important contributions to the regulation of inflammatory mediators outside a direct negative feedback loop. Because the induction of SOCS genes by one cytokine potentially modifies the duration and intensity of numerous cytokine signals, they are ideally situated to participate in cytokine signaling crosstalk, contributing to the overall regulation and control of the complex and redundant response that is the hallmark of an inflammatory response to injury.

SOCS Proteins, Cytokine Control and the Response to Hepatic IR Injury

The potential importance of SOCS proteins to both acute and chronic liver injury is apparent from studies in transgenic mice. SOCS1 -/- 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 SOCS1 -/- mice is markedly reduced in size and there is progressive loss of maturing B-lymphocytes in bone marrow, spleen, and peripheral blood. Animals lacking SOCS1 may be rescued by injection of antibodies to IFNγ, implying that an uncontrolled pro-inflammatory response mediated by IFNγ contributes to this phenotype. Mice lacking both SOCS1 and IFNγ however are viable and healthy. In vitro studies of SOCS1 overexpression by IFNγ offer evidence that functionally, SOCS1 appears to be primarily important to limiting the duration of response to cytokines, rather than the magnitude of the response. This is supported by experiments confirming that IL-6 induces normal STAT activation in SOCS1 deficient cells while IFNγ stimulation results in prolonged STAT-1 expression.

Just as IFNγ is a potent inducer of SOCS1, IL-6 is a potent inducer of SOCS3 and over-expression studies suggest that SOCS3 is a pleiotropic negative

regulator of cytokines. Like SOCS1, deletion of the SOCS3 gene (SOCS3 -/- mice) is a lethal defect, but comparative studies in conditional knock-out mice indicate that SOCS1 and SOCS3 each function in a remarkably specific manner. SOCS3 deficiency prolongs activation of STAT1 and STAT3 after IL-6 stimulation but activation of STAT1 after stimulation with IFNγ is normal. Although similar studies in mice with conditional-deletion of the SOCS1 gene are not completed, SOCS3 and SOCS1 appear to have reciprocal functions in IL-6 and IFNγ regulation and not only attenuate cytokine-specific intra-cellular signaling but also help to coordinate the biological responses by specific cytokines. Microarray analysis shows that IL-6 induces a pattern of gene expression in SOCS3 conditionally-deficient livers that mimics the pattern induced by IFNγ. Thus, both proteins may contribute to regulation of IFNγ and IL-6 signaling. While the role of SOCS1 and SOCS3 is to ensure the appropriate duration of cytokine signaling, like many body systems there appears to be redundancy between SOCS1 and SOCS3. Although not functionally interchangeable, these cytokine regulators represent overlapping potential mechanisms of cytokine control for a spectrum of disease.

In all, these data support the concept that it is the loss of balance between pro-inflammatory and negative control mechanism that tips the scales between acute fulminant liver injury and recovery. We hypothesize that SOCS proteins are at the fulcrum of the response to IR, such that, depending on the timing and intensity of a pro-inflammatory stimulus, the relative expression of SOCS proteins determines whether injury progresses or resolves. Thus the expression of SOCS proteins may represent an exploitable means of clinical injury control.

Our current work utilizes a murine model of hepatic IR to examine the role of SOCS proteins as critical modulators and gatekeepers of the phenotypic response to ischemia-reperfusion injury. In this model, mice undergo partial hepatic ischemia, retaining continuous perfusion to three small segments of the liver. As a first step, we have characterized compared injuries (histology, cytokine expression) and the pattern of SOCS expression in both previously ischemic and continuously perfused liver segments across a range of liver IR severity (20, 45, or 90 minutes of ischemia followed by variable periods of reperfusion). Table 1 summarizes these data. We have shown that SOCS3 appears to be induced as an early injury response gene, while SOCS1 expression

GPT (4 hours after reperfusion)	+		++		+++	
Isch		Perf	Isch	Perf	Isch	Perf
Neutrophils	–	–	+	–	++	–
Necrosis	–	–	+	–	++	–
SOCS3	++	++	++	++	++	++
SOCS1	–	–	+	+	++	++

TABLE 1: Summary of Increasing Injury Severity Effects
Mild Moderate Severe

is reserved as a second control mechanism, induced as an injury becomes increasingly severe.

Work to fully characterize the relationships between cytokine and chemokine expression, their cell signaling mechanisms and SOCS expression are ongoing. However, the severity of injury appears to be critical not only to the induction of pro-inflammatory mediators, but the timed expression, intensity and duration of potential injury control mechanisms. Interestingly, these effects are not limited to directly injured tissue. Continuously perfused liver invokes similar SOCS responses as tissue subjected to ischemic injury after a broad range of IR injury, likely due to the effects of circulating mediators. Confirmation of the central role of SOCS proteins to the control of IR injury, however, will require evidence that deletion of individual SOCS genes worsens injury and that early and/or sustained expression is protective.

To accomplish this, we are extending our murine IR model to transgenic mice with conditional deletion of SOCS genes. Given the peri-natal lethality of complete gene deletion, our collaborators in Australia have bred mice with SOCS deletion that is confined only to liver. This will allow us to test our hypotheses as to the relative importance of SOCS1 and/or SOCS3 to the evolution of liver IR injury. In wild type mice, a mild IR injury resolves without consequence, moderate IR should produce a more severe but potentially recoverable injury and prolonged ischemia should progress to irrevocable injury. With conditional deletion of SOCS3, significant worsening of mild injury (mimicking the severity of injury observed with longer periods of ischemia) would place SOCS1 and/or SOCS3 at the center of the early response to injury. Functional cell signaling redundancy between SOCS1 and SOCS3 may shift "responsibility" for injury control from one suppressor of cytokine signaling to another with conditional deletion of a single gene.

Alternatively, if SOCS3 and SOCS1 are "additive" mechanisms of control, conditional deletion

of SOCS1 will likely have a lesser overall effect on mild IR but dramatically undermine the capacity for recovery with a more severe IR injury characterized by further loss of hepatocytes with sustained GPT, prolonged cytokine and chemokine expression and early hepatic failure. The altered inflammatory milieu will also affect the more normal residual tissue (perfused lobes) due to an increase in circulating mediators, offering insights into the indirect effects of IR on hepatic reserve. As further proof of SOCS central role in IR control, we are also undertaking studies in which mice undergoing hepatic IR are stimulated with IL-6 or IFNγ, determining if overexpression of SOCS1 or SOCS3 is responsible for injury protection. In addition, we are addressing the interface between SOCS regulation and other inflammatory signaling pathways extending these studies to evaluate both local hepatic and secondary lung injury after hepatic IR.

In summary, our findings will not only further characterize the nature of injury control but support further study of SOCS proteins as novel potential therapies to improve outcomes after ischemia-reperfusion.

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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.

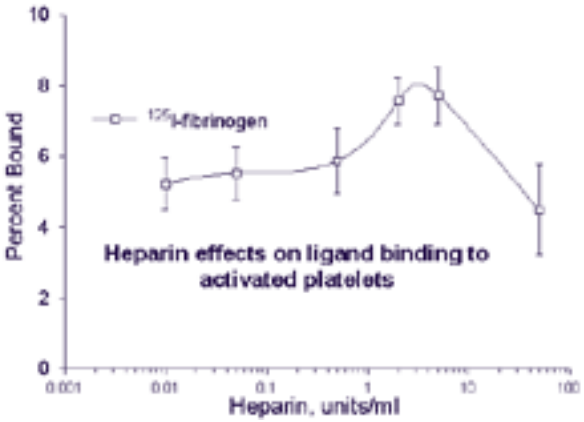

FIGURE 1: ^{125}I-Fibrinogen binding to thrombin-activated platelets was measured over a range of heparin concentrations. At concentrations of 2 and 5 units/ml heparin, fibrinogen binding was significantly increased.

> *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 subspecies 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.

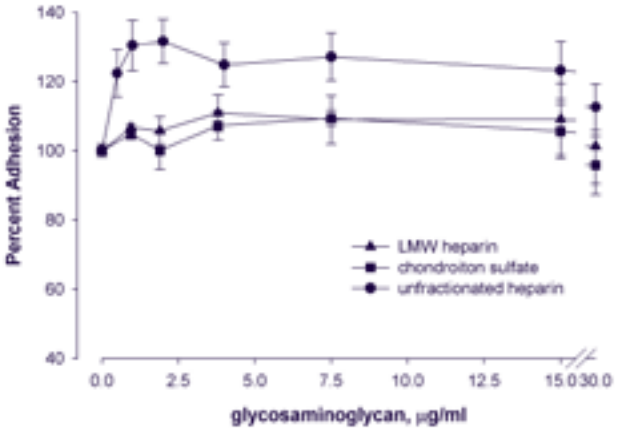

FIGURE 2: Thrombin activated platelets.

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, GpIIbIIIa (integrin $\alpha_{IIb}\beta_3$). 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).

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 (αIIbβ3), 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 (αIIbβ3, αV β3) 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.

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

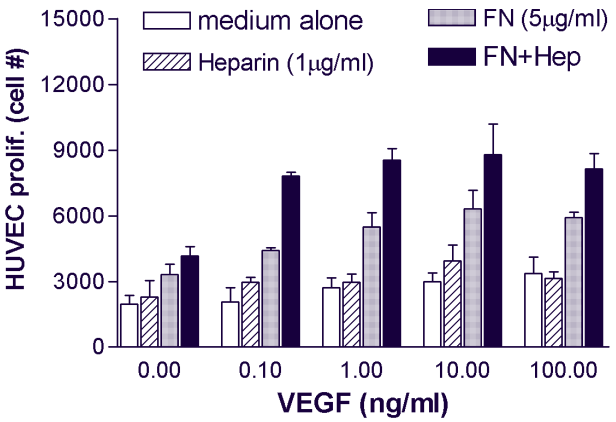

FIGURE 3: Adhesion of K562 $\alpha_v\beta_3$ cells to vitronectin. Unfractionated heparin enhances integrin-mediated adhesion, but other glycosaminoglycans do not.

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.

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VASCULAR SURGERY

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- Tissue Pulsatility Imaging for the Evaluation of Tissue Perfusion
- Tissue Pulsatility Imaging of Muscle
- Intracranial Tissue Pulsatility Imaging (TPI)
- Arteriolar and Venular Plethysmograpic Imaging
- TPI and Doppler Waveforms
- Real-Time Vibrometry
- Video Photoplethysmography
- Ultrasound Reading Center for Carotid Stents

FUNDING
National Institute on Biomedical Imaging and Bioengineering

Based on the pioneering work of D. Eugene Strandness, Jr., M.D. which continued for over forty years in the Department of Surgery, the noninvasive vascular laboratory has developed ultrasonic diagnostic methods used throughout the world. The Department of Surgery in collaboration with the new UW Center on Industrial and Medical Ultrasound and the Department of Bioengineering, is continuing to develop innovative ultrasound examination technologies for vascular diseases and abnormalities.

Tissue Pulsatility Imaging for the Evaluation of Tissue Perfusion

Plethysmography has been used for nearly a century to measure physiological changes in tissue volume with pulse and respiration. Strain gauge plethysmography has been used in the Department of Surgery for the diagnosis of tissue ischemia and cold sensitivity. Normal tissues expand 0.1% with the cardiac cycle (Figure 1) and 1% with the respiratory cycle.

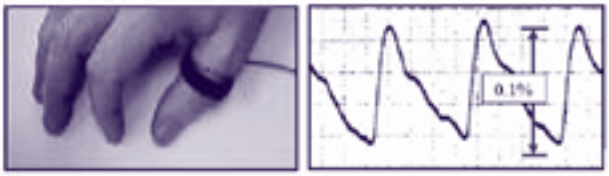

FIGURE 1: (Left) Mercury strain gauge plethysmograph around the thumb; (Right) three cardiac cycles of the plethysmographic waveform from the thumb.

The pulse plethysmographic waveforms associated with normal resting tissue differ from the waveforms associated with ischemic tissue and tissue in oxygen deficit which have "obstructive" waveforms (Figure 2). The expansion of the tissue is also called tissue strain.

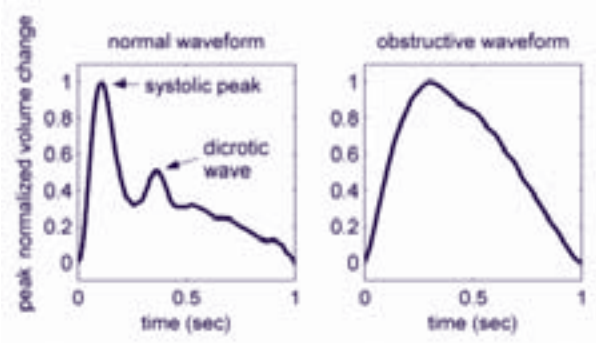

FIGURE 2: Normal and obstructive (oxygen deficit) plethysmographic waveforms shown for a single cardiac cycle.

We have developed a series of ultrasound instruments to measure plethysmographic waveforms in tiny volumes of tissue called voxels (0.01 cubic centimeters). These instruments are able to acquire tissue strain waveforms from 20,000 voxels from a single 2-dimensional image plane in tissue, by measuring the difference in motion between adjacent depths (Figure 3). The motion resolution is better than 0.1 micrometers producing a strain resolution of 0.01% or 1/10 of the normal arterial strain. Ultrasound systems allow 200 measurements along each image line. An image typically includes 100 lines, but a 3-dimensional space can include 10,000 lines including 2 million voxels.

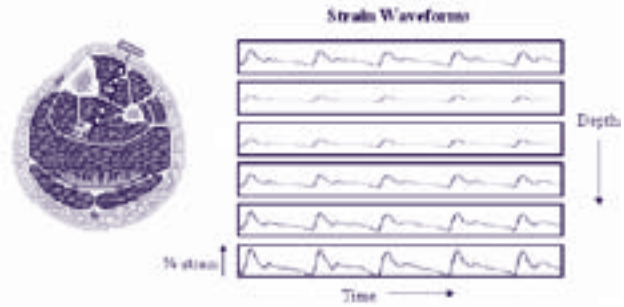

FIGURE 3: Tissue expansion (strain) measured at multiple depths in the anterior tibial muscle (schematic at left). The strain waveforms are from sample volumes separated by approximately 1 mm in depth. Notice the similarity of the waveshapes to the normal waveshape in Figure 2.

We call the results "Tissue Pulsatility Imaging" (TPI).

Tissue Pulsatility Imaging of Muscle

We have applied this method to several tissues including the muscles of the calf (Figure 4).

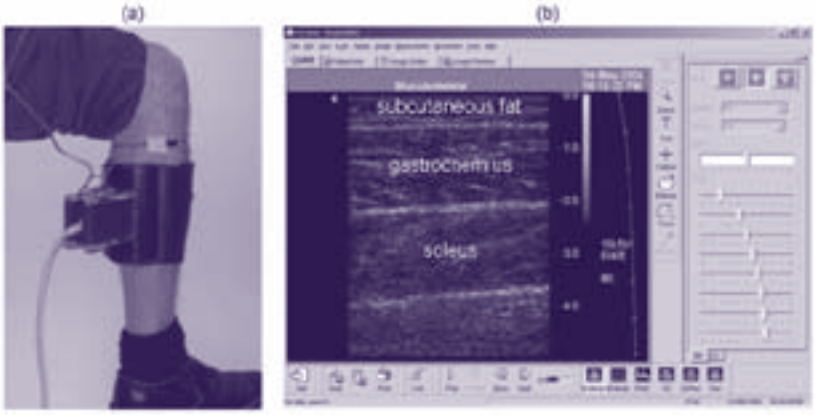

FIGURE 4: (a) Photograph of the ultrasound transducer and strain gauge around the leg. (b) Screen shot taken from the ultrasound scanner showing the muscle anatomy. Notice that the ultrasound scanhead must be held in a stable position during the 10 second periods of data acquisition.

At rest, the waveforms in the voxels of the soleus and gastrocnemius have a waveform that is similar to the "normal" resting waveform (Figure 5, "TPI before exercise"). However, with repeated "toe stand" exercise, the calf muscle becomes oxygen depleted so that in the post-exercise period, most of the voxels show waveforms similar to ischemic waveforms (Figure 5, "0:15 after"). After 5 minutes of rest, the muscles are still in the process of recovering (Figure 5, "4:45 after"). The "typical" resting TPI waveform (right edge of image) has a "dicrotic wave" (second peak) on the descending portion of the waveform (highest of 4) that indicates relative vasoconstriction. Immediately after exercise, the dicrotic wave is absent in the waveform (lowest of 4) indicating vasodilation, and slowly regains the dicrotic wave as the tissue oxygenation is restored.

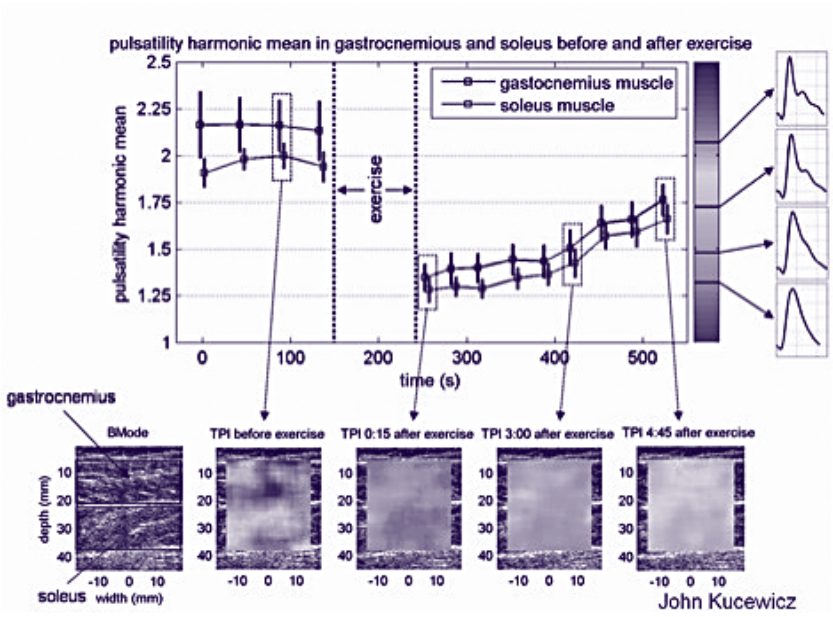

FIGURE 5: Results From a 2D Pulsatility Exercise Study. The "pulsatility harmonic mean" is a method of characterizing the waveform shape as a number. Higher values indicate that a dicrotic wave is present, consistent with vasoconstriction, lower values indicate that a dicrotic wave is absent indicating vasodilation.

Vasodilation in muscle can also be achieved by rapid breathing, lowering carbon dioxide in the blood (hypocapnia). In muscle, this causes vasodilation, in skin and brain, vasoconstriction is the result.

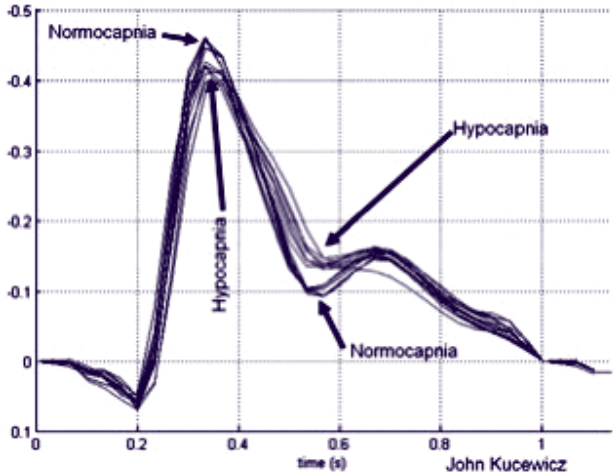

FIGURE 6: Effect of Respiratory Carbon Dioxide Pulsatile Waveforms in Muscle. Normocapnia Arterioles in the muscle are normally vasoconstricted.Hypocapnia With low levels of carbon dioxide, muscle arterioles are vasodilated.

Much of our effort in the past year has been to develop better measures of waveform shape that indicate the degree of vasoconstriction or vasodilation. These measures must not be corrupted by effects of irregular heart beats and must allow separation of venous effects.

Intracranial Tissue Pulsatility Imaging (TPI)

To demonstrate the measurement of vasodilation and vasoconstriction in the brain, a volunteer was asked to breath a air with elevated carbon dioxide. The decrease in the dicrotic wave indicating vasodilation was present throughout the brain. Vasodilation was represented in the image by a "vasodilation index" based on waveform shape (Figure 7).

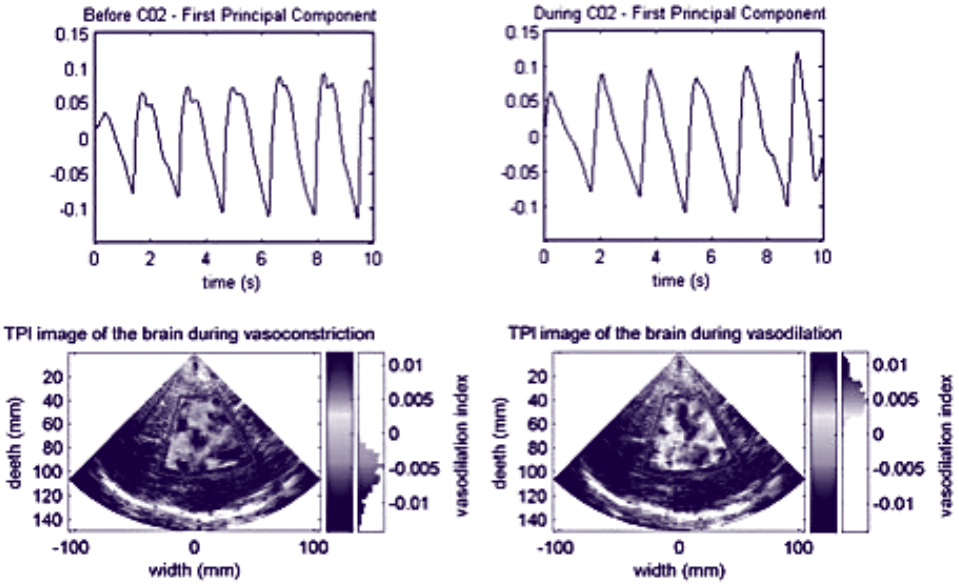

FIGURE 7: Brain Pulse Wave Shape Associated with Vasodilatation Due to CO_2 Exposure.
UPPER: Characteristic wave shape from "principle components" analysis using the individual wave shapes of 10,000 voxels in the image.
LOWER: Two-dimensional image based on the vasodilation index. (Left) Breathing normal air; (Right) Breathing air with elevated CO_2.

Arteriolar and Venular Plethysmograpic Imaging

It is generally accepted that the pulse waveform, which mimics the arterial pressure waveform, are primarily due to pulsatile expansion of the arterioles. Every plethysmographic waveform also includes expansion in synchrony with respiration, which is associated with venous pressure. These "respiratory waves" are primarily due to cyclic expansion of venules. The arteriolar and venular waveforms can be separated by a mathematical method called Independent Component Analysis (ICA). ICA reveals 2 sources of data from the data from the Soleus muscle (Figure 8).

By studying both the arteriolar and the venular waveforms, arterial hypoperfusion and venous hypertension can be evaluated in real time without waiting for tissue breakdown.

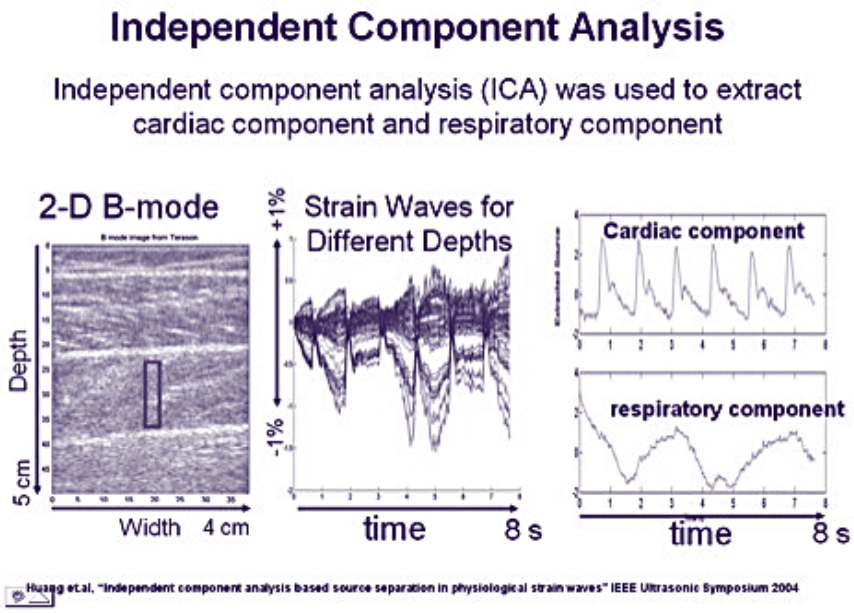

FIGURE 8: Independent Component Analysis applied to the Soleus Muscle. Extracted components including the arteriolar waveform and venular.

TPI and Doppler Waveforms

There is a direct mathematical relationship between the TPI arteriolar waveforms and conventional Doppler arterial waveforms (Figure 9): the TPI waveforms are the mathematical integral of the corresponding Doppler waveforms.

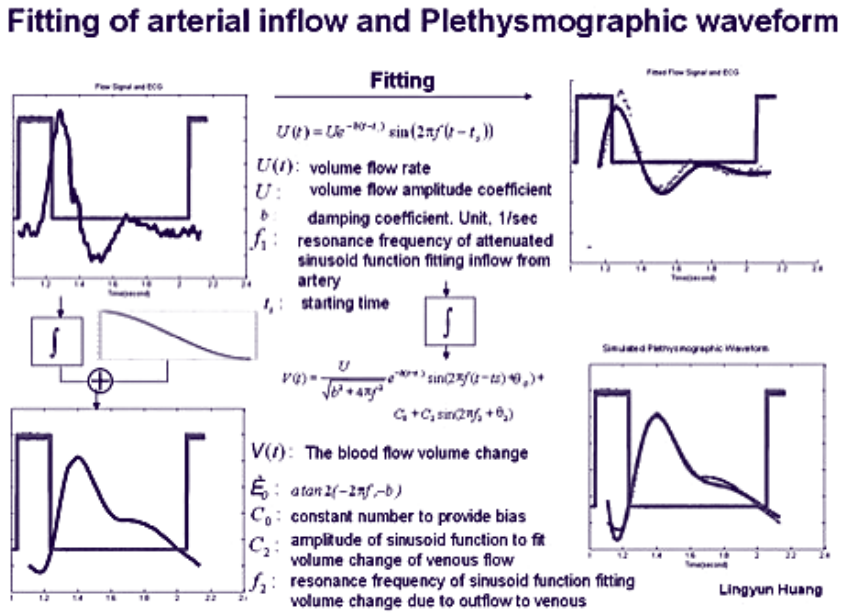

FIGURE 9: Deriving the plethsymographic waveform shape from the Doppler waveform.

Using such models, TPI studies can be done in patients with irregular heart rhythms. By using mathematical models of the vasculature supplying TPI voxels, statistical noise in the TPI images can be reduced to a minimum allowing the vascular supply to small regions of tissue to be evaluated. By studying both the arteriolar and the venular waveforms, arterial hypoperfusion and venous hypertension can be evaluated in real time without waiting for tissue breakdown.

Real-Time Vibrometry

Arterial stenoses cause bruits that can be heard with a stethoscope during physical examination. It is also possible to image those vibrations. A collaboration between the Department of Surgery and Bioengineering Image Computing Laboratory, a series of applications has been investigated. Vibrations have been imaged in peripheral arterial pathway (vein graft) flow (Figure 10).

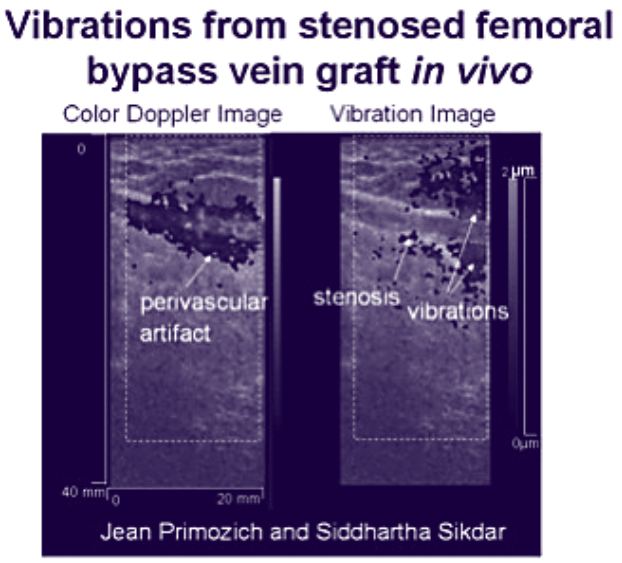

FIGURE 10: Vibration caused by Stenosis in a Vein Graft in an arterial pathway to the foot. Flow is from left to right. The amplitude of the vibrations caused by this stenosis bleed are about 2 micrometer.

Vibrations are generated by most arterial pathway stenoses. Vibrations can be detected in the heart muscle when a coronary artery stenosis is present (Figure 11).

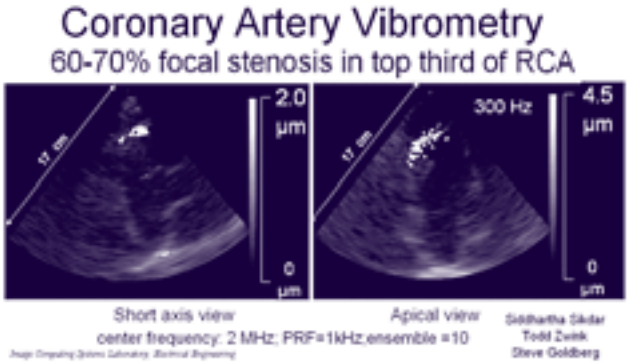

FIGURE 11: Coronary Artery Stenosis.

Vibrations can also be detected in bleeding organs.

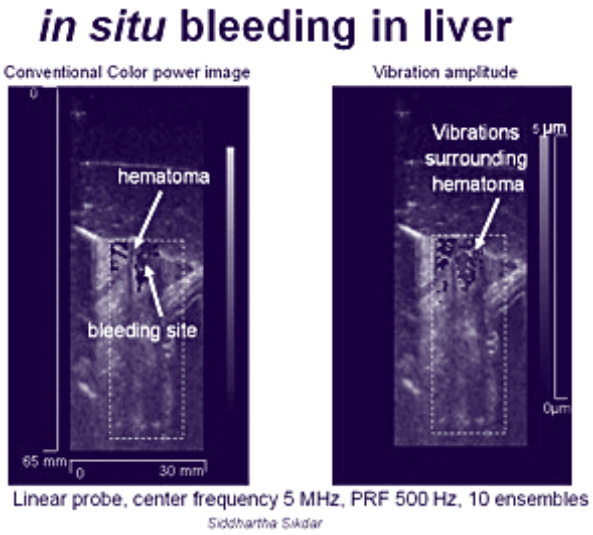

FIGURE 12: Vibration caused by Bleeding from a Lacerated Liver. Vibration amplitudes are 5 micrometers.

There are two advantages to imaging vibration to detect bleeding rather than imaging blood flow: 1) blood flow is present in normal tissues, vibrations are not, 2) the ultrasound signal strength from vibrating reflectors is 1000 times stronger than the ultrasound signals from flowing blood so that deep bleeds can be detected reliably.

Video Photoplethysmography

Another way to acquire plethysmography waveforms is by optical reflection or transmission. The method is called photo-plethysmography (PPG). This method looks at the changes in absorption of light by the tissues during the cardiac cycle as the blood fills and empties from the tissues. PPG can be used to differentiate normal blood supply from abnormal supply in the skin or oin the surfaces of organs. It is also the basis of pulse-oximetry, where two colors of light

(red and infra-red) are used to determine the oxygen content of the blood. Conventional PPG measures the absorption at one location, but using a video camera, an image of the pulsations in tissue can be created (Figure 13). If more than one light color is used, the tissue oxygenation can be determined.

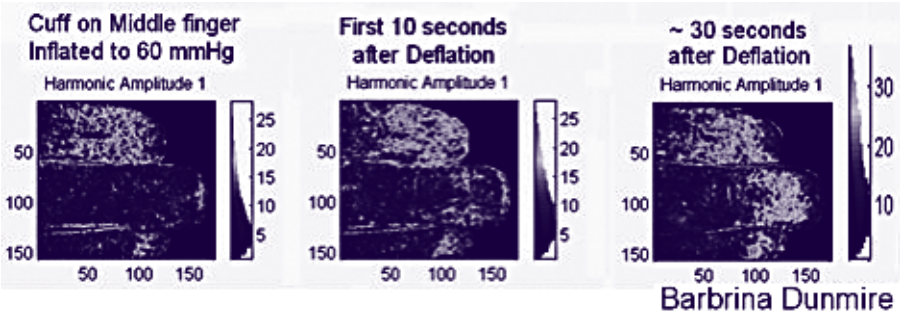

FIGURE 13: Video Photoplethysmography of the Fingers Showing Pulse Amplitude.

In Figure 13, the blood supply to the middle finger is occluded on the left, resulting in no pulsation in that finger. On the right, 30 seconds after the release of the blood pressure cuff, the blood pulsatility is restored to the middle finger. Note that the pulsatility is not restored during the first 10 seconds (middle image). In addition, note the change in the scale of the pulse amplitude (bar on right) which indicates that the pulse amplitude in the first finger is increased by 30% at 30 seconds over the baseline amplitude concurrent with the restoration of pulsation in the middle finger. In addition, the pulse amplitude in the proximal finger is generally low.

Ultrasound Reading Center for Carotid Stents

One of the major treatable causes of stroke is stenosis (narrowing) of a carotid artery in the neck. This artery supplies the majority of blood to the anterior part of the brain. The stenosis is usually formed by an atherosclerotic plaque. If left untreated, the plaque can fall apart sending debris to occlude smaller arteries in the brain causing stroke. Since 1960, removing the atherosclerotic plaque by surgical endarterectomy has been the treatment of choice. An alternative treatment, the placement of an artificial lining in the carotid to open the stenosis, has been under development since 1995. This artificial lining is called a stent. It is usually made of metal. It is placed by a catheter which is inserted in the artery in the leg, and threaded up though the body to the neck.

Stents are now used clinically in may arteries, but there effectiveness in the carotid arteries is still under investigation. The Ultrasound Reading Center (UWURC) in the department of Surgery provides quality assurance for following the patient by Doppler ultrasound after placement of a stent in the treatment group or carotid endarterectomy in the control group. The ultrasound reading center serves over 130 clinical sites in the United States, providing protocol manuals and providing evaluation of each Doppler/ultrasound study and statistical services for analysis of the results. The reading center has processed over 5,000 examinations.

The UWURC is the Ultrasound Core Laboratory for the Carotid Revascularization Endartectomy vs. Stenting Trial (CREST). The UWURC has completed analysis on 2443 studies for the CREST trial on a total of 1353 patients. Half of the patients have had only one ultrasound scan, a third have had two scans, the remaining 1/6 have had between 3 and 8 follow-up scans after stent placement. Many of the 618 pre-procedure scans, many also have pre-procedure angiographic studies which will allow a new analysis of the relationship between ultrasound Doppler blood velocity measurements and angiographic residual lumen and normal lumen diameter measurements.

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Alexander W. Clowes, M.D.

• Regulation of Vascular Smooth Muscle Cell Growth

AWARDS
National Heart, Lung, and Blood 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.

Recent studies in the laboratory have defined a novel pathway of smooth muscle cell activation which depends on these receptors. Thrombin can induce

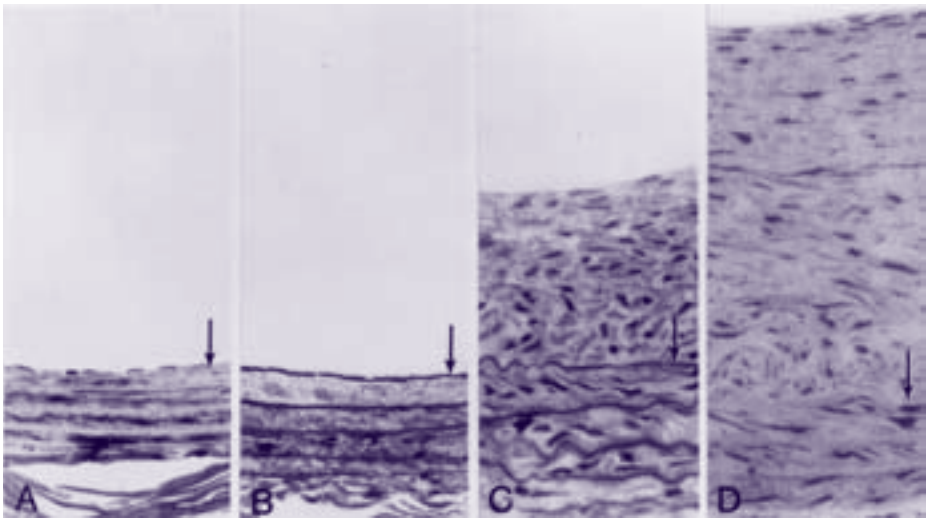

FIGURE 1: This series of photographs shows how a normal rat carotid artery (panel A-histologic cross-section) responds to injury. Angioplasty of the artery removes the surface endothelium (panel B). By two weeks (panel C), smooth muscle cells have migrated from the media into the intima (region above 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).

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.

Depending on the physiological state, endothelial cells can have a positive or a negative effect on smooth muscle cell growth.

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 immunoglobin; 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 neces-

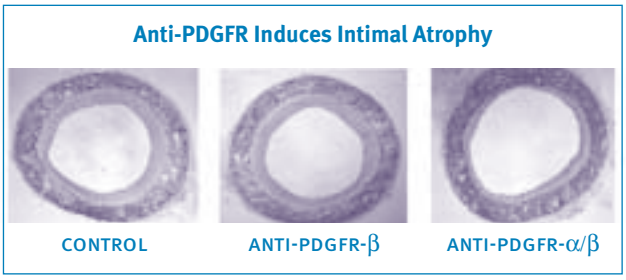

FIGURE 2: Histological cross-sections of normal flow PTFE grafts at 2 weeks following initiation of treatment with vehicle control, blocking antibodies to PDGFR-β, or blocking antibodies to both PDGFR-α and PDGFR-β. (H&E staining, 16X).

sary. 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.

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Günter Daum, Ph.D.

- Modulation of Growth Factor Signaling in Vascular Smooth Muscle Cells by Inflammatory Cytokines

FUNDING
National Institutes of Health

Restenosis is the cause for the unacceptably high failure rate 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 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.

The systemic use of a SMC growth inhibitor is most likely not a feasible approach to inhibit restenosis.

The systemic use of a SMC growth inhibitor, however, is most likely not a feasible approach to inhibit restenosis. One has to assume that patients undergoing treatment of an atherosclerotic lesion will have multiple asymptomatic lesions that do not restrict the vessel lumen. SMCs are part of the fibrous cap, which stabilizes these lesions. Thus, inhibiting proliferation and survival of these SMCs could promote plaque rupture and increase the risk of thrombotic events.

PDGF and Interleukin-1 Play a Role in Restenosis

Platelet deposition and degranulation following arterial injury is a crucial process for neointimal growth and PDGF is the major platelet-derived chemoattractant and mitogen for SMCs. The PDGF family consists of four members, PDGF-A, -B, -C, and -D, which bind as homodimers (AA, BB, CC, DD) or heterodimers (AB) to two PDGF receptors (alpha and beta). PDGF-A, B and C bind to PDGFRalpha, PDGF-B and -D bind to PDGFRbeta. PDGF-C stimulates PDGFRbeta in the context of a PDGFRalpha/beta heterodimer, but apparently does not bind to PDGFRbeta homodimers. The reason for this selectivity is unknown. The importance of PDGF in intimal growth has been demonstrated in various animal models including baboons. The principal effect of PDGF appears to be stimulation of SMC migration, although stimulation of SMC proliferation has also been suggested. PDGF effects in the response to vascular injury appear to be mainly mediated by PDGF receptor-beta. For instance, blockade of PDGF-receptor beta, but not alpha, inhibits intimal hyperplasia in the balloon-injured baboon carotid artery.

The proinflammatory cytokine IL-1 exists in two isoforms, IL-1alpha and IL-1beta. Both bind to the same receptor, which is a heterodimeric protein consisting of the type I IL-1 receptor (IL-1R1) and the IL-1 receptor accessory protein. Both IL-1 isoforms are synthesized as precursors and are cleaved by IL-1 converting enzyme, also called caspase-1, to yield the mature proteins, but only IL-1alpha is biologically active as a precursor. The IL-1 system also includes two antagonistically acting proteins, IL-receptor antagonist (IL-RA) and a second IL-1 receptor (IL-1R2), which lacks intracellular signaling domains. IL-RA functions as a competitive inhibitor for IL-1 binding whereas IL-1R2 is considered a decoy receptor, sequestering IL-1R1 ligands.

Several lines of evidence suggest a role for IL-1 in restenosis. In humans, a polymorphism in the IL-RA locus has been discovered, which may protect from restenosis after coronary angioplasty and after coronary stenting. Although the consequences for this polymorphism for IL-RA expression in the vessel wall is not known, observation in IL-RA-deficient mice point to an anti-inflammatory function of IL-RA in arteries. Animal models in which IL-1 has been investigated include pigs and mice. Chronic stimulation of non-diseased pig coronary arteries with IL-1 produces intimal lesions. In IL-1R1-deficient mice, intimal hyperplasia is reduced following carotid artery ligation when compared to wild type animals. Following balloon injury, expression of IL-1 is induced in the rat carotid and pig coronary artery. A well-defined mechanism of IL-1 is the induction of adhesion molecules in endothelial as well as smooth muscle cells. Blockade of NF-kappaB in the balloon-injured rat carotid decreased expression of ICAM (Intercellular adhesion molecule)-1 and VCAM (Vascular adhesion molecule)-1 and reduced the infiltration of media and neointima by macrophages and T-lymphocytes. In this study, blockade of NF-kappaB also decreased intimal growth. Together, these data suggest that IL-1 may contribute to intimal hyperplasia by induction of adhesion molecules that recruit inflammatory cells.

IL-1 Potentiates the Proliferative Response of SMCs to PDGF-BB

It is well documented that IL-1 elicits many responses only in cooperation with other stimuli. Because PDGF plays a central role in SMC activation, we have tested the possibility that these two factors act synergistically on SMC proliferation. In baboon SMCs, IL-1beta by itself is not mitogenic with or without cyclooxygenase inhibitors. When administered together with PDGF-BB, however, IL-1beta causes a 2-3 fold stimulation of PDGF-BB-induced SMC proliferation as determined by DNA synthesis as well as cell counts. Blockade of PDGF receptor-alpha, the sole receptor for PDGF-A, does not decrease the stimulatory effect of IL-1beta suggesting a mechanism for IL-1 other than induction of PDGF-A. Early PDGF-BB-induced signaling events, including PDGF receptor-beta phosphorylation and activation of extracellular signal-regulated kinases or protein kinase B, were not affected by IL-1beta. Analysis of cell cycle regulatory proteins showed that IL-1beta suppressed expression of cell cycle-dependent kinase (CDK) inhibitors, p21(WAF1/CIP1) and p27 (KIP1). Consistent with this observation is our finding that IL-1beta enhanced CDK2 activation by PDGF-BB. These data suggest that IL-1beta promotes proliferation of SMCs in the presence of PDGF-BB by inhibit-

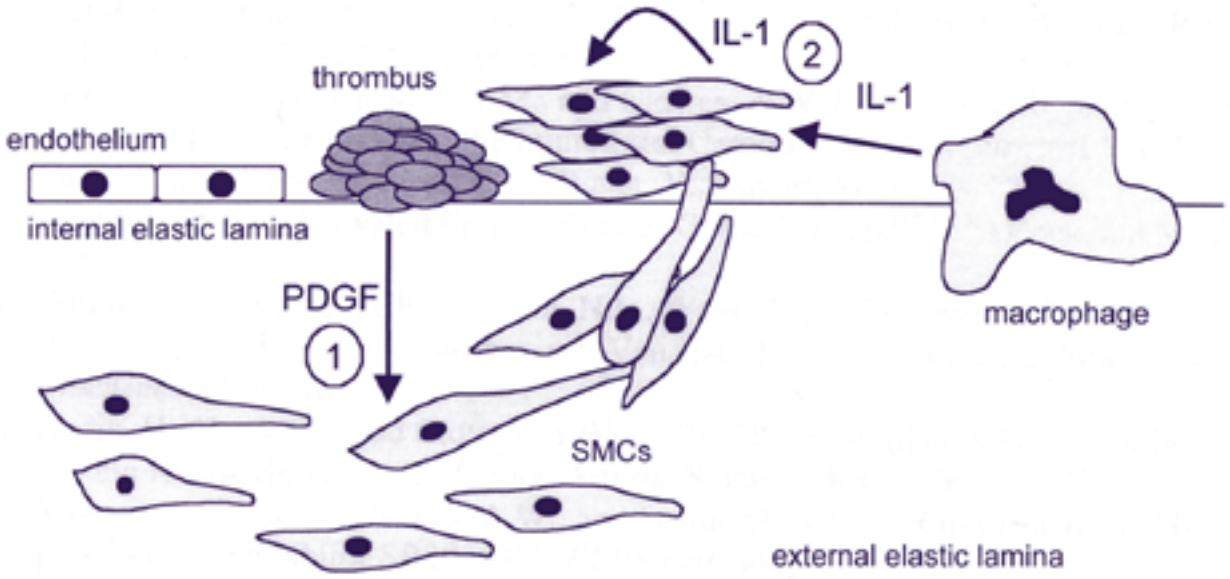

FIGURE 1: Potential Cross-Talk Between IL-1 and PDGF. 1) Following arterial injury, platelets deposit and release PDGF, which functions as a chemoattractant for SMCs. 2) IL-1 is released by the contracting thrombus and by adhering macrophages. 3) IL-1 promotes proliferation of intimal SMCs. Sources of IL-1 may be macrophages or intimal SMCs. This effect of IL-1 is mediated by suppression of the cell cycle inhibitors, p21 and p27. In addition, IL-1 may promote inflammation by inducing expression of adhesion molecules (ICAM-1, VCAM-1) and recruitment of macrophages and T-lymphocytes.

ing expression of CDK inhibitors. Experiments to address the question whether IL-1beta affects p21/p27 transcription, translation, or stability of message or protein, are currently under way in our laboratory.

Conclusion

IL-1 receptors, agonists, and antagonists appear to be expressed upon arterial injury in various animal models, strongly indicating a role for IL-1 in restenosis. Although many investigations in cultured cells support a function for IL-1 in intimal hyperplasia, such role for IL-1 has not yet been defined. Our work suggests that IL-1 cooperates with PDGF to stimulate SMC proliferation (see Figure 1). Whether this mechanism is important has to be addressed by *in vivo* experiments using specific inhibitors such as blocking antibodies to the IL-1 receptor type I, or neutralizing antibodies against members of the IL-1 family. Such studies are under way using a murine carotid injury model.

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Thomas Hatsukami, M.D.

- Magnetic Resonance Imaging of the High-Risk Atherosclerotic Plaque

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Over 16.7 million people die of cardiovascular disease (CVD) each year — one person every two 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 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.

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.

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

The mission of our research group is to advance high-resolution magnetic resonance imaging (MRI) technology for accurate, non-invasive examination of atherosclerosis.

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 neovasculature and inflammatory cellular infiltration of the plaque (Figure 5; Circulation 2003; 107:851 and *Radiology* 2005 in press) .

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 (R2) 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* 2005, in press).

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 –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).

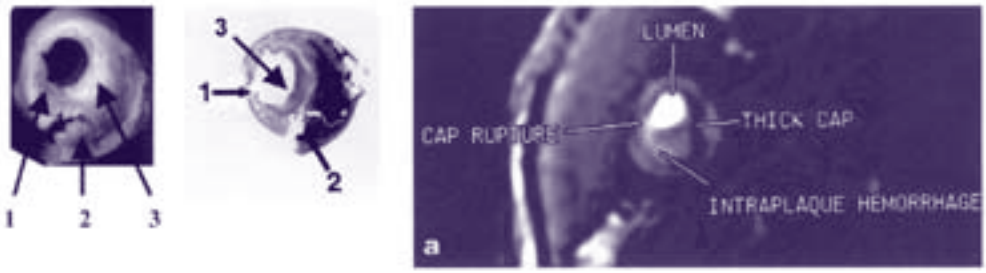

FIGURE 3: **Example of a common carotid plaque with fibrous cap rupture and intraplaque hemorrhage. Photo of gross section of common carotid artery (left panel), trichrome stained histological section (middle panel), and corresponding TOF MR image (right panel). Arrow 1 indicates an area of cap rupture, 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.**

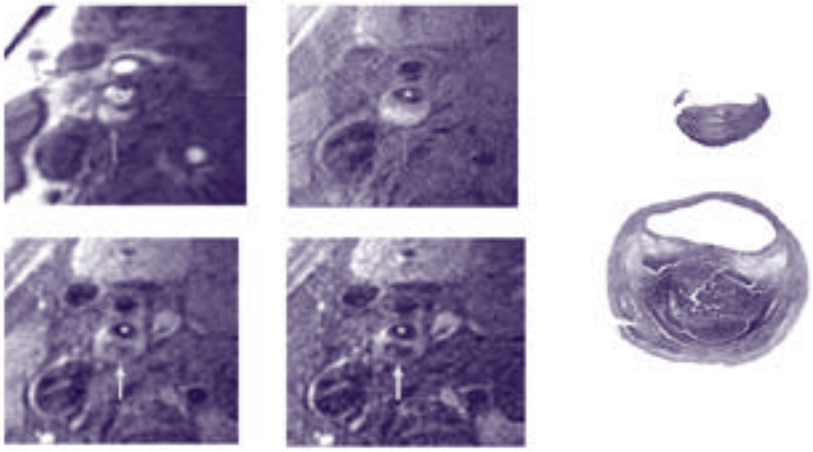

FIGURE 4: Example of an AHA Type VI (complicated) lesion with acute intraplaque hemorrhage. The asterisks indicate the lumen of the internal carotid artery. Early intraplaque hemorrhage, seen on the corresponding histological cross-section on the right, is identified by a hyperintense (bright) signal 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.

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; *Circulation* 2005; 112(17) suppl II-383 and *Stroke* 2005, submitted).

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 (*Circulation* 2005; 112 (17) suppl II-643; and *Journal of the American College of Cardiology*, 2005, submitted). Therefore, this prospective study confirms the hypothesis that compensatory enlargement of the outer boundary of the vessel wall (positive remodeling) accommodates 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).

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

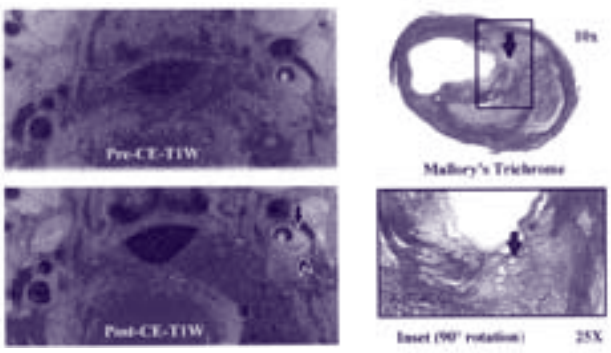

FIGURE 5: Pre-gadolinium contrast enhanced T1-weighted image of common carotid artery in left upper panel, post-contrast enhanced T1W image in left lower panel, and corresponding 10X and 25X trichrome stained histological sections. Note the enhancement seen in the shoulder region (arrow) in the post-contrast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

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 selective, appropriate intervention, and potentially lead to the development of novel methods for therapeutic intervention.

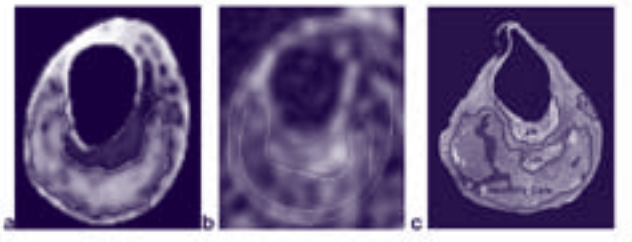

FIGURE 6: Scatter plot demonstrating high correlation (R = 0.80, p < 0.001) between fractional blood volume in plaque wall on contrast-enhanced MRI and size/concentration of plaque neovasculature (fractional vascular area) in corresponding histological cross-sections.

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Ted R. Kohler, M.D.

- Endovascular Therapy
- Effect of Blood Flow on Intimal Hyperplasia and Access Graft Failure
- Dialysis Access Grafts

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Northwest Kidney Foundation

Endovascular Therapy

Endovascular therapy is an exciting new approach to aneurysm repair that 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 early clinical trials and are soon to be approved for market release by the FDA. It remains to be seen, however, if these devices 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 hospital. We are one of several centers in the country participating in an FDA-sponsored trial of the AneuRx endovascular graft. Dr. Kohler was on the planning committee for the VA Cooperative Trial of Open versus Endovascular Repair of abdominal aortic aneurysms. This trial began in October, 2002 at the Seattle VA.

Effect of Blood Flow on Intimal Hyperplasia and Access Graft Failure

Vascular surgery has made tremendous advances in the last few decades. Bypass grafts, angioplasty, and stents are now standard treatment for arterial insufficiency and aneurysm disease in peripheral arteries. However, long-term success of these procedures is limited by a process of wound healing called intimal hyperplasia, in which wall thickening from smooth muscle cell proliferation narrows the lumen.

Intimal hyperplasia causes failure of almost one-third of all vascular reconstructions. Much research has been devoted to understanding the cellular pathology of this process and to developing ways to combat it with drugs, new devices, and genetic modification of the cells involved. Our laboratory is studying the effects of altered blood flow on intimal hyperplasia, and is evaluating new vascular devices to reduce restenosis.

Re-operation for failed access is a major cause of morbidity, prolonged hospital stay, and increased cost in the treatment of renal failure.

Dialysis Access Grafts

Effective renal dialysis requires several hundred cc's per minute of blood flow. To accomplish this, a fistula is created between an artery and vein, typically in the arm. 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.

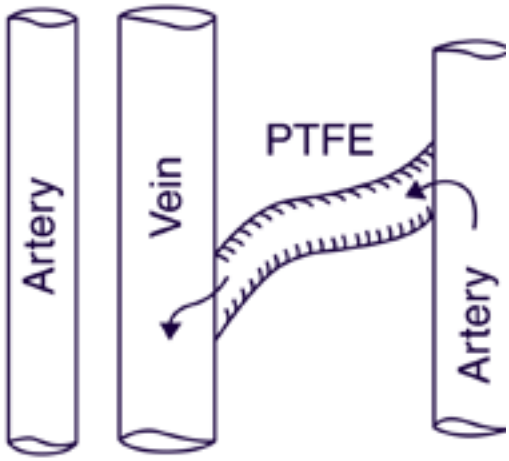
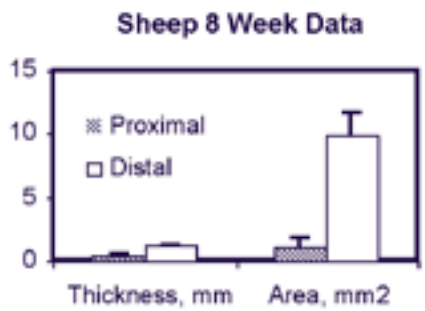


FIGURE 1: Fistula created between artery and vein to provide high-flow conduit

We are studying this problem in an animal model. Polytetrafluoroethylene (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 (see graph). Active thrombosis along the graft surface, particularly at the venous end, appears to be a 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.



GRAPH 1: Sheep Eight Week Data

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 immunohistochemistry. 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 2, showing a proliferating-cell-nuclear-protein (PCNA)-positive nucleus marked by an arrow.

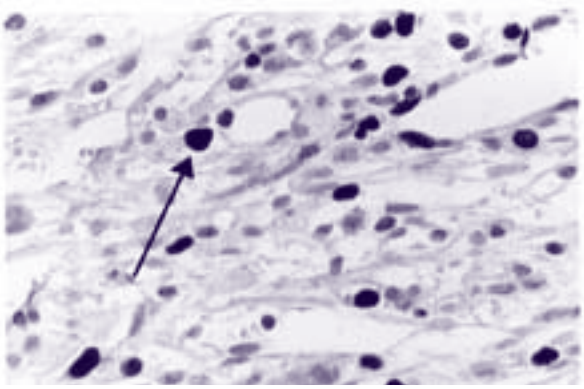


FIGURE 2: PCNA-positive nucleus

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.

We have determined that tissue factor (a stimulant of thrombosis) is increased along the length of the access graft, at both early and late times, possibly in response to this injury (Table 1). Elevated levels of this clotting factor may explain the thrombosis we have observed. Studies are underway to determine the cellular source of this enzyme and whether local drug infusion can block its production and therefore the development of intimal hyperplasia.

LOCATION	TISSUE FACTOR ACTIVITY (+/-SD)	TISSUE FACTOR PROTEIN	FIBRIN
Normal Artery	22.0 +/– 18.0	–	–
Graft near Artery	113.5 +/– 10.9 *	++	+
Graft near Vein	194.5 +/– 15.2 *	+++	+
Normal Vein	32.0 +/– 1.5	–	–

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

TABLE 1: Tissue Factor Levels in Sheep Access Grafts

We will compare standard PTFE grafts with grafts that are more porous. Increased porosity allows ingrowth of capillaries across the graft to the lumen, where they spread and form an endothelial lining that may protect against thrombosis and intimal hyperplasia. We are also using this model to study the use of arterial grafts to deliver gene therapy.

PTFE grafts are seeded with smooth muscle cells that have been transduced with the erythropoietin (epo) gene (Dr. William Osborne, PI). Erythropoietin, normally made by the kidney, stimulates production of red blood cells. Patients with renal failure do not make enough of this hormone and as a result are anemic. We will use a uremic sheep model to find out if epo made by cells placed in dialysis access grafts can reverse the anemia of chronic renal failure.

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- Vein Graft Surveillance Using 3D Ultrasound Imaging
- Measurement of Abdominal Aortic Aneurysms with 3D Ultrasound
- Measurement of Flow-Mediated Vessel Dilation

FUNDING
Environmental Protection Agency
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Quantitative evaluation of anatomy from medical images has applications in clinical diagnosis, monitoring, drug development and research. Ultrasound is a safe, non-invasive and relatively inexpensive imaging modality that produces a tomographic image of a thin tissue slice within a region of interest. Standard real-time ultrasound systems, however, do not maintain a record of the spatial relationship between sequential 2D images. Therefore, measurements of size and shape are often based on geometric assumptions and may be operator dependent.

We use a custom ultrasound imaging system that preserves the relationship of the 2D image planes in space, thereby allowing reconstruction of structures in a 3D coordinate system. Accurate 3D reconstructions provide better quantification of geometric parameters, enhancing comparisons of data both over time and between imaging modalities. In addition, realistic and intuitive displays can assist in the transfer of information between the multiple groups often involved in patient care.

Vein Graft Surveillance Using 3D Ultrasound Imaging

Vein grafts are placed to bypass diseased arteries in the lower limb when symptoms such as pain during walking, rest pain, and tissue necrosis occur. While vein grafts provide effective relief of lower extremity ischemia for the majority of patients, approximately 30-40% of these grafts fail due to focal stenoses caused by myointimal hyperplasia. Because these lesions can be effectively corrected, their early detection is crucial.

Our laboratory is developing 3D ultrasound imaging techniques for vein graft monitoring. Arteriograms and conventional ultrasound imaging produce only 2D views of vessels. Lesions at sites of complex geometry are difficult to monitor with 2D methods, and spatial relationships over time are not preserved. Three-dimensional imaging, however, can produce a full representation of the vessel geometry, allowing assessment of changes over time at specific sites.

Our 3D ultrasound imaging system is based on a standard ultrasound imager modified with a magnetic tracking system to register 2D ultrasound images in a 3D coordinate system. The tracking system records the location and orientation of the ultrasound scanhead during imaging, from which a 3D computer reconstruction of the vessel can be derived. Cross-sectional area measurements in planes normal to the center axis of the vessel are calculated from the 3D surface reconstructions.

We are using the 3D imaging methods to quantitatively track size changes in vein grafts over time. In a study of patch angioplasty revisions, lumenal narrowing documented by 3D scanning was not associated with consistent velocity changes on conventional duplex graft surveillance scans. Therefore, the 3D method provides documentation of anatomical

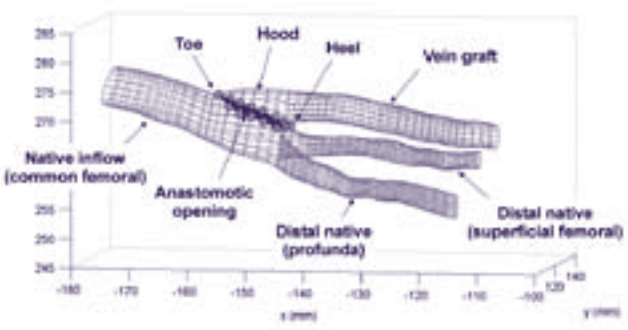

FIGURE 1: Components of a vein graft proximal anastomosis that are used for analysis of the 3D geometry. The anastomosis geometry is quantified by 1) the cross-sectional areas of the graft, native inflow artery, and the combined native/hood segment, 2) the cross-sectional area and shape of the anastomotic opening, which is the 3D polygon defined by the spheres superimposed on the mesh reconstruction, and 3) the 3D angle between the native inflow artery and the vein graft.

While vein grafts provide effective relief of lower extremity ischemia for the majority of patients, approximately 30–40% of these grafts fail due to focal stenoses caused by myointimal hyperplasia.

changes in areas of complex geometry where velocity measurements are difficult to perform and interpret. Quantitative monitoring of vein graft morphology may provide a means to distinguish normal remodeling from pathologic changes that threaten vein graft patency.

The attachment sites of lower extremity bypass vein grafts are known to exhibit a wide range of geometries. We have compiled statistics from 46 patients on geometrical parameters that describe the proximal anastomoses of vein grafts, including sizes and angles of the native vessel and graft (Figure 1). The 3D imaging method has also allowed us to make quantitative measurements of the size of the surgical opening at the graft attachment site, which we refer to as the anastomotic opening.

Together these measurements provide a method to study anastomosis configurations associated with particular graft types or outcomes. Significant stenoses developed at 8 of the 46 proximal anastomoses, and the stenosis group had significantly smaller elevation angles than the normal group. The elevation angle is the angle of graft in a longitudinal plane perpendicular to the plane of the anastomotic opening. Potential applications of these data are 1) prediction of stenosis risk based on geometry, and 2) recommendation of anastomosis construction methods that are more favorable to long-term patency.

The 3D tracking method also allows us to register and display blood velocity measurements from Doppler spectral waveforms with 3D surface reconstructions along the entire length of the vein graft (Figures 2 and 3). Spectral waveforms are used to reconstruct the representative flow dynamics that are spatially related to the anatomic reconstructions. The spectral waveforms over a single cardiac cycle are used to create a 3D surface that combines time in the cardiac cycle, axial location along the vessel, and blood velocity. In Figure 3, flow disturbances associated with the dilated site of a vein patch angioplasty revision are evident in the velocity map. This technique allows us measure changes in blood velocity over time at specific locations in a graft.

We are developing additional imaging methods to integrate both geometry and dynamics in vein graft surveillance. Figures 4 and 5 demonstrate coordi-

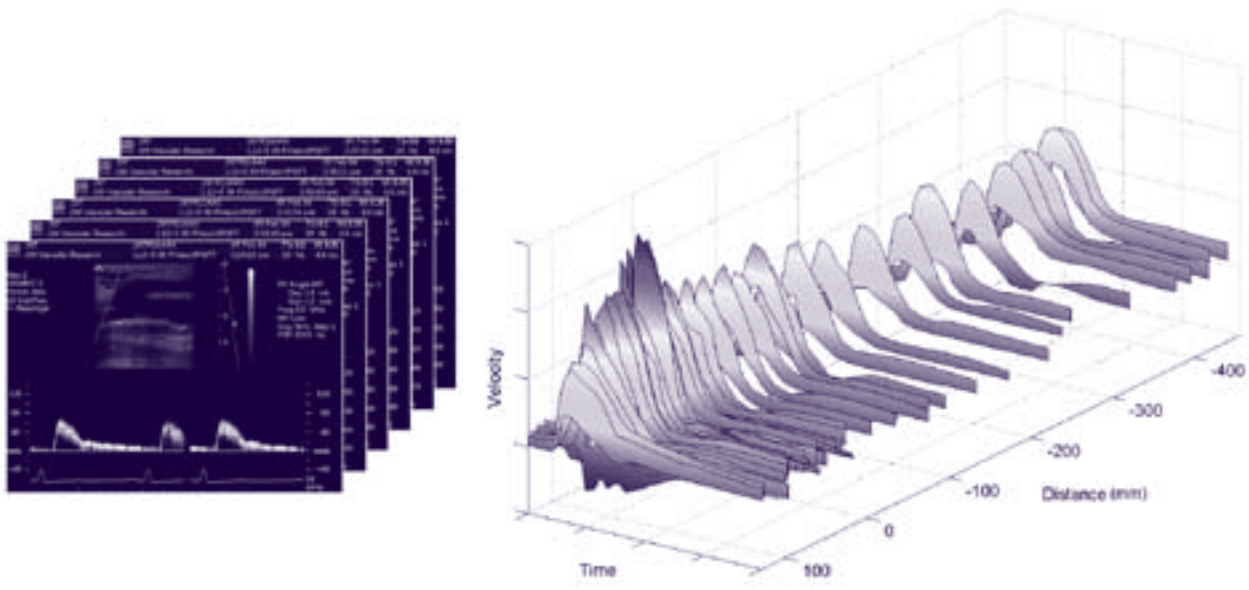

FIGURE 2: Doppler spectral waveforms for a single cardiac cycle are outlined on a series of 2D duplex ultrasound images of the vein graft (left). The waveform outlines are then spatially registered along the length of the vein graft (right). The registered waveforms show the velocity as a function of time at a series of points within the graft. The first time point for each outline corresponds to the R-wave of the ECG signal.

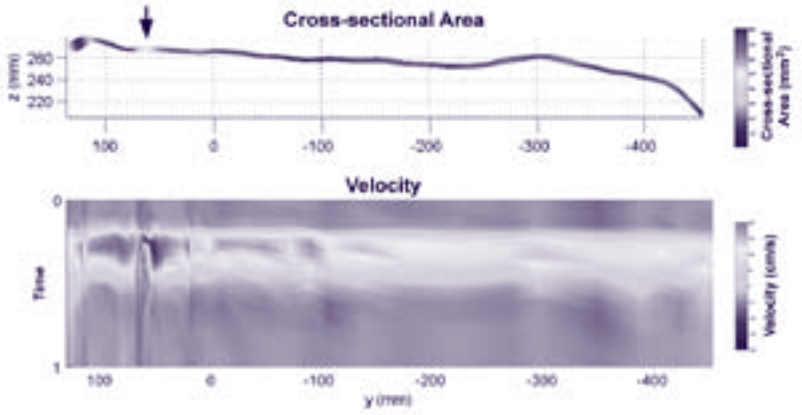

FIGURE 3: The 3D waveform plot from Figure 2 is interpolated and displayed as a 2D projection, showing velocity (represented by the gray scale intensity) as a function of time and space for the entire length of the vein graft. Time zero corresponds to the ECG R-wave. A registered 3D surface reconstruction of the lumen of the bypass graft is shown at the top (proximal anastomosis at the right), with the graft cross-sectional area mapped to the surface according to the brightness scale at the right. A site of revision by vein patch angioplasty is indicated by the arrow at the top left.

nated 3D imaging data for a vein graft that includes lumen size, wall distensibility, vector blood flow, and wall thickness. The patient has a reversed saphenous vein graft with a stenosis at the proximal end of a vein patch angioplasty. The size of the lumen proximal and distal to the stenosis is shown in Figure 4a, reconstructed from a 3D ultrasound scan. Additional imaging scans of this segment were performed to measure distensibility by tissue Doppler processing of the wall motion (Figure 4b), and blood flow patterns by vector Doppler ultrasound (Figure 4c). A segment of the graft centered on the stenosis is shown in Figure 5 with graft wall thickness measured by MRI.

Measurement of Abdominal Aortic Aneurysm with 3D Ultrasound

Abdominal aortic aneurysms (AAAs) are dilations of the aorta occurring between the renal and the iliac arteries. Reliable quantitative evaluation of AAAs is required both for diagnosis and in the follow-up studies needed to avoid life-threatening rupture. Small aneurysms enlarge at an average rate of 0.5 cm in diameter per year, and they require close tracking by serial measurements to assure suitable treatment before risk of rupture is significant. A recent development in AAA treatment is endovascular repair, which is a minimally-invasive procedure to exclude the aneurysm from the circulation.

In contrast to the traditional open surgery, an endovascular graft is deployed using a catheter system passed into the aorta through the femoral arteries. This procedure is associated with significantly reduced morbidity and recovery time. However, extended post-treatment monitoring is generally required to ensure that the endograft is stable and that there are no leaks. While decrease in aneurysm

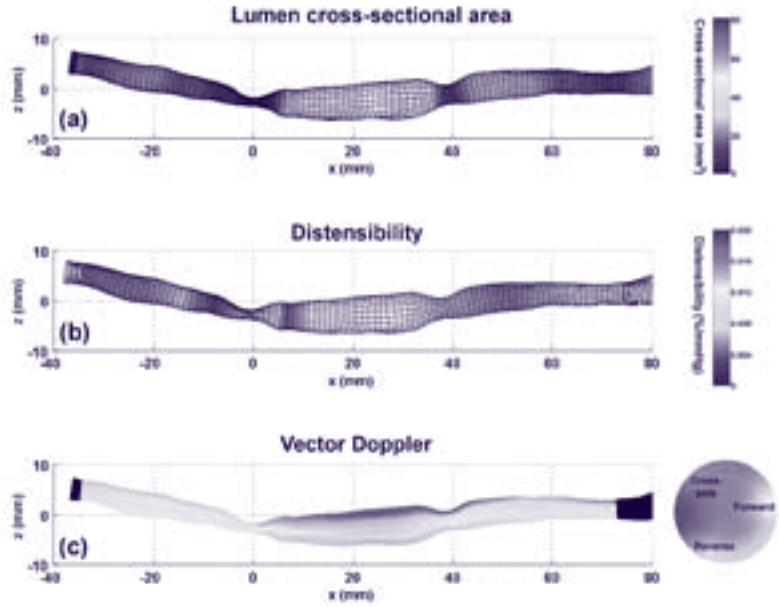

FIGURE 4: 3D reconstruction of a vein graft segment with a stenosis (x = 0) at the proximal end of a patch angioplasty site (between x = 0 and x = 40). (a) Cross-sectional area measurements mapped to a surface reconstruction of the vein graft lumen. (b) Distensibility (compliance normalized by the cross-sectional area) measured by tissue Doppler processing. The distensibility is lowest at the stenosis site. (c) Classification of flow regimes based on vector Doppler measurements. Laminar flow dominates the center of the lumen, but reverse and cross-axis flow are detected distal to the stenosis, particularly along the superficial wall.

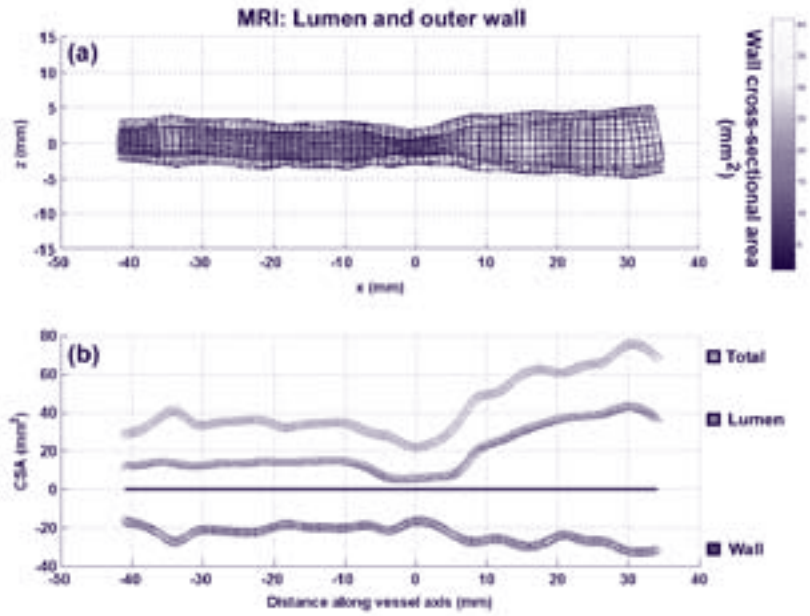

FIGURE 5: Graft size measurements based on an MRI scan of the vein graft shown in Figure 4. The origin of the x-axis is assigned to the stenosis site (x = 0) to register the MRI and ultrasound data. (a) 3D reconstructions of the lumen (surface) and the graft outer wall (mesh) based. The surface is shaded according to the cross-sectional area of the vessel wall at each point along the axis. (b) Cross-sectional area measurements of the total vessel (outer wall), the lumen, and the vessel wall are plotted as a function of distance along the graft center axis. Negative remodeling is observed at the stenosis: the outer wall cross-sectional area at the stenosis is reduced relative to the normal segment proximal to the stenosis.

size indicates its successful exclusion from the circulation, post-implant expansion indicates the presence of a leak and a risk of aneurysm rupture.

Ultrasound is an attractive imaging modality for screening and monitoring AAA patients since it does not involve radiation or contrast agents. However, dimensional measurements made with conventional 2D ultrasound are sensitive to image plane orientation. In addition, the orientation and placement of the imaging planes change from visit to visit, which contributes to measurement variability in studies over time. Therefore, we are using the 3D ultrasound imaging system described above to generate computer reconstructions of the aorta from which quantitative measurements can be extracted. Computer reconstructions of an AAA are presented in Figure 6 for a series of 3D ultrasound studies after endovascular repair, showing shrinkage of the aneurysm sac. We are currently investigating automatic computer segmentation methods to improve the potential for practical application of the 3D ultrasound imaging method.

Measurement of Flow-Mediated Vessel Dilation

Ultrasound measurement of flow-mediated vessel dilation has been proposed as a means to assess changes in endothelial function associated with atherosclerosis, hypertension and heart failure. Typically, the diameter of the brachial artery is measured at a single time point after release of a blood pressure cuff to quantify the flow-mediated response to temporary ischemia. This measurement, however, does not necessarily represent the point of maximum dilation. As part of research studies in the Department of Obstetrics and Gynecology and the Department of Environmental & Occupational Health Sciences, we have developed an image capture and analysis method to measure the response of the vessel as a function of time after transient ischemia (Figure 7). This method provides documentation of the vessel response without assumptions regarding the time of maximum dilation.

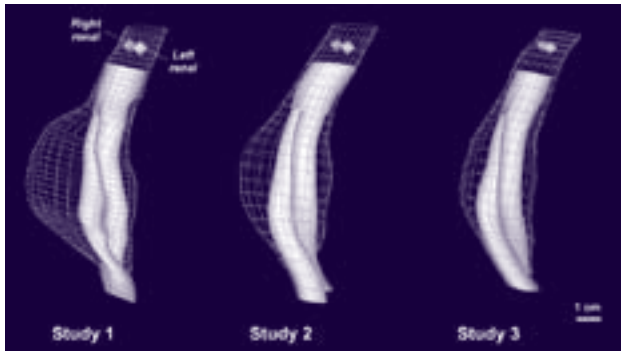

FIGURE 6: Serial study of an AAA repaired by an endovascular graft. The 3D reconstructions show both the aneurysm (outer mesh) and the graft (inner surface). The studies were performed 1) 2 weeks, 2) 6-months, and 3) 1 year after graft placement. Diamond markers show the origins of the renal arteries.

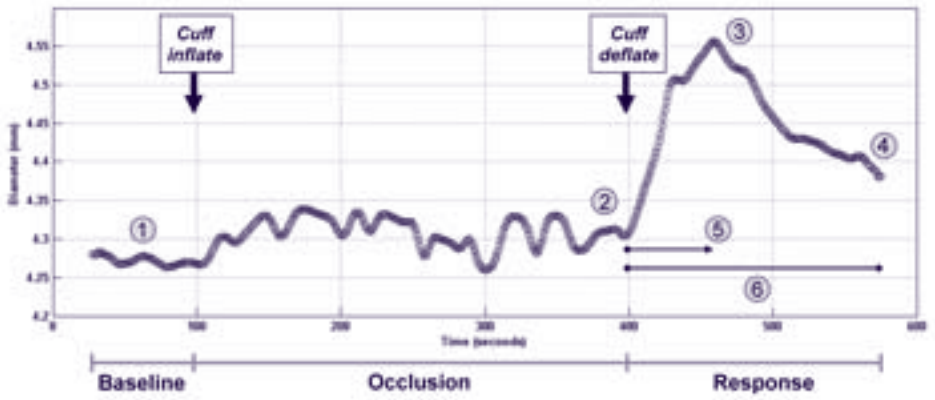

FIGURE 7: Flow-mediated response of the brachial artery, with diameter plotted as a function of time. The following measurements are used to characterize the response: 1) baseline diameter, 2) diameter after 5 minutes of occlusion, 3) maximum diameter after cuff release, 4) diameter 3 minutes after cuff release, 5) time of maximum diameter relative to cuff release, and 6) area under the response curve.

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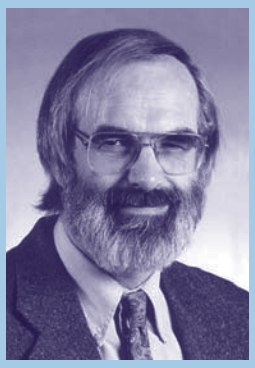

- Tissue Pulsatility Imaging for the Evaluation of Tissue Perfusion
- Tissue Pulsatility Imaging of Muscle
- Intracranial Tissue Pulsatility Imaging (TPI)
- Arteriolar and Venular Plethysmograpic Imaging
- TPI and Doppler Waveforms
- Real-Time Vibrometry
- Video Photoplethysmography
- Ultrasound Reading Center for Carotid Stents

FUNDING
National Institute on Biomedical Imaging and Bioengineering

Based on the pioneering work of D. Eugene Strandness, Jr., M.D. which continued for over forty years in the Department of Surgery, the noninvasive vascular laboratory has developed ultrasonic diagnostic methods used throughout the world. The Department of Surgery in collaboration with the new UW Center on Industrial and Medical Ultrasound and the Department of Bioengineering, is continuing to develop innovative ultrasound examination technologies for vascular diseases and abnormalities.

Tissue Pulsatility Imaging for the Evaluation of Tissue Perfusion

Plethysmography has been used for nearly a century to measure physiological changes in tissue volume with pulse and respiration. Strain gauge plethysmography has been used in the Department of Surgery for the diagnosis of tissue ischemia and cold sensitivity. Normal tissues expand 0.1% with the cardiac cycle (Figure 1) and 1% with the respiratory cycle.

The pulse plethysmographic waveforms associated

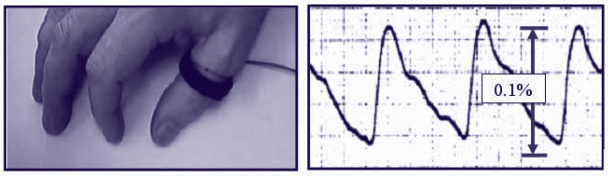

FIGURE 1: (Left) Mercury strain gauge plethysmograph around the thumb; (Right) three cardiac cycles of the plethysmographic waveform from the thumb.

with normal resting tissue differ from the waveforms associated with ischemic tissue and tissue in oxygen deficit which have "obstructive" waveforms (Figure 2). The expansion of the tissue is also called tissue strain.

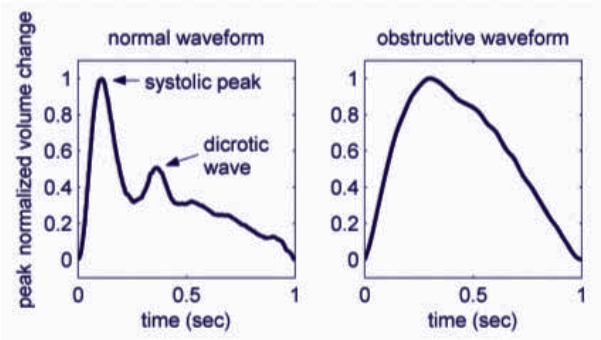

FIGURE 2: Normal and obstructive (oxygen deficit) plethysmographic waveforms shown for a single cardiac cycle.

We have developed a series of ultrasound instruments to measure plethysmographic waveforms in tiny volumes of tissue called voxels (0.01 cubic centimeters). These instruments are able to acquire tissue strain waveforms from 20,000 voxels from a single 2-dimensional image plane in tissue, by measuring the difference in motion between adjacent depths (Figure 3). The motion resolution is better than 0.1 micrometers producing a strain resolution of 0.01% or 1/10 of the normal arterial strain. Ultrasound systems allow 200 measurements along each image line. An image typically includes 100 lines, but a 3-dimensional space can include 10,000 lines including 2 million voxels.

We call the results "Tissue Pulsatility Imaging" (TPI).

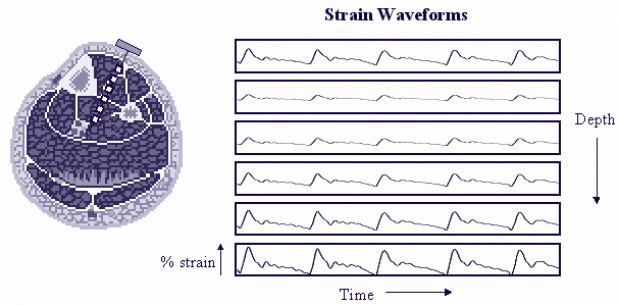

FIGURE 3: Tissue expansion (strain) measured at multiple depths in the anterior tibial muscle (schematic at left). The strain waveforms are from sample volumes separated by approximately 1 mm in depth. Notice the similarity of the waveshapes to the normal waveshape in Figure 2.

FIGURE 4: (a) Photograph of the ultrasound transducer and strain gauge around the leg. (b) Screen shot taken from the ultrasound scanner showing the muscle anatomy. Notice that the ultrasound scanhead must be held in a stable position during the 10 second periods of data acquisition.

Tissue Pulsatility Imaging of Muscle

We have applied this method to several tissues including the muscles of the calf (Figure 4).

At rest, the waveforms in the voxels of the soleus and gastrocnemius have a waveform that is similar to the "normal" resting waveform (Figure 5, "TPI before exercise"). However, with repeated "toe stand" exercise, the calf muscle becomes oxygen depleted so that in the post-exercise period, most of the voxels show waveforms similar to ischemic waveforms (Figure 5, "0:15 after"). After 5 minutes of rest, the muscles are still in the process of recovering (Figure 5, "4:45 after"). The "typical" resting TPI waveform (right edge of image) has a "dicrotic wave" (second peak) on the descending portion of the waveform (highest of 4) that indicates relative vasoconstriction. Immediately after exercise, the dicrotic wave is absent in the waveform (lowest of 4) indicating vasodilation, and slowly

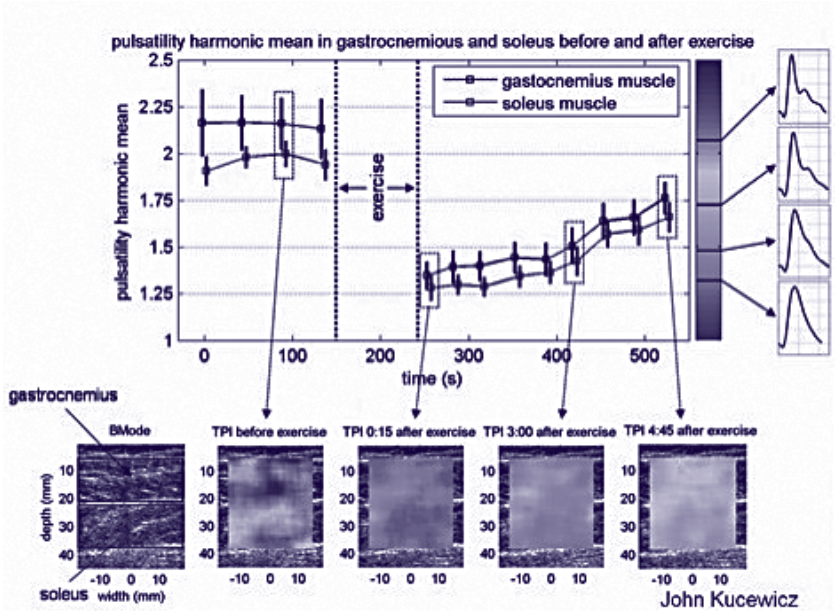

FIGURE 5: Results From a 2D Pulsatility Exercise Study. The "pulsatility harmonic mean" is a method of characterizing the waveform shape as a number. Higher values indicate that a dicrotic wave is present, consistent with vasoconstriction, lower values indicate that a dicrotic wave is absent indicating vasodilation.

By studying both the arteriolar and the venular waveforms, arterial hypoperfusion and venous hypertension can be evaluated in real time without waiting for tissue breakdown.

regains the dicrotic wave as the tissue oxygenation is restored.

Vasodilation in muscle can also be achieved by rapid breathing, lowering carbon dioxide in the blood (hypocapnia). In muscle, this causes vasodilation, in skin and brain, vasoconstriction is the result.

Much of our effort in the past year has been to develop better measures of waveform shape that indicate the degree of vasoconstriction or vasodilation. These measures must not be corrupted by effects of irregular heart beats and must allow separation of venous effects.

Intracranial Tissue Pulsatility Imaging (TPI)

To demonstrate the measurement of vasodilation and vasoconstriction in the brain, a volunteer was asked to breath a air with elevated carbon dioxide. The decrease in the dicrotic wave indicating vasodilation was present throughout the brain. Vasodilation was represented in the image by a "vasodilation index" based on waveform shape (Figure 7).

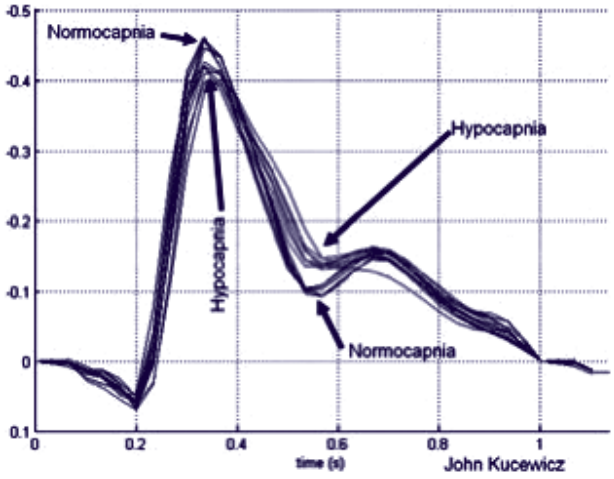

FIGURE 6: Effect of Respiratory Carbon Dioxide Pulsatile Waveforms in Muscle. Normocapnia Arterioles in the muscle are normally vasoconstricted.Hypocapnia With low levels of carbon dioxide, muscle arterioles are vasodilated.

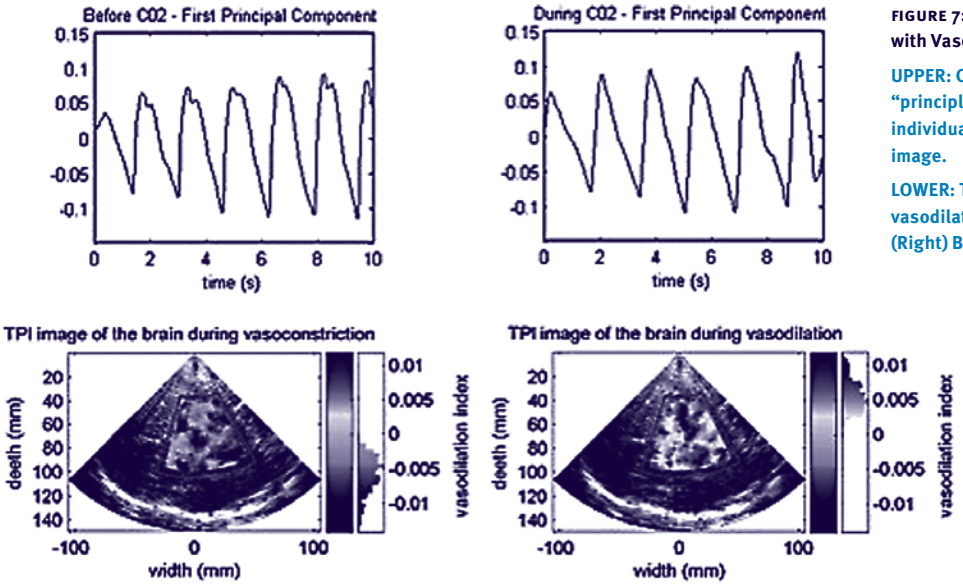

FIGURE 7: Brain Pulse Wave Shape Associated with Vasodilatation Due to CO_2 Exposure.

UPPER: Characteristic wave shape from "principle components" analysis using the individual wave shapes of 10,000 voxels in the image.

LOWER: Two-dimensional image based on the vasodilation index. (Left) Breathing normal air; (Right) Breathing air with elevated CO_2.

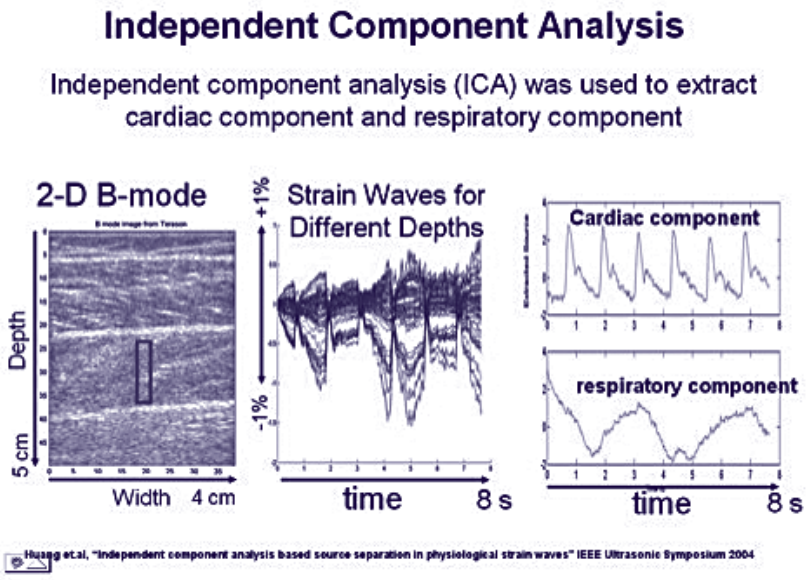

FIGURE 8: Independent Component Analysis applied to the Soleus Muscle. Extracted components including the arteriolar waveform and venular.

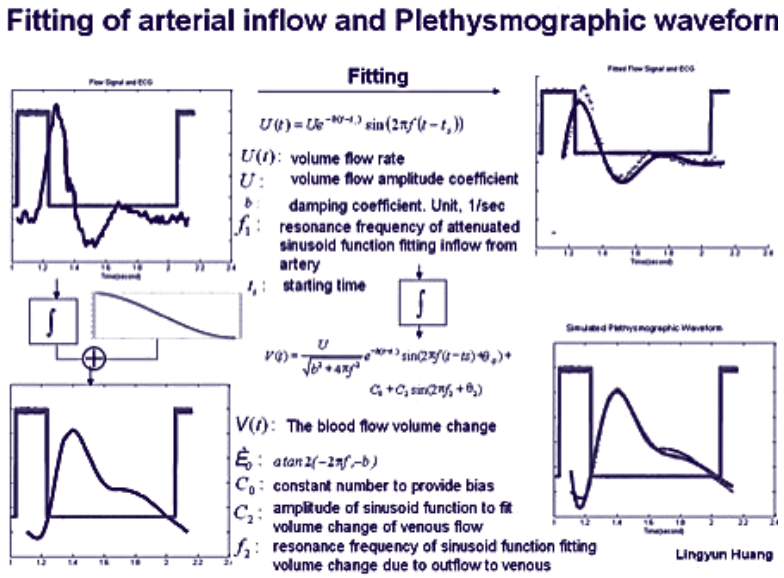

FIGURE 9: Deriving the plethsymographic waveform shape from the Doppler waveform.

Arteriolar and Venular Plethysmograpic Imaging

It is generally accepted that the pulse waveform, which mimics the arterial pressure waveform, are primarily due to pulsatile expansion of the arterioles. Every plethysmographic waveform also includes expansion in synchrony with respiration, which is associated with venous pressure. These "respiratory waves" are primarily due to cyclic expansion of venules. The arteriolar and venular waveforms can be separated by a mathematical method called Independent Component Analysis (ICA). ICA reveals 2 sources of data from the data from the Soleus muscle (Figure 8).

TPI and Doppler Waveforms

There is a direct mathematical relationship between the TPI arteriolar waveforms and conventional Doppler arterial waveforms (Figure 9): the TPI waveforms are the mathematical integral of the corresponding Doppler waveforms.

Using such models, TPI studies can be done in patients with irregular heart rhythms. By using mathematical models of the vasculature supplying TPI voxels, statistical noise in the TPI images can be reduced to a minimum allowing the vascular supply to small regions of tissue to be evaluated. By studying both the arteriolar and the venular waveforms, arterial hypoperfusion and venous hypertension can be evaluated in real time without waiting for tissue breakdown.

Real-Time Vibrometry

Arterial stenoses cause bruits that can be heard with a stethoscope during physical examination. It is also possible to image those vibrations. A collaboration between the Department of Surgery and Bioengineering Image Computing Laboratory, a series of applications has been investigated. Vibrations have been imaged in peripheral arterial pathway (vein graft) flow (Figure 10).

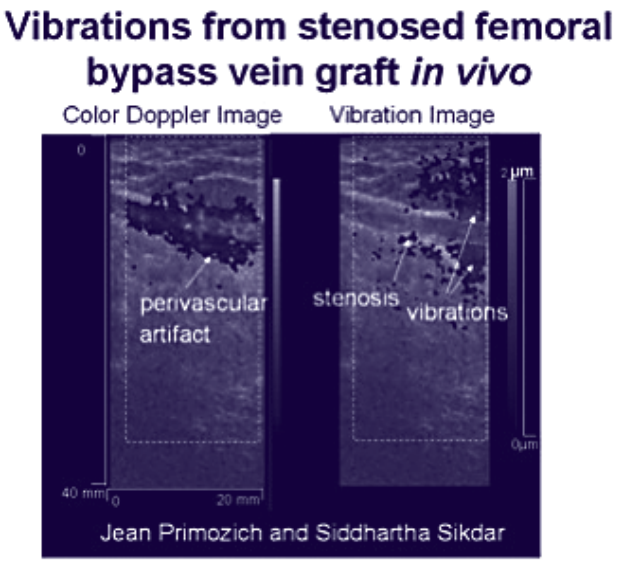

FIGURE 10: Vibration caused by Stenosis in a Vein Graft in an arterial pathway to the foot. Flow is from left to right. The amplitude of the vibrations caused by this stenosis bleed are about 2 micrometer.

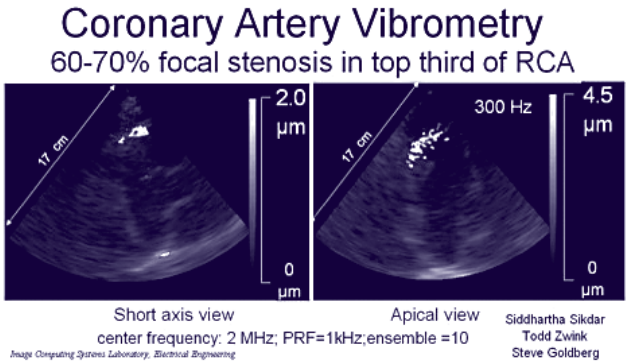

FIGURE 11: Coronary Artery Stenosis.

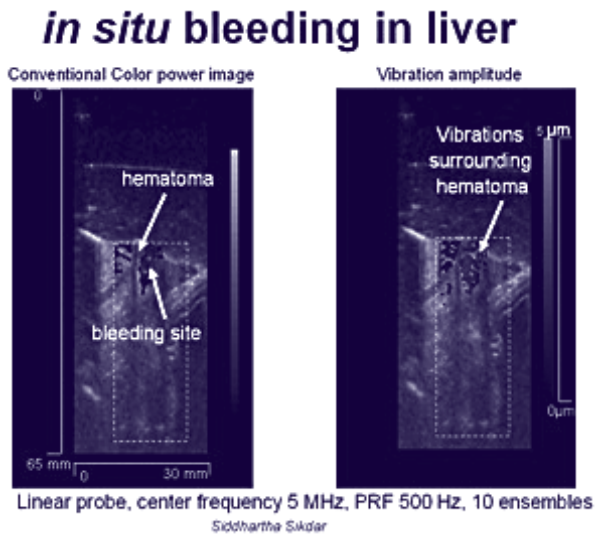

FIGURE 12: Vibration caused by Bleeding from a Lacerated Liver. Vibration amplitudes are 5 micrometers.

Vibrations are generated by most arterial pathway stenoses. Vibrations can be detected in the heart muscle when a coronary artery stenosis is present (Figure 11).

Vibrations can also be detected in bleeding organs.

There are two advantages to imaging vibration to detect bleeding rather than imaging blood flow: 1) blood flow is present in normal tissues, vibrations are not, 2) the ultrasound signal strength from vibrating reflectors is 1000 times stronger than the ultrasound signals from flowing blood so that deep bleeds can be detected reliably.

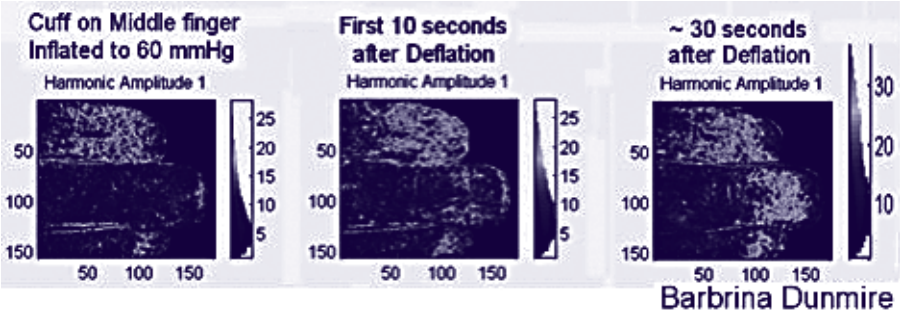

FIGURE 13: Video Photoplethysmography of the Fingers Showing Pulse Amplitude.

Video Photoplethysmography

Another way to acquire plethysmography waveforms is by optical reflection or transmission. The method is called photo-plethysmography (PPG). This method looks at the changes in absorption of light by the tissues during the cardiac cycle as the blood fills and empties from the tissues. PPG can be used to differentiate normal blood supply from abnormal supply in the skin or oin the surfaces of organs. It is also the basis of pulse-oximetry, where two colors of light (red and infra-red) are used to determine the oxygen content of the blood. Conventional PPG measures the absorption at one location, but using a video camera, an image of the pulsations in tissue can be created (Figure 13). If more than one light color is used, the tissue oxygenation can be determined.

In Figure 13, the blood supply to the middle finger is occluded on the left, resulting in no pulsation in that finger. On the right, 30 seconds after the release of the blood pressure cuff, the blood pulsatility is restored to the middle finger. Note that the pulsatility is not restored during the first 10 seconds (middle image). In addition, note the change in the scale of the pulse amplitude (bar on right) which indicates that the pulse amplitude in the first finger is increased by 30% at 30 seconds over the baseline amplitude concurrent with the restoration of pulsation in the middle finger. In addition, the pulse amplitude in the proximal finger is generally low.

Ultrasound Reading Center for Carotid Stents

One of the major treatable causes of stroke is stenosis (narrowing) of a carotid artery in the neck. This artery supplies the majority of blood to the anterior part of the brain. The stenosis is usually formed by an atherosclerotic plaque. If left untreated, the plaque can fall apart sending debris to occlude smaller arteries in the brain causing stroke. Since 1960, removing the atherosclerotic plaque by surgical endarterectomy has been the treatment of choice. An alternative treatment, the placement of an artificial lining in the carotid to open the stenosis, has been under development since 1995. This artificial lining is called a stent. It is usually made of metal. It is placed by a catheter which is inserted in the artery in the leg, and threaded up though the body to the neck.

Stents are now used clinically in may arteries, but there effectiveness in the carotid arteries is still under investigation. The Ultrasound Reading Center (UWURC) in the department of Surgery provides quality assurance for following the patient by Doppler ultrasound after placement of a stent in the treatment group or carotid endarterectomy in the control group. The ultrasound reading center serves over 130 clinical sites in the United States, providing protocol manuals and providing evaluation of each Doppler/ultrasound study and statistical services for analysis of the results. The reading center has processed over 5,000 examinations.

The UWURC is the Ultrasound Core Laboratory for the Carotid Revascularization Endartectomy vs. Stenting Trial (CREST). The UWURC has completed analysis on 2443 studies for the CREST trial on a total of 1353 patients. Half of the patients have had only one ultrasound scan, a third have had two scans, the remaining 1/6 have had between 3 and 8 follow-up scans after stent placement. Many of the 618 pre-procedure scans, many also have pre-procedure angiographic studies which will allow a new analysis of the relationship between ultrasound Doppler blood velocity measurements and angiographic residual lumen and normal lumen diameter measurements.

RELATED PUBLICATIONS

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