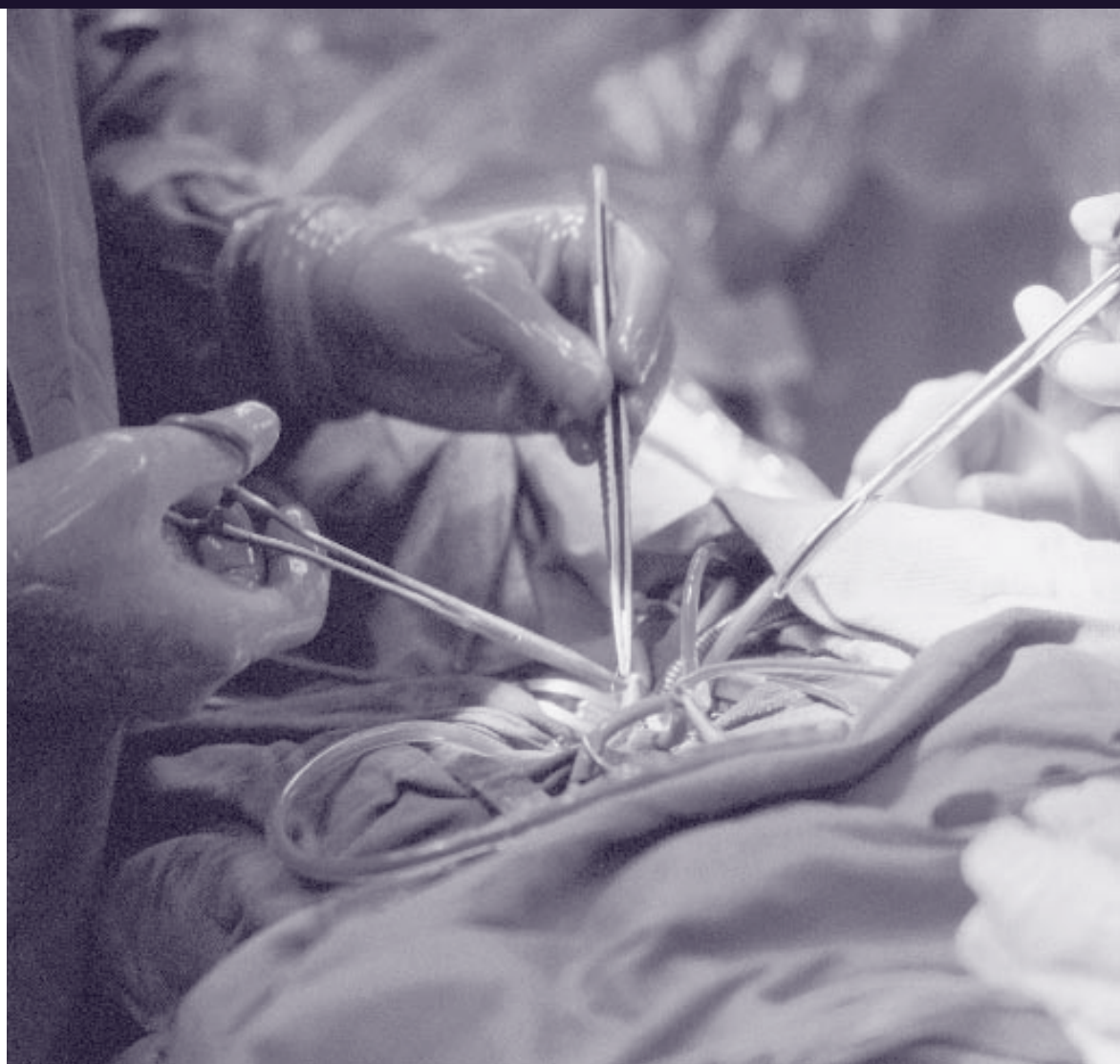
SURGERY RESEARCH

DEPARTMENT OF SURGERY 2005 REPORT



UW Medicine
SCHOOL OF MEDICINE

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

2005 REPORT

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COVER PHOTOS: DAN LAMONT, PHOTODISC

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Clinical Treatment Begins in the Research Laboratory

Medical research unravels the mysteries of life and helps us understand how even the smallest components of the body work and how the body reacts to the environment. However important that is, research really "hits home" when its findings can be applied directly to improve our ability in preventing, diagnosing, or curing disease and, thus, directly helping people improve their lives. This is frequently referred to as *translational research* — the clinical application of new knowledge. Certainly there is no specific line separating basic science research from translational research as, by its very nature, basic science research eventually provides the foundation for the translation into clinical applications. The scientific community holds our department investigators' research in high regard and I can easily point to an impressive array of credentials, honors, awards, and peer-reviewed funding sources as testimony of this esteem. Yet the real reason I know that our work matters is because I see and hear from the people whose lives have been transformed because of it. Their stories confirm that our work makes a difference.

The benefits of research are most obvious and valuable in those areas of life over which we have no control. For example, we are all getting older and it is a well-documented fact that the incidence of vascular disease increases with age. The intimate pathophysiology of atherosclerosis, the formation and evolution of plaque, and the natural history of revascularization are processes that have been studied for years by our vascular surgeons. They have found many answers which have brought substantial acknowledgment to this group from within and outside of the University of Washington. More recently, they have been recognized by their integration into a major vascular research initiative at the new UW research campus. This state-of-the-art facility concentrates all the UW's research expertise in vascular disease in one location at South Lake Union. These new arrangements will foster greater interdisciplinary collaboration and benefit the aging patient population with innovative treatment protocols.

Nevertheless, research possibilities do not exist in a vacuum. Technological advances offer choices and demand decisions that were unheard of only a few years ago; at the same time, fewer dollars are available to support such services. Societal pressures have led us more and more into the field of quality of care, which is broadly defined as doing what is right for the right individual at the right time and within the economic constraints of society. Consequently, we are actively involved in the emerging field of outcomes research. This discipline addresses the impact of surgery on an individual's life and on society as a whole. It paints an overview that helps the surgeon and patient determine what *should* be done instead of what *could* be done. A valuable tool when used properly, outcomes research ensures that investigative endeavors continue to provide the very best in comprehensive patient care. Under David Flum's leadership our department is developing a major center dedicated to Outcomes Research which should be fully developed by mid 2005.

Above all, our primary mission is to provide you and your loved ones with the very best in surgical care. I invite you to learn how traumatic injury and other illnesses will be treated tomorrow by reading this report in which our faculty explain some of their groundbreaking studies. Our investigators are listed in alphabetical order by division to make it easier for you to locate either a particular individual or specialty. If you are interested in supporting our efforts to discover tomorrow's cures, please contact Eileen Herman, Public Information Specialist, at (206) 522-7076, email: emherman@u.washington.edu.

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

RESEARCH HONORS & AWARDS

ALEXANDER W. CLOWES, M.D., CHIEF, DIVISION OF VASCULAR SURGERY: winner of *The Sheen Award*. Nominated by the American College of Surgeons and reviewed by a committee of physicians from Atlantic County, NJ, this illustrious award honors doctors who have made outstanding contributions to mankind through the fields of medicine and medical research. The award includes funding of $25,000.

THOMAS HATSUKAMI, M.D.; ASSOCIATE PROFESSOR: awarded the prestigious *Royal College of Surgeons Traveling Fellowship*. He spent four weeks in the United Kingdom discussing many aspects of his research, learning new techniques, and establishing ties with our British colleagues.

RONALD V. MAIER, M.D.; PROFESSOR AND HMC SURGEON-IN-CHIEF: received the *Distinguished Scientist Award* from the Shock Society for a lifetime of dedication and commitment to understanding the mechanisms of shock. Dr. Maier was recognized for his research work in innate immunity and the inflammatory process in the host response to injury and sepsis. The Shock Society is a national organization dedicated to prevention, treatment and cure of diseases resulting from shock

ARSHAD MUZAFFAR, M.D.; ASSISTANT PROFESSOR: was awarded *The 2004 American Society of Maxillofacial Surgeons Traveling Fellowship*. This highly competitive award is designed to further the education of young craniofacial surgeons and provide for their travel to major craniofacial centers in North America. The award comes with a $5,000 stipend.

KIMBERLY RIEHLE, M.D.; (THEN) R3: awarded a two-year, $30,000 American College of Surgeons research scholarship for work entitled, "Liver Regeneration in SOCS-3 Knockout Mice." Dr. Riehle will work in the laboratory of Dr. Nelson Fausto, Professor & Chairman of the Department of Pathology.

MATHEW ROSENGART, M.D.; (THEN) SURGICAL CRITICAL CARE FELLOW: won the Best Clinical Paper in the American College of Surgeons National Committee on Trauma resident paper competition for work entitled, "An Evaluation of the Effect of State Firearm Legislation on Firearm Mortality."

ERIK VAN EATON, M.D.; (THEN) R3: awarded a $41,796 National Library of Medicine Postdoctoral Research Fellowship for bioinformatics work entitled, "Clinical Informatics Applications for Improving Patient Safety, Continuity of Care, and Clinician Workflow."

EDWARD D. VERRIER, M.D.; THE K. WILLIAM EDMARK PROFESSOR IN CARDIOTHORACIC SURGERY: received a $2.2 million, five-year renewal of his National Institutes of Health grant to study the molecular biology of myocardial ischemia/reperfusion.

DAVID FLUM, M.D.; ASSISTANT PROFESSOR & **BRANT OELSCHLAGER, M.D.;** ASSISTANT PROFESSOR: were each selected by the editorial board of the *Archives of Surgery* as having published one of the top 10 clinical research articles in 2003. Dr. Flum wrote about his work in intraoperative imaging during gall bladder surgery. Dr. Oelschlager wrote about the effectiveness of laparoscopic anti-reflux surgery in the treatment of Barrett's esophagus.

CARDIOTHORACIC SURGERY

GABRIEL S. ALDEA, M.D.

RIYAD KARMY-JONES, 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
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 (CABG) 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 neurologic and neuropsychological function. Alternatives to traditional cardiac surgical methods, including "minimally invasive" techniques, are being developed to limit morbidity associated with conventional CABG.

Many of the complications of CABG are related to the biologic 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. Although these responses are usually short lived and leave no residual deficits, they can lead to long-lasting cardiac, pulmonary, renal and neurologic dysfunction in a subset of patients.

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.

Alternatives to traditional cardiac surgical methods, including "minimally invasive" techniques, are being developed to limit morbidity associated with conventional CABG.

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). 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 tech-

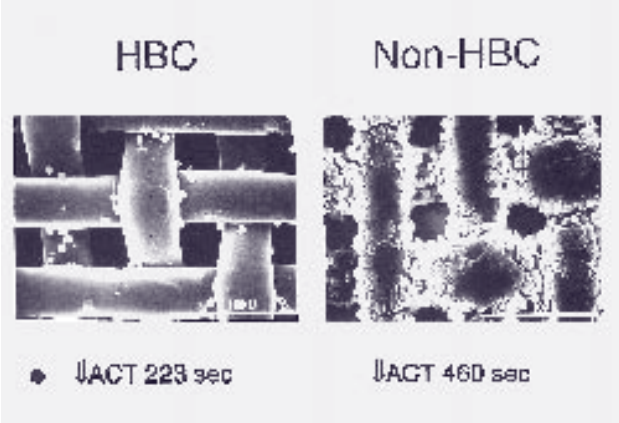

FIGURE 1: Scanning electron micrographs at 200 fold magnification of arterial filter. Lowest activating times (ACT) in seconds are noted. HBC= heparin-bonded circuits. Non-HBC- control non-heparin-bonded circuits.

niques (the effects of cardiotomy suction vs. use of cell saver technology) on markers of hemostasis, inflammation, neurological and neuropsychological deficits. Although both result in blood conservation, one (cardiotomy suction) reinfuses 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 reinfusion. These different approaches result in markedly different effects on inflammation and thrombin generation during artificial perfusion. This research may lead to changes in both the design and application of this technology.

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

Furthermore, we are becoming more aware of differences and individual variability between individual patients in expressing such responses to CPB with some

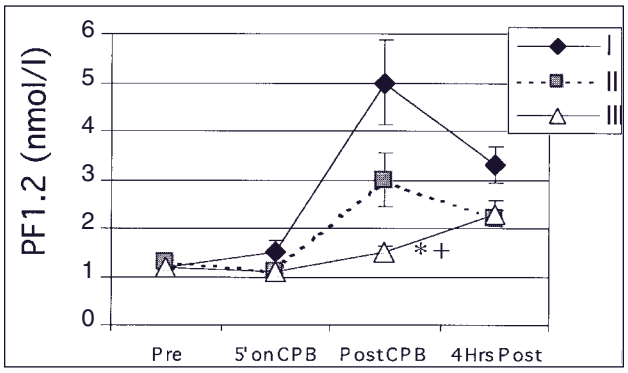

FIGURE 2: PF1.2 for thrombin generation.

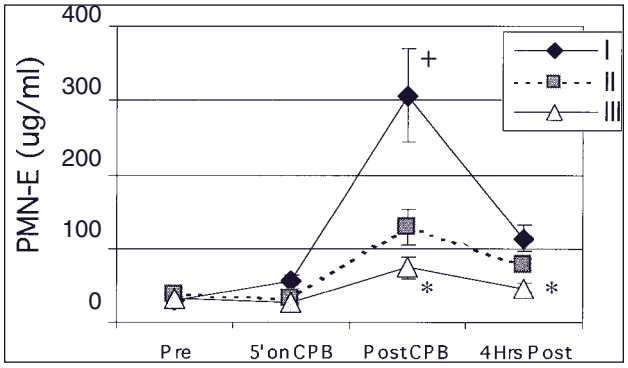

FIGURE 3: PMN-E for elastase.

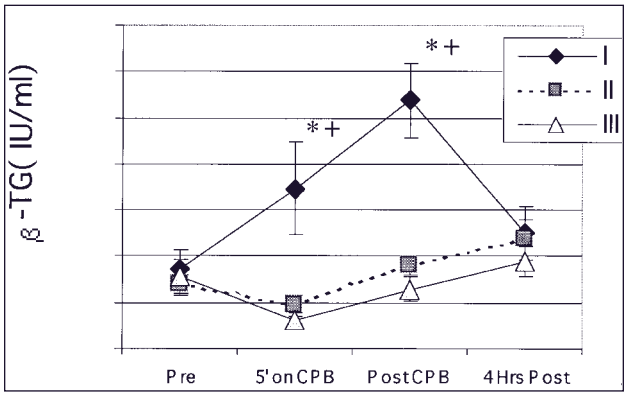

FIGURE 4: β-Thromboglobulin for platelet activation.

patients having a minimal response and others having a very accentuated response to CPB. In collaboration with labs of Drs. Mulligan, Rosengart and Chandler, we are trying to determine ways to identify individual biological susceptibility prior to surgery so we can alter surgical technique (either avoid CPB altogether or use a combination of altered equipment, techniques and pharmacological therapy). We hope to develop reliable specific biological essays to predict an individual patient's response to artificial perfusion and direct clinical therapy.

RELATED PUBLICATIONS

1. McKenney PA; Apstein CS; Mendes LA; Connelly GP; Aldea GS; Shemin RJ; Davidoff R. Immediate effect of aortic valve replacement for aortic stenosis on left ventricular diastolic chamber stiffness. *Am J Cardiol,* 1999; 84:914-18.
2. Aldea GS. Heparin-bonded circuits decrease thromboembolic complications in patients undergoing CABG. *Seminars in Cardiothor Vasc Anesth,* 1999; 3(1):9-16.
3. Shapira OM; Xu A; Aldea GS; Vita JA; Shemin RJ; Keaney JF. Enhanced nitric oxide-mediated vascular relaxation in the radial artery compared to internal mammary artery and saphenous vein. *Circ,* 1999; 100(Suppl II):322-27.
4. Shapira OM; Alkon JD; Macron DSF; Keaney JF; Vita JA; Aldea GS; Shemin RJ. Nitroglycerin in preferable to Diltiazem for prevention of coronary bypass conduit spasm – A prospective randomized study. *Ann Thor Surg,* 2000; 70(3):883-9.
5. Aldea GS; Mori H; Husseini WK; Austin RE; Hoffman JIE. Effects of increased pressure inside and outside the ventricles on total and regional myocardial blood flow. *Am J Physiol,* 2000; 279:H2927-38.
6. Slade P; Sanchez P; Townes B; Aldea GS. The use of neurocognitive tests in evaluating the outcome of cardiac surgery: Some methodological considerarions. *J Cardiothorac Vasc Anesth,* 2001; 15(1):4-9.
7. Vander Salm TJ; Kip KE; Jones RH; Schaff HV; Shemin RJ; Aldea GS; Detre KM. What constitutes optimal surgical revascularization? Answers from the Bypass Angioplasty Revascularization Investigation (BARI). *J Am Coll Cardiol,* 2002; 39:565-72.
8. Aldea GS; Soltow LO; Chandler WL; Triggs CM; Vocelka CR; Crockett GI; Shin YT; Curtis WE; Verrier ED. Limitation of thrombin generation platelet activation and inflammation by elimination of cardiotomy suction in patients undergoing coronary artery bypass grafting treated with heparin-bonded circuits. *J Thorac Cardiov Surg,* 2002; Apr 123(4):742-55.
9. Aldea GS; Goss R; Boyle EM et al. Use of off-pump and on-pump CABG strategies in current clinical practice: the Clinical Outcomes Assessment Program of the State of Washington. *J Card Surg,* 2003;18:206-215.
10. Dabal RJ; Goss R; Maynard C; Aldea GS. The effect of left internal mammary artery ustilization on short-term outcomes after coronary revascularization. *Ann Thorac Surg,* 2003; 76:464-70.

DEPARTMENT CO-INVESTIGATORS

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OTHER CO-INVESTIGATORS

Wayne Chandler, M.D.; UW Department of Laboratory Medicine / **Terry Gernsheimer, M.D.;** UW Department of Medicine

Riyad Karmy-Jones, M.D.

- Indications and Outcome Following Thoractotomy
- Traumatic Aortic Rupture
- Lung Inflammation

AWARDS
Resident Teaching Award

FUNDING
Defense R&D, Canada
U.S. Department of Defense
U.S. Office of Naval Research
Zymogenetics

Chest injury is responsible for at least 25% of deaths following trauma, and plays a major factor in a further 25% of deaths. Our research has been focused in three areas: Indications for and outcomes of thoracotomy following trauma; aortic trauma; ischemia-reperfusion injury and lung inflammation.

Indications and Outcome Following Thoracotomy

The timing of thoracotomy following injury has been based on historical data originating during the Vietnam War. Indications have been "1500 cc of blood output on initial placement of chest tube or 200-300 cc/hour of output for several hours." This has been particularly problematic following blunt injury, when thoracotomy has been delayed in hopes that "output is decreasing." We conducted a multi-center retrospective study within the Western Trauma Association. When considering patients who underwent thoracotomy for bleeding (as opposed to specific diagnosis or because of shock), we documented that for each 500 cc of blood loss prior to thoracotomy, mortality increased 60% and that this increased risk of death was independent of mechanism (Figure 1).

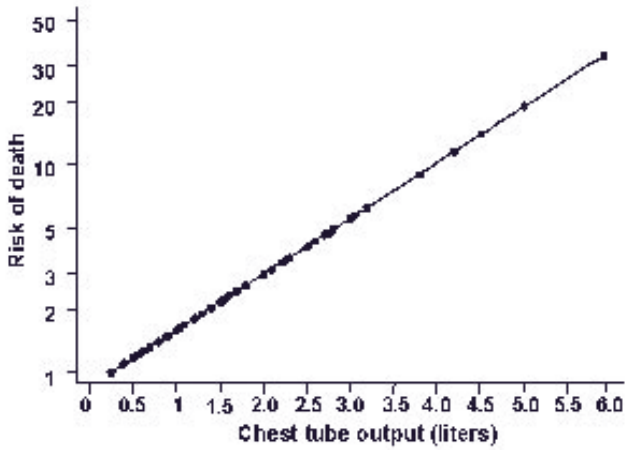

FIGURE 1: Risk of death and volume chest tube output.

Harborview is unique in that it has one of the largest volumes of aortic trauma in the nation... We are currently leading a multi-center prospective study of non-operative management of aortic trauma.

This has prompted a multi-center prospective study, with change in practice so that once 1500 cc has been reached within 24 hours, thoracotomy or thoracoscopy should be considered. Preliminary data suggests a 50% reduction in mortality.

We have also reviewed specific techniques of lung resection following traumatic injury. Considering all mechanisms of injury, there is an incremental risk of death with progressively complex resections (Figure 2). A related review focusing on patients with penetrating injury alone found that anatomic resections were associated with a lower incidence of septic complications compared to "stapled" approaches. The implications of this work are that a) lesser resections are favored, including damage control techniques but b) surgeons must be facile in all possible methods and be ready to progress to more complex operations without delay.

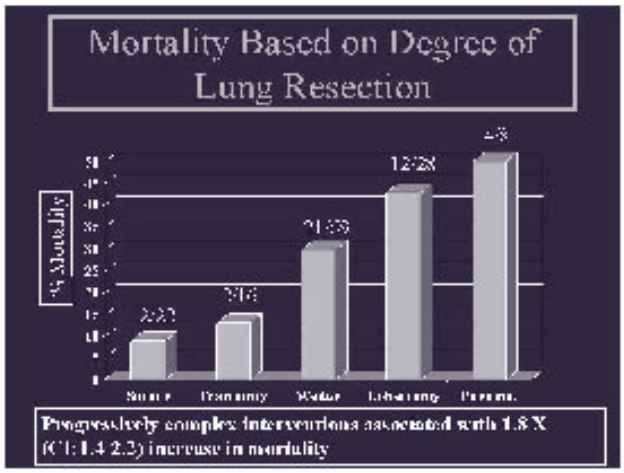

Figure 2: Mortality based on degree of lung resection.

Traumatic Aortic Rupture

Harborview is unique in that it has one of the largest volumes of aortic trauma in the nation. The average trauma center treats on average less than two patients/year with aortic trauma while HMC, on average, treats 15. This volume, coupled with the ability to utilize the trauma database, has allowed us to study a number of questions. An important anatomic detail is the location of the injury relative to the left subclavian artery (LSCA). In 91 cases the exact location of the injury in patients who underwent operative repair could be determined. Forty-one were ≤ 1 cm from the junction of the LSCA and the thoracic aorta, 49 more distal. The more proximal injuries were associated with increased mortality (43% vs. 22%, p=0.04), intra operative rupture (17% vs. 2%, p=0.003) and cross clamp time (39.5±21.9 minutes vs. 28.4±13 minutes, p=0.04). Three ruptures occurred while obtaining proximal control in patients with injuries close to the LSCA. We advocate instituting bypass before attempting proximal control to avoid the risk of rupture before bypass can be instituted.

Also, the operative outcomes of 120 patients were reviewed relative to presentation. Patients were classified as "unstable" if presenting systolic blood pressure was < 90 mm Hg or if it decreased to < 90 mm Hg after admission. Operative mortality was significantly higher in unstable patients (62%) vs. stable patients (17%,p=0.001), and patients with cardiac ischemia or contusion (71%) vs. those without (24%,p=0.001). Free rupture was the cause of hypotension in only 25% of unstable patients, the remainder being due to other causes. Although the use of mechanical circulatory support (MCS) appeared to reduce the risk of paralysis, (0/59 cases with MCS vs. 8/61 without MCS), logistic regression analysis found that only preoperative instability was a significant independent predictor of paralysis (risk increased 5.5 times, confidence intervals 3.3-10). Currently, patients with closed head injury are not excluded from operative repair, but patients with severe lung injury or depressed cardiac function are managed by alternative means.

One such approach for patients who may not be operative candidates is blood pressure control with *B*-blockers. We were able to review and follow the course of 30 cases managed non-operatively who had serial radiological exams. Three patients exhibited progression of injury size within 5 days of injury, one of whom experienced free rupture (and was not managed with *B*-blockers). Our data demonstrated that *B*-blockers can significantly reduce the risk of rupture, that the risk of rupture is greatest within the first five days of injury, and that psueodoaneurysms that persist greater than two weeks will not resolve and will ultimately follow the pattern of chronic thoracic aneurysms. We are currently leading a multi-center prospective study of non-operative management of aortic trauma.

An exciting option in the management of thoracic vascular diseases is the role of endovascular stent grafts (EVSG). A review of 50 angiograms of patients with aortic rupture identified some key anatomic details with regards to modeling grafts. The mean distance along the lesser curve to the superior aspect of the tear was 5.8 mm, aortic diameter 19 mm and mean degree of curvature 27°. The majority of aortic tears will require the origin of the subclavian to be covered. We have

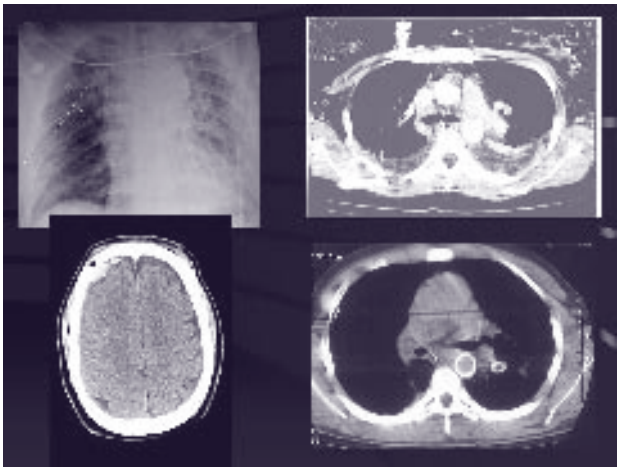

Figure 3: 18 year old female involved with MVC. Patient sustained closed head injury with elevated ICP, severe pulmonary contusion and required splenectomy. Her pelvis was unstable and angiographic embolisation needed for arterial bleeding. EVSG was utilized to control her TRA.

treated 10 patients with this approach (Figure 3) and have made the following observations: Commercially available grafts are superior to "home made ones"; most aortic injuries can be treated by commercially available "cuff-extenders"; tears near the aortic curvature of > 1.5 cm are associated with "telescoping" resulting in increased risk of endoleak. The ideal thoracic grafts are not yet available, but our group is leading a trial with the Talent device which we have used on one occasion and appears to be ideally suited to the thoracic aorta.

Lung Inflammation

Although direct ischemia-reperfusion injury has been an area of great interest to the transplant community, indirect mechanisms of lung injury may be more important following trauma. We used a model of hilar occlusion to demonstrate that ischemia-reperfusion to one lung results in an indirect injury to the contralateral lung with release of NFkB (Figure 4).

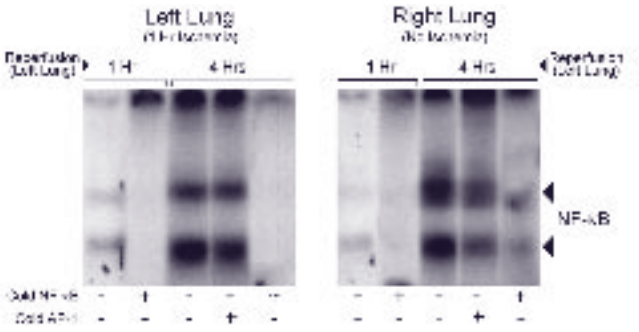

Figure 4: Left lung with ischemia compared to right lung with no ischemia

We are also involved with determining the role of novel cytokines, including Interleukin 20, in parenchymal disorders. Preliminary data suggest that patients with degrees of fibrosis and active inflammation have increased expression, while patients with cancer have much lower expression of this cytokine and its mediators.

Related Publications

1. Karmy-Jones R; Vallieres E; Harrington R. Surgical management of necrotizing pneumonia. *Clin Pul Med,* 2003; 10:17-25.
2. Cheng SG; Glickerman DJ; Karmy-Jones R; Borsa JJ. Traumatic sternomanubrial dislocation with associated bilateral internal mammary artery occusion. *AJR,* 2003; 180:810.
3. Karmy-Jones R; Hoffer E; Meissner M; Bloch RD. Management of traumatic rupture of the thoracic aorta in pediatric patients. *Ann Thorac Surg,* 2003; 75:1513-1517.
4. Karmy-Jones R; Vallieres E. Non-small cell lung cancer with chest wall involvement. *Chest,* 2003; 123:1323-1325.
5. Karmy-Jones R; DuBose R; King S. Traumatic rupture of the innominate artery. *Eur J Card Thorac Surg,* 2003; 23:782-787.
6. Karmy-Jones R; Avansino J; Stern EJ. CT of blunt tracheal rupture. *AJR,* 2003; 180:1670.
7. Hoffer E; Karmy-Jones R. Rupture of the common carotid in a helicopter crash in a patient with aberrant right subclavian artery. *Ann Thorac Surg,* 2003; 75:1977.
8. Karmy-Jones R; Hoffer E; Meisnner M; Nicholls S; Mattos M. Endovascular stent grafts and aortic rupture: A case series. *J Trauma,* 2003; 55:805-810.
9. Karmy-Jones R; Nathens AB; Jurkovich GJ; Shatz DV; Brundage S; Wakk MJ, Jr; Engelhardt S; Hoyt DB; Holcroft J; Knudson MM; Michaels A; Long W. Urgent and emergent thoracotomy for penetrating chest trauma. *J Trauma,* 2004; 56:664-669
10. Dunham MB; Zygun D; Petrasek P; Kortbeek JB; Karmy-Jones R; Moore RD. Endovascular stent grafts for acute blunt aortic rupture. *J Trauma,* 2004; 56:1173-8.
11. Karmy-Jones R. The impact of homelessness on the ability to provide resection as an option in the management of lung cancer. Current Medical Literature: Respiratory Medicine 2004; 18:81-4.
12. Karmy-Jones R; Jurkovich GJ. Blunt chest injury: Current problems in surgery 2004; 41:223-380.

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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, Prize, 2000
Seattle Surgical Society, Best Presentation, 1999
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 β chemokines have two adjacent cysteine residues, and function primarily as monocyte and lymphocyte chemotactic agents. Members of this family include MCP-1, RANTES and MIP-1α, MIP-1β, 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-1β, PAF) as important mediators in these models and to a lesser degree, in lung reperfusion injury.

Likewise three C-C chemokines, MCP-1, MIP-1α, 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-1α 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 1a, 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 recipient 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.

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.

A technically simple model for airway transplantation with histopathologic features of obliterative bronchiolitis has gained acceptance.

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.

RELATED PUBLICATIONS

1. Mulligan MS; Lentsch AB; Huber-Lang M; Ren-Feng G; Sarma V; Wright CD; Ulich TR; Ward PA. Anti-inflammatory effects of mutant forms of secretory leukocyte protease inhibitor. *Am J Pathol,* 2000; 156(3):1033-1039.
2. Mulligan MS; Warner RL; Rittershaus C; Thomas LJ; Ryan US; Foreman KE; Crouch LD; Till GO; Ward PA. Endothelial targeting and enhanced anti-inflammatory effects of complement inhibitors possessing sialyl lewis x moieties. *J Immunol,* 1999; April 15; 162(8):4952-9.
3. Mulligan MS; Lentsch AB; Shanley TP; Miyasaka M; Johnson KJ; Ward PA. Cytokine and adhesion molecule requirements for lung injury induced by anti-glomerular basement membrane antibody. *Inflammation,* 1998; 22(4):403-417.
4. Mulligan MS; Warner RL; Lowe LB; Smith PL; Suzuki Y; Miyasaka M; Yamaguchi S; Ohta Y; Tsukada Y; Kiso M; Hasegawa A; Ward PA. *In vitro* and *in vivo* selectin-blocking activities of sulfated lipids and sulfated sialyl compounds. *Intl Immunol,* 1998; 10(5):569-575.
5. Mulligan MS; Miyasaka M; Suzuki Y; Kawashima H; Iizuka M; Suzuki T; Hasegawa A; Kiso M; Warner RL; Ward PA. Anti-inflammatory effects of sulfatides in selectin-dependent acute lung injury. *Intl Immunology,* 1998; 7:10, pp1107-1113.
6. Mulligan MS; Schmid E; Till GO; Friedl HP; Hugli TE; Roth RA; Ward PA. C5a-dependent upregulation of p-selectin *in vivo. J Immunol,* 1997; Feb. 15; 158(4):1857-61
7. Mulligan MS; Shanley T; Foreback J; Warner R; Ward PA. Protective effects of IL-4, IL-10, IL-12 and IL-13 in IgG immune complex-induced lung injury. *J Immunol,* 1997; 159:3483-3489.
8. Mulligan MS; Schmid E; Beck-Schimmer B; Till GO; Friedl HP; Rauer RB; Hugli TE; Miyasaka M; Warner RL; Johnson KJ; Ward PA. Requirement for and role of C5a in acute inflammatory lung injury in rats. *J Clin Invest,* 1996; 98:503-12.
9. Mulligan MS; Warner RL; McDuffie JE; Bolling SF; Sarma V; Ward PA. Regulatory roles of the Th-2 cytokines IL-4 and IL-10 in cardiac allograft rejection. *J Exp Mol Pathol,* 2000.
10. Mulligan MS; McDuffie JE; Shanley TP; Guo RF; Sarma JV; Warner RL; Ward PA. Role of rantes in experimental cardiac allograft rejection. *J Exp Path* (in press).

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- Cellular and Molecular Mechanisms of Ischemia-Reperfusion Injury

AWARDS

American College of Surgeons
- National Committee on Trauma Competition, First Place
- Region X Committee on Trauma Competition, Finalist
- Washington State Chapter Henry Harkins Resident Research Award, First Place
- Washington State Chapter Henry Harkins Scientific Presentation Award

American Heart Association
- Vivian Thomas Young Investigators Award in Cardiothoracic Surgery, Finalist

Helen and John Schilling Resident Research Symposium Awards
Thoracic Surgery Foundation For Research and Education Fellowships
Western Thoracic Surgery Association Sampson Resident Research Award

FUNDING

3M/Surgical Infection Society
Bayer Corporation
ICOS
National Institutes of Health
National Science Foundation
NovoNordisk Pharmaceuticals
Q-pharma, Inc.
Thoracic Surgery Foundation
ZymoGenetics, Inc.

The vascular endothelium has a principal role in several of the biologic events that affect the preoperative, operative, and postoperative course of nearly all surgical patients. In response to injury, endothelial cells become activated, releasing or expressing a number of inflammatory mediators that enhance leukocyte adhesion, promote coagulation and induce vasoconstriction. These responses to injury are beneficial to the patient when limited to localized areas of infection or tissue disruption. During severe systemic illness (for example, cardiopulmonary bypass, sepsis, or shock), inflammatory reactions may become generalized, however, initiating a distinct pathologic state called the "Systemic Inflammatory Response Syndrome" (SIRS). Systemic inflammatory reactions in general cause damage to tissue, which leads to organ dysfunction.

In our laboratory, we have examined the molecular mechanisms of *regional* I/R injury that complicates cardiothoracic surgical procedures, and *systemic* I/R injury that is the result of resuscitation in the trauma patient with hemorrhagic shock. The scope of our research includes the study of basic biologic processes at molecular, cellular and physiologic levels, and the examination of the pathophysiologic mechanisms of I/R injury. Our goal is to translate an understanding of the molecular mechanisms of I/R injury into applications for clinical practice.

Thrombin is generated during reperfusion and may mediate reperfusion injury.

Ischemia-reperfusion injury: Ischemia/reperfusion (I/R) injury contributes significantly to morbidity and mortality in surgical patients. I/R injury is the principal pathogenetic event in stroke, complications of peripheral vascular disease, hemorrhagic shock, and early transplant graft dysfunction. Paradoxically, reperfusion of oxygen-deprived tissue, the mainstay of therapy for ischemia, causes further tissue injury by inciting a deleterious inflammatory reaction in and around 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 important consequence of reperfusion.

The cellular and molecular mechanisms of endothelial cell activation during I/R injury are complex. These mechanisms result in tissue factor expression (leading to microvascular thrombosis and disseminated intravascular coagulation [DIC]); neutrophil adhesion secondary to upregulation of neutrophil adhesion molecules on activated endothelium (for example, E-selectin); and leukocyte activation and chemotaxis caused by the release from I/R injured endothelium of

chemokines (for example, Interleukin-8) and growth factors. One component of our research is based on the transcription factor, NF-κB, that regulates transcription and expression of the genes that encode these proteins.

NF-κB is composed of subunits from the NF-κB/Rel family of proteins. Five distinct DNA-binding proteins of the family, p50, p52, p65 (also known as RelA), c-Rel, and RelB, are involved in mammalian transcription. Members of this family are defined by the presence of a highly conserved region of approximately 300 amino acids called the "Rel homology domain," which bears the DNA binding site, located in the amino-terminus half of the domain.

During I/R injury, NF-κB activation may be preceded by signal transduction through a cytoplasmic molecule, p38, of the MAP kinase family of signal transduction proteins. Once activated in response to environmental changes surrounding a cell, p38 in turn activates transcription factors leading to changes in gene expression in cardiac cells, endothelial cells of coronary vessels, or inflammatory cells such as macrophages.

Thrombin is generated during reperfusion and may mediate reperfusion injury. Thrombin interacts with a specific cell receptor, protease-activated receptor-1 (PAR-1), present on endothelial cells, cardiac myocytes, and macrophages, signaling changes in gene expression in these cells. Complement chemotactic fragments, C3a and C5a, are also generated during reperfusion of ischemic tissue and, with thrombin, may be the initiating signals of I/R injury.

The specific aims of our research are: (1) Determine the molecular pathways that lead to NF-κB activation during ischemia and reperfusion; (2) determine the role of NF-κB-mediated gene transcription in regional and systemic I/R injuries; and (3) identify novel therapies that block NF-κB activation only during I/R injury, preserving the capacity of the cell, and the patient within whom it resides, to respond to other injuries (e.g., sepsis).

Experimental techniques: We utilize cultured cells to examine molecular mechanisms that are involved in the response to I/R injury. Although cell culture is a highly artificial system, it allows us to examine in precisely controlled conditions specific questions about the effects of hypoxia and reoxygenation on molecular pathways in human cells.

In addition, cell culture gives us the capability to move DNA sequences into human cells in a controlled fashion to deduce cellular mechanisms of activation based on the effect of the protein encoded by the transfected DNA on cellular function. 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. Interpretations of findings *in vitro* are provisional, however, until they can be confirmed *in vivo*.

We have developed several animal models of regional and systemic inflammatory responses induced by I/R injury. We have also included in our experimental repertoire *ex vivo* perfusion of hearts by the method of Lagendorf. Recently we have found that rabbit hearts made ischemic by transient coronary artery ligation, express large amounts of tissue factor after release of the ligature and reperfusion of the ischemic segment. Furthermore, we have recently reported that inhibition of IL-8 significantly blocks myocardial I/R injury.

We have developed and utilize a mouse model of myocardial I/R injury. A well-defined I/R injury is induced in mouse hearts by transient occlusion of the left anterior descending coronary artery. Following reperfusion we determine the size of the infarcted region to quantify the magnitude of cardiac I/R injury. Although the mouse myocardial I/R injury model is technically challenging and is performed in only two other laboratories in the U.S., use of transgenic or gene knockout strains allows us to examine the effect of specific genotypic changes (and thus phenotypic changes) on myocardial I/R injury.

For example we have examined mice that have been genetically engineered to lack PAR-1 (PAR-1 knockouts; or PAR-1 -/-). Compared to wild-types, PAR-1 knockouts develop a significantly smaller infarct after myocardial I/R injury — confirming, as we have postulated, that thrombin (through its interaction with PAR-1) plays a necessary role in the pathogenesis of I/R injury. Furthermore, based on evidence we have developed with regard to signaling pathways involved in myocardial I/R injury, we have been able to pharmacologically reduce infarct size in our mouse model of I/R injury. Blockade of p38 activity with a proprietary compound significantly attenuates infarct size after ischemia and reperfusion compared to mice treated with vehicle alone. Thus, we have been able to apply what we have determined about the basic science of myocardial I/R injury to potential clinical development.

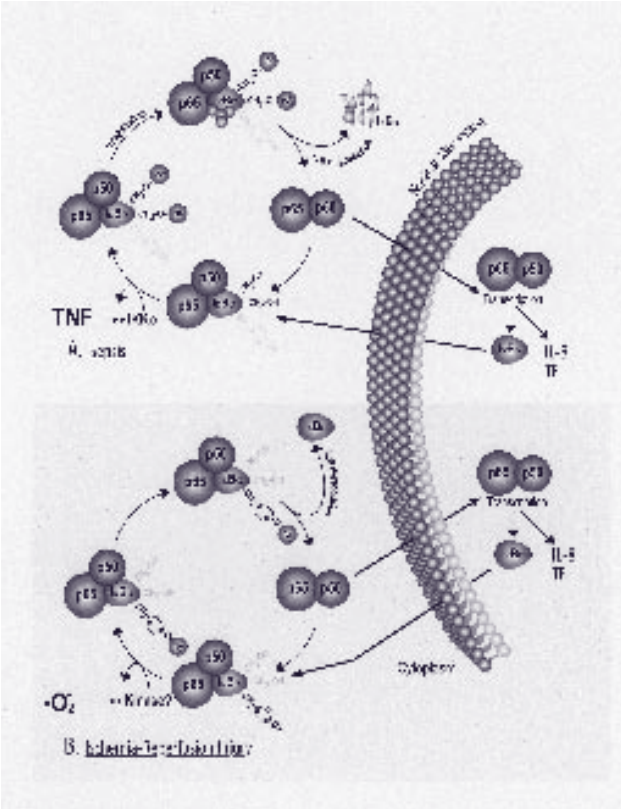

FIGURE 1: Parallel pathways of NF-κB activation. In (A), septic stimuli, such as TNF-α (or, IL-1 or LPS), activate transmembrane signaling pathways in responsive cells leading to the phosphorylation of a serine/threonine kinase, IKKα. This kinase in turn phosphorylates IκBα on serine residues 32 and 36. IκBα is an inhibitor of NF-κB, but upon phosphorylation undergoes degradation. Degradation of Ser-phosphorylated IκBα requires the addition of ubiquitin molecules that target proteins for degradation in proteasomes.

After IκBα degradation, NF-κB, consisting of two subunits, p65 and p50, then translocates to the nucleus, where it binds to specific DNA sequences in the 5'-flanking region of several genes that encode proteins mediating inflammatory reactions (for example, IL-8), coagulation (for example, tissue factor [TF]), and immunologic reactions. Of interest, NF-κB regulates transcription of new IκBα, which functions in a negative feedback loop to down-regulate this particular cellular response.

In (B), reactive oxygen intermediates activate signaling pathways yet to be determined that lead to tyrosine phosphorylation of IκBα. The tyrosine kinase (or kinases) responsible for this reaction has not yet been identified. Tyr-phosphorylated IκBα, in contrast to Ser-phosphorylated I IκBα, dissociates from NF-κB without degradation. NF-(B subsequently translocates to the nucleus to promote transcription of a similar set of genes as shown in (A), including IκBα.

This figure shows the molecular basis for the inflammatory reaction induced by ischemia-reperfusion injury, or any other injury in which reactive oxygen intermediates are formed. The figure also indicates that it may be possible to suppress an inflammatory reaction associated with ischemia-reperfusion injury that may be detrimental to the patient, without blocking the patient's ability to generate an inflammatory reaction when required to contain microbial invasion.

RELATED PUBLICATIONS

1. Griscavage, JM; Wilk, S; Ignarro, LJ. Inhibitors of the proteasome pathway interfere with induction of nitric oxide synthase in macrophages by blocking activation of transcription factor NF-κB. *Proc Natl Acad Sci USA,* 1996; 93:3308-3312.
2. Boyle EM, Jr; Kovacich JC; Canty TG, Jr; Morgan EN; Verrier ED; Pohlman TH. Inhibition of NF-κB nuclear localization prevents E-selectin expression and the systemic inflammatory response. *Circulation,* 1998; 98(19 Suppl):II282-8.
3. Boyle EM, Jr; Kovacich JC; Hèbert C; Canty TG; Jr., Morgan EN; Pohlman TH; Verrier ED. Inhibition of interleukin-8 blocks myocardial ischemia-reperfusion injury. *J Thor Cardio Surg,* 1998; 116:114-121.
4. Sato TT; Kovacich JC; Boyle EM, Jr; Haddix TL; Weintraub A; Pohlman TH. CD14-dependent activation of human endothelial cells by bacteroides fragilis outer membrane. *J Surg Res,* 1998; 74(2):103-11.
5. Kovacich JC; Boyle EM, Jr; Morgan EN; Canty TG, Jr; Farr AE; Caps M; Frank N; Pohlman TH; Verrier ED. Inhibition of the transcriptional activator protein nuclear factor κB prevents hemodynamic instability associated with the whole-body inflammatory response syndrome. *J Thor Cardio Surg,* 1999; 118(1):154-62.
6. Canty TG, Jr; Boyle EM, Jr; Farr AL; Morgan EN; Verrier ED; Pohlman TH. Oxidative stress-induced NF-κB nuclear localization without IκBα degradation: An alternative pathway for human endothelial cell activation. *Circulation,* 1999; 100(19 Suppl):II361-4.
7. Morgan EN; Boyle EM, Jr; Yun W; Griscavage-Ennis JM; Farr AL; Canty TG, Jr; Pohlman TH; Verrier ED. An essential role for NF-κB in the cardioadaptive response to ischemia. *Ann Thorac Surg,* 1999; 68:377-382.
8. Morgan EN; Boyle EM, Jr; Yun W; Kovacich BS; Canty TG, Jr; Chi E; Pohlman TH; Verrier ED. Platelet-activating factor acetylhydrolase prevents myocardial ischemia-reperfusion injury. *Circulation,* 1999; 100(19 Suppl):II365-8.
9. Pohlman TH; Harlan JM. Adaptive responses of the endothelium to stress. *J Surg Res,* 2000; 89(1):85-119.
10. Erlich JH; Boyle EM; Labriola J; Kovacich JC; Santucci RA; Fearns C; Morgan EN; Yun W; Luther T; Kojikawa O; Martin TR; Pohlman TH; Verrier ED; Mackman N. Inhibition of the tissue-factor-thrombin pathway limits infarct size after myocardial ischemica-reperfusion injury by reducing inflammation. *Am J Pathol,* 2000; 157(6): 1849-62.

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- Hypertonic Resuscitation for Blunt Trauma
- Prehospital Airway Management & Treatment for Traumatic Brain Injury
- Immunomodulation of the Alveolar Macrophage
- The Cytokine Profile of Burn Patients Receiving Plasmapheresis
- Rib Fracture Management
- Variations in the Care of Head Injured Patients
- The Use of Anabolic Steroids in the Chronically Ventilated Surgical Patient

AWARDS

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

Seattle Surgical Society Award

Shock Society
- Young Investigator Award, Finalist

FUNDING

American Association for the Surgery of Trauma
Research Scholarship

Brain Trauma Foundation

Clinical Nutrition Research Unit, University of Washington
- Pilot & Feasibility Award

Medic One Foundation

National Institutes of Health

Washington State Council of Firefighters

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, 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. Nicole Gibran seeks to explore the cytokine physiology associated with the response to plasmapheresis in the severely burned patient. Additional clinical trials address the pain management options for patients with rib fractures, the use of anabolic steroids during critical illness and variability in the care of patients with traumatic brain injury.

Hypertonic Resuscitation for Blunt Trauma

An evolving body of evidence suggests that resuscitation with hypertonic fluids following injury may improve outcome. The potential benefits of hypertonic resuscitation include more rapid restoration of tissue perfusion, preservation of cerebral perfusion while lowering intra-cranial 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 are embarking on a clinical trial to answer these questions. We will randomize patients to receive 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 is ARDS within 28 days. Secondary outcomes include mortality, infectious complications, multiple organ dysfunction, and long term neurologic function for patients with traumatic brain injury. We intend to enroll 400 patients over a 2.5 year time period. Without the support of the Seattle/King County Medic One program and Airlift Northwest, this study would not be possible. It is our hope that the lessons learned from this trial will support a multicenter trial of hypertonic resuscitation in the future.

The NIH has recently released a request for applications to establish a clinical research consortium to conduct multi-center trials for the acute resuscitation of both cardiac arrest and traumatic injury. They have committed $43 million over 5 years, toward funding seven regional clinical centers and a data coordinating center to conduct these studies. With the support of the Medic One Program, Dr. Peter Kudenchek (Principal Investigator) and myself and Dr. Tom Rea (Co-PIs) are submitting an application for Harborview Medical Center to be a regional clinical center within this consortium. If funded, this will provide the infrastructure and support to conduct a number of clinical trials with the large patient population available from multiple centers.

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.

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. In addition, in collaboration with Drs. Jerry Jurkovich and Fred Rivara, co-PIs on the National Study of Costs and Outcome for Trauma (NSCOT), we have been utilizing data collected from 14 geographic regions in the US to assess the variability in prehospital care provided to victims of traumatic injury. 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.

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 this in mind we have initiated a project supported by the Brain Trauma Foundation to investigate the relationship between prehospital interventions and outcome following TBI. We are currently in the process of linking prehospital data from the Seattle Fire Dept and King County EMS to trauma registry data from Harborview Medical Center to address these issues.

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 recently 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.

The Cytokine Profile of Burn Patients Receiving Plasmapheresis

Burn mortality has dramatically decreased over the past twenty years due to improvements in ICU management and better skin coverage. However, patients with large burns still face a high mortality during the first 48 hours of resuscitation. Severe burn injury is associated with a systemic inflammatory response which results in increased capillary permeability. As a result, these patients require a massive fluid resuscitation.

Several formulas have been developed to help estimate the fluid requirements during the first 24 hours, however, some patients, especially those with large, deep burns or inhalation injury, exceed these estimates and thus have evidence of ongoing inflammation which is not self-limiting. These patients have a higher mortality. Anecdotal experience suggests that these patients benefit from a plasma exchange which results in cessation of the capillary leak and decreased fluid requirements after therapy. In collaboration with Dr. Nicole Gibran, we are investigating the cytokine profile and degree of oxidative stress of these patients, both before and after plasmapheresis, to better define the mechanism responsible for the clinical improvement seen with this therapy.

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%.

One of the key strategies in the management of these patients involves the ability to obtain adequate pain control to optimize pulmonary status. To further investigate the issues surrounding pain management for these patients, we are currently enrolling patients into a prospective, randomized trial of thoracic epidural vs. intravenous narcotics. A preliminary analysis of this data suggests that the use of epidural analgesia decreased the subsequent rate of pneumonia and shortens the duration of mechanical ventilation. We hope to use this data to develop an optimal management strategy for these patients.

Variations in the Care of Head Injured Patients

In 1995 the Brain Trauma Foundation compiled a series of evidenced-based guidelines for the care of the head injured patient. That same year, a survey of the clinical management of the head injured patient, nationwide, revealed considerable variation in care.

In this study we sought to determine the current status of variations in care, since these guidelines have been widely distributed, with a particular focus on the controversy surrounding intracranial pressure monitoring. We have analyzed data from 34 academic trauma centers of the University HealthSystem Consortium regarding the management of patients with severe brain injury (GCS < 8). Centers were classified as "aggressive" if they placed intracranial pressure monitors in more than 50% of those patients meeting the Brain Trauma Foundation guidelines for monitoring. We have found that management at "aggressive" centers is associated with a significant reduction in mortality.

The Use of Anabolic Steroids in the Chronically Ventilated Surgical Patient

Multisystem traumatic injury results in a hypermetabolic state which leads to a stress-induced catabolism and the accelerated breakdown of protein stores. If this process continues unchecked it results in loss of lean body mass which can lead to muscle weakness and depression of the immune response, making the patient more susceptible to infectious complications. Weakness of the respiratory musculature can inhibit ventilator weaning and lack of protein leads to significant impairment in wound healing. These complications are observed with a loss of only 10-15% of lean body mass. A loss of lean body mass greater than 40% is usually fatal due to infectious complications.

Recognition of these concerns has led to an emphasis on early nutritional support including replacement of protein losses. Despite this approach, however, several studies have shown that aggressive nutritional support alone does not prevent substantial body protein loss during the catabolic state of severe illness. As a result, attention has turned to the development of adjuvant nutritional therapies which when administered, in conjunction with aggressive protein support, will help reverse the catabolic state. These include the use of recombinant human growth hormone and anabolic steroids.

Oxandrolone is an oral anabolic steroid with enhanced anabolic activity and minimal androgenic activity when compared to testosterone. In chronically malnourished patients including renal dialysis patients, COPD patients, and HIV patients, anabolic steroids, in combination with an enhanced protein diet, have been shown to significantly improve lean body mass and muscle strength. In burn patients, oxandrolone use has yielded improvements in lean body mass and strength training during the rehabilitation phase.

Based on these studies, oxandrolone has achieved FDA approval as an adjunctive therapy to promote weight gain after extensive surgery, chronic infections, and severe trauma. Despite this approval, this agent has not been well studied in the acute trauma population. We hypothesized that post-surgical or trauma patients who require a prolonged period of mechanical ventilation (>7 days) may benefit from oxandrolone therapy. We have recently completed a prospective, randomized controlled trial of oxandrolone in this population and although we demonstrated improvement in the nutritional parameters for patients given oxandrolone we were unable to identify any improvement in clinical outcome.

RELATED PUBLICATIONS

1. Bulger EM; Smith DG; Maier RV; Jurkovich GJ. Fat embolism syndrome: A ten year review. *Archives of Surgery,* 1997; 132:435-439.
2. Bulger EM; Helton WS; Clinton CM; Roque RP; Garcia I; Maier RV. Enteral vitamin E supplementation inhibits the cytokine response to endotoxin. *Archives of Surgery,* 1997; 132:1337-1341.
3. Bulger EM; Garcia I; Maier RV. Dithiocarbamates enhance tumor necrosis factor-production by rabbit alveolar macrophages, despite inhibition of NF-B. *Shock,* 1998; 9(6):397-405.
4. Bulger EM; Arneson MA; Mock CM; Jurkovich GJ. Rib Fractures in the elderly. *J Trauma*, 2000; 48(6):1040-1046.
5. Bulger EM; Arbabi S; Garcia I; Maier RV. The macrophage response to endotoxin requires platelet activating factor. *Shock*, 2002; 17(3):173-179.
6. Bulger EM; Garcia I; Maier RV. Intracellular antioxidant activity is necessary to modulate the macrophage response to endotoxin. *Shock*, 2002; 18(1):58-63.
7. Bulger EM; Nathens AB; Rivara FP; Moore M; MacKenzie EJ; Jurkovich GJ. Management of severe head injury: Institutional variations in care and effect on outcome. *Critical Care Medicine*, 2002; 30(8):1870-1876.
8. Bulger EM; Copass MK; Maier RV; Larsen J; Knowles J; Jurkovich GJ. An analysis of advanced prehospital airway management. *J Emerg Med,* 2002; 23:183.
9. Bulger EM; Gourlay D; Cuschieri J; Jelacic S; Staudenmeyer K; Garcia I; Maier RV. Platelet Activating Factor Acetylhydrolase inhibits alveolar macrophage activation, *in vivo. Shock*, 2003; 20:17-22.
10. Bulger EM; Garcia I; Maier RV. Induction of heme-oxygenase 1 inhibits the proinflammatory response of endothelial cells. *Surgery*, 2003; 134:146-52.

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Joseph Cuschieri, M.D.

- Toll-Mediated Signaling
- Priming of Mononuclear Cells
- Immunomodulation of Mononuclear Cells

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

Inflammation-induced tissue injury remains a leading cause of morbidity and mortality following trauma. This occurs through an exaggerated pro-inflammatory cell-mediated response to inflammatory stimuli such as endotoxin from the cell wall of gram-negative bacteria. Although many components of the innate immune response are involved to various degrees, the monocyte and tissue-fixed macrophage appears to be responsible for the orchestration of this exaggerated pro-inflammatory state. My laboratory efforts are to elucidate the cellular mechanisms involved in mononuclear cell priming in patients suffering from multiple organ dysfunction syndrome (MODS) and acute respiratory distress syndrome (ARDS) following trauma. If this were 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

Toll-mediated signaling is an important pathway involved in the activation of the monocyte and tissue-fixed macrophage. Activation of the macrophage by this pathway leads to sequential activation of a number of intracellular kinases and nuclear factors, eventually resulting in the production of inflammatory mediators. The production of these mediators is central to activation of the innate immune response, which in turn results in the remote tissue injury accompanying trauma.

Endotoxin or lipopolysaccharide (LPS) binds to lipopolysaccharide-binding protein (LBP) and thus is able to interact with the toll-4 receptor (TLR4) complex formed on lipid rafts, composed of the TLR4 receptor, CD14 and MD2. This in turn activates a series of intracellular signaling cascades and the eventual production of various anti- and pro-inflammatory cytokines and chemokines required for regulation of the innate immune response.

Although these pathways are critical for bacterial clearance, it is apparent that mononuclear cell priming results in an exaggerated pro-inflammatory response. It is, therefore, the overall goal of our laboratory to determine the factors activated following trauma that lead to priming of endotoxin-mediated signaling. To this end, we have focused on the calcium regulated kinase, CaMK II, and kinases assembled on lipid rafts that form "focal adhesion like complexes" that lead to cellular kinase pre-assembly and priming (Figure 1).

Priming of the Mononuclear Cells

Trauma is known to result in cellular reprogramming. This reprogramming has been termed *priming* and results in altered responses to subsequent stimuli. Although priming leads to cellular activation within mononuclear

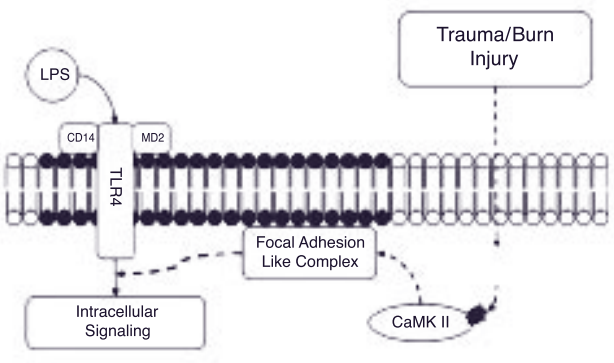

FIGURE 1: **Proposed mechanism of priming of TLR4-mediated signaling following trauma and burn injury.**

cells, pro-inflammatory mediator production does not occur. Rather, cellular priming initiates a process in which these mononuclear cells demonstrate an enhanced and exaggerated response to secondary inflammatory insults, such as endotoxin from the cell wall of gram-negative bacteria. This reprogrammed or primed response appears to be central to the development of inflammation-induced acute lung injury (ALI), ARDS, and MODS. Thus, our research efforts are focused on the cell types known to be primed following trauma, the peripheral blood monocyte and tissue-fixed alveolar macrophage.

mechanism in which intracellular calcium increases following PAF and oxidant stress remains poorly elucidated, but remains a major focus of our ongoing laboratory work. Despite clear evidence *in vitro* demonstrating this calcium flux during priming, identification of increased intracellular calcium *in vivo* has been highly variable following traumatic injury. These data, however, may be explained in part by the transient, rather than sustained, nature of the calcium flux.

Since the CaMK family of kinases are activated by calcium and regulate subsequent intracellular signaling, we have investigated *in vitro* the effect of priming factors

Trauma is known to result in cellular reprogramming. This reprogramming has been termed priming and results in altered responses to subsequent stimuli.

Although the cellular mechanism(s) underlying this response remain ill defined, factors such as platelet activating factor (PAF) and oxidants, which are released following trauma, result in cellular reprogramming and priming. Common to both factors *in vitro* are the initial mobilization of intracellular calcium and the activation of the calcium dependent protein kinase, CaMK II. As a result of this activation, "focal adhesion like complex" assembly occurs on lipid rafts leading to enhanced cellular responsiveness to insults such as endotoxin through the Toll-mediated signaling cascade.

Intracellular calcium is the critical intracellular messenger essential to mononuclear cell reprogramming. Although intracellular calcium levels are not substantially altered by inflammatory stimuli such as endotoxin, we have demonstrated that inhibition of calcium release does affect subsequent endotoxin-mediated cytokine release and mortality. This finding is intriguing since it suggests that calcium flux occurs within mononuclear cells and is important to optimal activation.

In addition to the potential role calcium plays in endotoxin activation, calcium appears to play a significant role in priming. This notion is based on the finding that there is an immediate but non-sustained increase in intracellular calcium within mononuclear cells induced by PAF and oxidant stress. Although these factors activate the macrophage by different mechanisms, we have demonstrated that resulting changes in intracellular calcium is essential to priming. The

on the activation of the CaMK family. Within mononuclear cells, the CaMK family is composed of CaMK II and CaMK IV. We have been able to demonstrate that reprogramming of mononuclear cells following exposure to priming factors occurs through CaMK II, while endotoxin signaling occurs through CaMK IV. Although the mechanism(s) by which this event leads to priming is unknown, it is our hypothesis that this occurs through enhanced spatial organization and the formation of focal adhesion like kinases on lipid rafts.

Lipid rafts are microdomains of the plasma membrane that contain high concentrations of cholesterol and glycosphingolipids. These microdomains appear to be small in size, but constitute a relatively large fraction of the plasma membrane. These cholesterol rich microdomains appear to function as platforms for signal transduction within a number of cell types. However, the role of lipid rafts within mononuclear cells during priming and endotoxin-mediated signaling was incompletely understood. As a result, a series of experiments were performed that demonstrated that members of the regulatory SRC kinase family were constitutively found on lipid rafts. The members consisted of SRC and HCK. However, as a result of a priming stimulus, SRC became activated leading to the lipid raft translocation and activation of cytoskeletal kinases consisting of Pyk2 and paxillin. Additionally, PKC-z, a kinase, necessary for endotoxin-mediated assembly of the TLR4 receptor complex, was found to be associated with lipid rafts during priming. Formation of this complex during

priming was found to be dependent of both cytoplasmic calcium and the activation of CaMK II.

In addition to the role lipid rafts played as platforms for the formation of focal adhesion like kinases, lipid rafts appear to be an important source of cytoplasmic calcium during oxidant induced priming. Treatment of cells with MbCD, a cholesterol-depleting agent, results in a marked attenuation of oxidant induced calcium flux and subsequent CaMK II activation. Therefore, this data provides the first *in vitro* evidence that lipid rafts play prominent roles in priming and could be modulated by cholesterol depletion. As a result of these findings, we hypothesize that similar assembly kinase assembly occurs, and that modulation of lipid raft cholesterol content could provide a unique therapeutic target to limit trauma induced priming.

Immunomodulation of Mononuclear Cells

These translational studies have been performed to determine the potential cellular mechanisms responsible for any clinically relevant changes found in inflammatory organ injury and nosocomial infection following trauma. Critically injured patients are known to have significant alterations in their responsiveness to subsequent infections as previously defined as priming. As a result of this *in vitro* data suggesting that the activation of CaMK II and the formation of focal adhesion like complexes on lipid rafts are essential to priming we have explored potential mechanisms that may disrupt these events. These potential mechanisms include cholesterol depletion, slow-channel calcium blockade, and hypertonic resuscitation. Thus, this project sets out to determine if similar activation and assembly of these kinases is not only predictive for the development of ARDS and MODS following trauma, but if modulation of these factors could result in attenuation of the development of these pro-inflammatory states.

RELATED PUBLICATIONS

1. Cuschieri J; Gourlay D; Bulger E; Garcia I; Jelacic S; Maier RV. Platelet Activating Factor (PAF) priming of inflammatory cell activity requires cellular adherence. *Surgery,* 2002; 132:157-166.
2. Cuschieri J; Gourlay D; Garcia I; Jelacic S; Maier RV. Hypertonic preconditioning inhibits macrophage responsiveness to endotoxin. *J Immuno,* 2002; 168(3):1389-96.
3. Cuschieri J; Gourlay D; Garcia I; Jelacic S; Maier RV. Slow channel calcium inhibition blocks pro-inflammatory gene signaling and reduces macrophage responsiveness. *J Traum,* 2002; 52(3):434-442.
4. Cuschieri J; Gourlay D; Garcia I; Jelacic S; Maier RV. Modulation of endotoxin-induced endothelial activity by microtubule depolymerization. *J Traum,* 2003; 54(1):104-112.
5. Cuschieri J; Gourlay D; Garcia I; Jelacic S; Maier RV. Modulation of sepsis induced endothelial function by calcium/calmodulin-dependent protein kinase. *Shock,* 2003; 20(2):176-182.
6. Cuschieri J; Gourlay D; Garcia I; Jelacic S; Maier RV. Stress-induced endothelial cell proinflammatory phenotypic differentiation requires stress fiber polymerization. *Shock,* 2003; 19(5):433-439.
7. Cuschieri J. Implications of lipid raft disintegration: Enhanced anti-inflammatory macrophage phenotype. *Surgery,* 2004; 136(2):169-75.
8. Cuschieri J; Bulmus V; Gourlay D; Garcia I; Stayton P; Maier RV. Modulation of macrophage responsiveness to LPS by IRAK-1 manipulation. *Shock,* 2004; 21(2):182-8.
9. Cuschieri J; Gourlay D; Garcia I; Jelacic S; Maier RV. Implications of proteasome inhibition: Enhanced anti-inflammatory macrophage function. *Cell Immuno,* 2004; 227(2):140-7.
10. Cuschieri J; Umanskiy K; Solomkin J. Protein kinase-zeta is essential toward endotoxin-induced macrophage activation. *J Surg Res,* 2004; 121(1):76-83.

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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

These findings are important because itching, which is mediated by neuropeptides, is a major complaint of patients with thermal injuries.

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 focused on intracellular signaling pathways that mediate substance P mediated changes to the endothelial cell.

Activated endothelial cells stimulate reinnervation of the injury site. We have defined this process to be a neuro-endothelial axis and believe that it may contribute to the pathophysiology of hypertrophic scar formation. Our latest effort has been to determine the mechanism by which substance P upregulates an inflammatory response. We have evidence that change in substance P-induced cell shape with the accompanying reorganization of the cytoskeleton may be an intermediary step. Most recently we have focused on the role of nitric oxide synthase and the EGFr as means of mediating substance P activity. These studies have been funded by the NIH.

RELATED PUBLICATIONS

1. Cole J; Tsou R; Wallace K, et al. Early gene expression profile of human skin to injury using high-density cDNA microarrays. *Wound Repair Regen,* 2001; 9(5):360-70.
2. Matsumura H; Engrav LH; Gibran NS, et al. Cones of skin occur where hypertrophic scar occurs. *Wound Repair Regen,* 2001; 9(4):269-77.
3. Tsou R; Cole JK; Nathens AB, et al. Analysis of hypertrophic and normal scar gene expression with cDNA microarrays. *J Burn Care Rehabil,* 2000; 21(6):541-50.
4. Spenny M; Maungman P; Olerud J, et al. Neutral endopeptidase inhibition improves wound repair in diabetic mice (abstract). *J Invest Dermatol,* 2001; 117:441.
5. Underwood RA; Gibran NS; Muffley LA, et al., Color subtractive-computer-assisted image analysis for quantification of cutaneous nerves in a diabetic mouse model. *J Histochem Cytochem,* 2001; 49(10):1285-91.
6. Antezana M; Sullivan S; Usui M, et al. Neutral endopeptidase activity is increased in the skin of subjects with diabetic ulcers. *J Invest Dermatol,* 2002; 119(6):1400-4.
7. Gibran NS; Jang YC; Isik FF, et al. Diminished neuropeptide levels contribute to the impaired cutaneous healing response associated with diabetes mellitus. *J Surg Res,* 2002; 108(1):122-8.
8. Spenny ML; Muangman P; Sullivan SR, et al. Neutral endopeptidase inhibition in diabetic wound repair. *Wound Repair Regen,* 2002; 10(5):295-301.
9. Muangman P; Spenny ML; Tamura RN, et al. Fatty acids and glucose increase neutral endopeptidase activity in human microvascular endothelial cells. *Shock,* 2003; 19(6):508-12.
10. Gibran NS; Tamura R; Tsou R, et al. Human dermal microvascular endothelial cells produce nerve growth factor: Implications for wound repair. *Shock,* 2003, 19(2):127-30.

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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 two 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 the three-year, $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 initial goal of the study was to determine whether functional outcomes of seriously injured trauma patients are better in Level I trauma centers than among patients treated in non-trauma centers, the costs of this care, and an assessment of the cost-effectiveness of care in trauma centers compared to non trauma centers.

We therefore very carefully selected the regions of the country and the hospitals from which we recruited patients. We obtained hospital discharge data from 21 states across the country, linked this to the Area Resource File, the American Hospital Association, and trauma center designation databases to identify 14 regions of the country that had high volume trauma and non-trauma centers. We then selected and recruited 80 hospitals representing 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.

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

sampling algorithm and instituted this to identify 8000 trauma patients for the study.

We have 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 them using the UB-92 standard format. To supplement the CDC funds for this project, we wrote a grant and were funded by the National Institute on Aging to obtain Medicare data on the study patients aged 65 and older. We obtained data from MarketScan to determine national data on professional fee costs for trauma. The products from this study will be remarkable. Just a few of them are:

- Determination 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. We have over 5000 trauma patients enrolled with one-year follow up data available, and are in the early stages of analysis and data publication.

Washington State Trauma Registry and Central Region CQI

As Washington State continues to evolve and expand its statewide trauma system, hospitals and pre-hospital agencies that are designated patient care providers are required to submit information to a statewide trauma registry. Central Region (conforming geographically to King County) is one of eight designated trauma and emergency medical regions in the state, and has been collecting such information for the past four 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, located primarily 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, pediat-

rics, 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. Prospective cohort studies suggest that between 13-35% of injured children and adolescents ages 7-21 may go on to develop PTSD over the course of the year after injury . Other posttraumatic behavioral and emotional disturbances such as depression and substance abuse are also common occurrences. Furthermore, between 10-20% of the parents of injured youth may also develop posttraumatic distress.

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. The Surgeon General in a recent report on child mental health has recommended that primary care providers be educated regarding psychiatric disorders in children and adolescents. There are, however, no investigations that have systematically described the detection and treatment of posttraumatic behavioral and emotional disturbances in adolescents by surgical inpatient or pediatric outpatient providers. The Centers for Disease Control's Acute Care Division has made the development of screening and intervention protocols for patients with psychiatric and substance abuse disorders in the acute care setting a tier 1 research priority.

This investigation is a prospective cohort study of posttraumatic stress disorder (PTSD), substance use, functional impairment, and health service utilization among traumatically injured adolescents and their parents. The investigation will prospectively follow 110 randomly selected hospitalized injured adolescents ages 12-18. Adolescents and their parents will be interviewed during the inpatient surgical hospitalization and again at 1, 4 and 12 months after the injury. We hypothesize that adolescents with PTSD will demonstrate clinically and statistically significant functional impairments even after adjusting for other injury and demographic characteristics. 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.

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.

RELATED PUBLICATIONS

1. Arbabi S; Jurkovich GJ; Rivara FP; Demarest G; Moore M; Maier RV. Patient outcomes in academic medical centers: Influence of fellowship programs and in-house on-call attending surgeon. *Arch.Surg,* 2003; 200(138):47-51.
2. MacKenzie EJ; Hoyt DB; Sacra JC; Jurkovich GJ; Carlini AR; Teitelbaum SD; Teter H, Jr. National inventory of hospital trauma centers. *JAMA,* 2003; 289:1515-1522.
3. Segui-Gomez M; Chang D; Paidas C; Jurkovich GJ; MacKenzie E; Rivara F. Pediatric trauma care: An overview of pediatric trauma systems and their practices in 18 US States. *J Ped Surg,* 2003; 38(8):1162-69.
4. Nathens AB; Jurkovich GJ; MacKenzie EJ; Rivara FP. A resource-based assessment of trauma care in the United States. Accepted, *J Trauma,* 1.03.
5. Donovan DM; Dunn CW; Rivara FP; Jurkovich GJ; Ries RR; Gentilello LM. Comparison of trauma center patients self-reports and proxy reports on the alcohol use identification test (AUDIT). *J Trauma*, 2004; 56(4):873-882.
6. Nathens AB; Jurkovich GJ; MacKenzie EJ; Rivara FP. A resource-based assessment of trauma care in the United States. *J Trauma*, 2004; 56(1):173-178.
7. Acierno S; Jurkovich GJ; Nathens A. Is pediatric trauma still a surgical disease? Patterns of urgent operative intervention in the injured child. *J Trauma*, 2004; 56(5):960-66.
8. Arbabi S; Jurkovich GJ; Wahl WL; Franklin GA; Hemmila MR; Taheri PA; Maier RV. A comparison of prehospital and hospital data in trauma patients. *J Trauma*, 2004; 56(5):1029-32.
9. Mosenthal AC; Livingston DH; Lavery RF; Knudson MM; Lee S; Morabito D; Manley G; Nathens A; Jurkovich GJ; Hoyt DB; Coimbra R. The effect of age on functional outcome in mild traumatic brain injury: 6-month report of a prospective multicenter trial. *J Trauma,* 2004; 56(5):1042-48.
10. Jurkovich GJ; Rivara FP; Johansen JM; Maier RV. CDC injury research agenda: identification of acute care research topics of interest to the CDC-NCIPC. *J Trauma,* 2004; 56(5):1166-70.
11. Zatzick DF; Roy-Byrne P; Russo J; Rivara FP; Droesch R; Wagner A; Dunn C; Jurkovich GJ; Uehara E; Katon W. A randomized effectiveness trial of stepped collaborative care for acutely injured trauma survivors. *Arch Gen Psychiatry*, 2004; 61(5):498-506.
12. Cornejo C; Vaezy S; Jurkovich GJ; Paun M; Sharar SR; Martin RW. High intensity ultrasound treatment of blunt abdominal solid organ injury: An animal model. *J Trauma*, 2004; 57(1):152-156.
13. Bulger EM; Jurkovich GJ; Farver CL; Klotz P; Maier RV. Oxandrolone does not improve outcome of ventilator dependent surgical patients. (*American Surgical,* April, 2004) *Ann Surg*, 2004; 240(3):472-80.
14. Zatzick D; Jurkovich GJ; Russo J; Roy-Byrne P; Katon W; Wagner A; Dunn C; Wisner D; Grossman D; Rivara F. Posttraumatic distress, substance intoxication, and recurrent trauma across Level 1 trauma center sites. *J Trauma*, 2004; 57(2):360-366.

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Ronald V. Maier, M.D.

- Harborview Injury Prevention & Research Center
- Clinical Trials in the Surgical Intensive Care Unit
- Modulation of the Excessive Inflammatory Response to Biomaterials
- Elucidation and Modulation of the Trauma-Related Macrophage Inflammatory Response to Prevent ARDS, MOFS and Death in the Severely Injured and Septic Patient

AWARDS

National Institutes of Health
- NIGMS Institutional NRSA

Shock Society
- Young Investigator of the Year

American Trauma Society
- William S. Stone Award

FUNDING

Centers for Disease Control
- National Center for Injury Prevention and Control

Engineering Research Center (ERC)

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

National Science Foundation

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

The Effect of Interfacility Transfer on Outcome in an Urban Trauma System

Triage decisions are made by emergency medical care providers to distinguish patients that require care at a fully equipped trauma center from those whose injuries are less extensive. Transporting all trauma patients to regional trauma centers is inefficient; however, the bypass of near, non-designated hospitals in deference to regional trauma centers decreases mortality in the severely injured. One approach to improving efficacy is to allow the initial assessment of selected patients at lower-level (Level III/IV) designated centers. We are currently evaluating whether patients initially assessed at these lower level centers and then transferred to a Level I facility are adversely affected by delays to the definitive care center. Using retrospective cohort evaluations of patients being initially assessed at a Level III or IV trauma center prior to transport, the outcomes investigated are mortality, length of stay and hospital charges.

Preliminary evaluation shows that interfacility transfers in a mature, urban trauma system do not appear to have a negative impact on clinical outcome. However, transfer patients appear to use significantly greater resources as measured by hospital charges. This effect appears to be due to the recognition by referring hospitals of the increased severity and resource requirements of those patients needing transfer to the definitive care center.

Relationship Between Trauma Center Volume and Outcome

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

Current projects include identifying the risk factors for injury while developing new techniques for the application of epidemiology in the field of trauma research.

Clinical Trials in the Surgical Intensive Care Unit at Harborview Medical Center

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 may exacerbate or perpetuate lung injury and, in contrast, the use of lower tidal volumes during ventilation may reduce or prevent 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 than 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 of 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. Preliminary 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 one-month study of new 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.

An HMC study demonstrated that supplementing 3 grams/day of Vitamin C and 3 grams/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 plasma and tissue Vitamin C and E concentrations are significantly low in patients admitted to the intensive care units at HMC and that routine supplementation of Vitamin C and E will elevate levels. Elevated levels of these two potent antioxidants may well protect against oxidant-induced injury in these severely injured and stressed patients, and avoid the diffuse insult predisposing to ARDS and other organ dysfunction, and also to 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 international units of Vitamin E, divided over three doses per day, started at the time of admission. Otherwise, care was standard and the populations were followed to determine the incidence of ARDS, duration in the ICU, mortality and infectious complications. In addition, we studied BAL samples for evidence of oxidant injury and cytokine production. The results show that the treatment with anti-oxidant supplementation on admission to the surgical ICU produced a 50% reduction in the 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 have demonstrated that adherence by the macrophage to various surfaces primes the macrophage for activation. Subsequent steps in the inflammatory response lead to multi-nucleated giant cell formation and subsequent capsule formation, secretion of extracellular matrix, vascular budding, and fibroblast proliferation with thick collagen deposition. Prevention of the pro-inflammatory phenotype may

well equate with prevention of foreign body reaction. In current studies, we are investigating coating of biomaterials with various molecules. These include osteopontin and various anti-inflammatory agents, such as anti-oxidants, including Vitamin E and components of the extracellular matrix, such as hyaluronic acid derivatives, to test the subsequent response of adherent macrophages to inflammatory stimuli, such as endotoxin.

In addition, we are studying materials of various selected pore sizes to minimize cell spreading and to test environmental 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.

In additional experiments, we will test the effect of end products of macrophage activation and modulation of macrophage activation. Using a chorioallantoic membrane fractal dimension and grid intersection assay, we monitor angiogenesis as a crucial component of both normal and abnormal wound healing and incorporation, or "healing," of biomaterials. 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 p38. In addition, we are investigating signaling processes activated through formation of focal adhesion complexes induced by adherence of the monocyte/macrophage as critical to the host inflammatory cell response. A major focus is on the ability of anti-oxidants, such as vitamin E, or cytoskeletal 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.

RELATED PUBLICATIONS

1. Hassantash SA; Afrakhteh M; Maier RV. Causalgia: A meta-analysis of the literature. *Arch Surg,* 2003; 138:1226-31.
2. Maier RV. Commentary: Seattle's Harborview Medical Center, 1877-2003. *Arch Surg,* 2004; 139:14-5.
3. Cuschieri J; Bulmus V; Gourlay D; Garcia I; Stayton P; Maier RV. Modulation of macrophage responsiveness to Lipopolysaccharide by IRAK-1 manipulation. *Shock,* 2004; 21(2):182-88.
4. "US Trauma Center Crisis: Lost in the Scramble for Terror Resources." Board of Directors, National Foundation for Trauma Care. May 2004.
5. Rosengart MR; Maier RV. Electrolyte Disorders. In" Cameron JL (Ed.) *Current Surgical Therapy,* 8th Edition. Philadelphia, PA: Elsevier Mosby, pp. 1152-9, 2004.
6. Arbabi S; Jurkovich GJ; Wahl WL; Franklin GA; Hemmila MR; Taheri PA; Maier RV. A comparison of pre-hospital and hospital data. *J Trauma,* 2004; 56:1029-32.
7. Jurkovich GJ; Maier RV; Rivara F; Johansen J. CDC Injury Research Agenda: Identification of acute care research topics of interest to the CDC-NCIPC. *J Trauma,* 2004; 56:1166-70.
8. Bowen SL; Garcia IA; Jelacic S; Maier RV. LPS stimulation of monocytes causes decreased long term adhesion and increased cytokine production. *JURIBE,* 2004; 4(1):19-24.

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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

Ruth B. Sauber Distinguished Medical Alumni Lectureship
- Brown University Medical School Commencement

FUNDING

Atlantic Philanthropies

Medic One Foundation

National Highway Traffic Safety Administration (NHTSA)

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 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 found 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 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.

Co-workers from several countries and I 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 UW Department of Surgery has served as my home base. The advice and expertise of colleagues in the Department

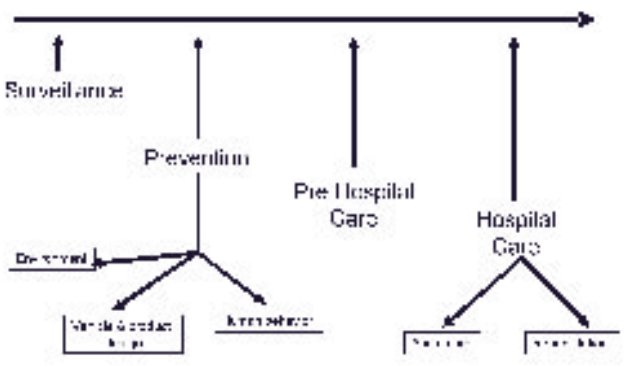

FIGURE 1. Spectrum of Injury Control

and at the Harborview Injury Prevention and Research Center has 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 statistic 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 has 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 has 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, pre-hospital 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 lays.

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, 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

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.

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 has been used by the National Road Safety Committee in its educational campaigns and has 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.

Pre-hospital 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, which 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 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.

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.

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 I 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 participated 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 I, 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. Research is currently underway to assess how the material taught in this course has been used by course participants and whether the course has improved the process of trauma care in rural hospitals.

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

	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.

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 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 the Hanoi area in Vietnam and nationwide in Ghana and Mexico.

2. The State Government of Gujarat, India, along with the WHO Gujarat sub-country office co-sponsored a meeting in April 2003 to adapt the Guidelines to local circumstances and to develop implementation strategies.

3. The Mexican Association for the Medicine and Surgery of Trauma has officially endorsed the Guidelines for use in Mexico. The national Ministry of Health and the Mexico WHO country office sponsored a meeting to adapt the Guidelines to local circumstances and to develop implementation strategies. This meeting included representatives of several health care systems in Mexico and leaders of eight professional organizations that deal with trauma (e.g. general surgeons, orthopedists, intensivists, and emergency physicians, among others). Most participants felt that this was the highest-level attention given to trauma care in the country ever.

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, in September 2003, 4 faculty members from UW, along with a representative of the CDC in Atlanta, conducted a two-week training course on injury control for 24 participants from throughout Vietnam. These individuals came from 20 different institutions in 12 different locations from throughout Vietnam. They represented the leadership of injury control efforts in Vietnam. This course also provided considerably publicity and stature to injury control efforts in that country. Similar collaborative work is planned over the next 3 years.

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 is fed back to NHTSA to help with the development of safety regulations and to the automobile manufacturers to help with safety engineering design.

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.

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 currently 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 over-estimated 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 under-estimated 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 pick up 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 causing. 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.

RELATED PUBLICATIONS

1. Mock CN. Trauma system development in ghana. Invited editorial commentary. *Ghana Medical Journal,* Volume 35, 2001; pp. 1-3.
2. London JA*; Mock CN; Quansah RE; Abantanga FA; Boateng KA. Using mortuary statistics in the development of a trauma surveillance system in Ghana. *Bulletin of WHO,* 2002; 80: 357-364.
3. Mock CN; Tiska M; Adu-Ampofo M; Boakye G. Improvements in prehospital trauma care in an African country with no formal emergency medical services. *Journal of Trauma,* 2002; 53: 90-97.
4. Nirula R*; Mock CN; Kaufman R; Rivara FP; Grossman D. Correlation of head injury to vehicle contact points using Crash Injury Research and Engineering Network (CIREN) data. *Accident Analysis and Prevention,* 2003; 35:201-210.
5. Mock C; Arreola-Risa C; Quansah R. Strengthening the care of injured patients in developing countries: A case study of Ghana and Mexico. *Injury Control and Safety Promotion,* 2003; 10:45-51.
6. Mock CN; Boland E; Acheampong F; Adjei S. Long-term injury related disability in Ghana. *Disabil Rehabil.* 2003; 25(13):732-741.
7. Mock CN; Arreola-Risa C; Trevino-Perez R; Almazan-Saavedra V; Zozaya-Paz J; Gonzalez-Solis J; Simpson K; Rodriguez-Romo L; Hernandez-Torre M. Injury prevention counseling to improve safety practices by parents in Mexico. *Bulletin of the World Health Organization,* 2003; 81(8):591-598.
8. Acierno S*; Kaufman R; Rivara F; Grossman D; Mock C. Vehicle mismatch: Injury patterns and severity. *Accident Analysis and Prevention,* 2004; 36:761-772.
9. Mock C; Lormand JD; Goosen J; Joshipura M; Peden M. *Guidelines for Essential Trauma Care.* Geneva: World Health Organization, 2004.
10. Mock C; Quansah R; Krishnan R; Arreola Risa C; Rivara F. Strengthening the prevention and treatment of injuries worldwide. *The Lancet,* 2004; 363:2172-2179.

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

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- Effect of Organized Systems of Trauma Care on Motor-Vehicle Crash Mortality
- Relationship Between Trauma Center Volume and Outcomes
- A Resource-Based Assessment of Trauma Care in the United States
- The Effect of Interfacility Transfer on Outcome in an Urban Trauma System
- Randomized, Prospective Trial of Antioxidant Supplementation in Critically Ill Surgical Patients

AWARDS

American College of Surgeons, Committee on Trauma Region X
- Best clinical paper, 1999
- Best clinical paper, 2000

FUNDING

Centers for Disease Control and Prevention
National Institute of Health, National Heart, Lung and Blood Institute
National Institute of Sciences, National Institute of General Medical Sciences
Surgical Infection Society

Effect of Organized Systems of Trauma Care on Motor-Vehicle Crash Mortality

During the period 1976 through 1995, 22 states developed organized systems of trauma care with the intent of reducing injury-related mortality. Despite calls for wider national implementation, the effectiveness of an integrated approach to trauma care at a regional or state level remains unproven.

This study was designed to assess the impact of trauma system implementation on mortality due to motor-vehicle crashes across the United States between 1979 and 1995. The primary endpoint was the rate of death of front-seat occupants of passenger vehicles aged 15 through 74. Crash rates were compared before and after trauma system implementation in states with organized systems of trauma care. After controlling for secular trends in crash mortality and implementation of traffic safety laws (restraint laws, maximum posted speed limits, laws designed to limit drinking and driving), trauma systems had a significant impact on deaths due to traffic crashes. Eight years following initial trauma system implementation, mortality due to traffic crashes began to decline; about 15 years following trauma system implementation, mortality was reduced by 9% (95% CI, 2-15%) (Figure 1).

By contrast, legislative initiatives geared toward enforcing restraint laws result in an early reduction in crash mortality of 13% (95% CI, 9-16%) while relaxation of state speed limits increased mortality by 6% (95% CI, 3-9%). These data suggest that implementation of an organized system of trauma care reduces deaths due to motor-vehicle crashes. The effect takes several years to manifest, a finding that is consistent with the maturation and development of trauma triage protocols, inter-hospital transfer agreements, organization of trauma centers, and ongoing quality assurance.

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 study evaluated the effect of trauma center volume in two distinct cohorts of patients admitted to one of 31 academic trauma centers across the country. These cohorts included patients with isolated penetrating abdominal trauma and patients with a combination of lower extremity long bone fractures and closed head injury.

The greatest benefits to these high-risk patients occurred when they were cared for in centers with greater than 650-700 major trauma admissions per annum.

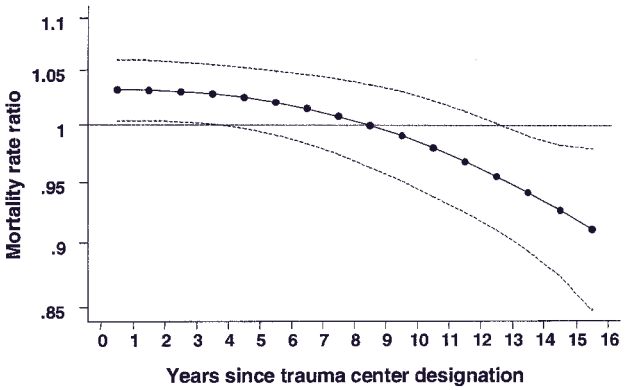

FIGURE 1: Adjusted mortality rate ratio attributable to a trauma system as a function of time from first trauma center designation. The dashed lines represent upper and lower bands of the 95% confidence interval.

The relationship between trauma center volume and outcome depended on the severity of illness. For example, there was no association between volume and outcome in penetrating abdominal trauma patients without shock or in blunt multisystem trauma patients without coma. However, in patients with shock or coma there was a marked reduction in the risk of death (Figure 2).

Similar advantages were also evident when hospital lengths of stay were assessed. The greatest benefits to these high-risk patients occurred when they were cared for in centers with greater than 650-700 major (ISS>15) trauma admissions per annum.

In summary, these data provide further support emphasizing the importance of regionalization of trauma care, and provide guidelines for estimating the number of trauma centers required per unit population. Trauma system care should ensure triage of the most severely injured patients to relatively few dedicated trauma centers. Consideration should be given to consolidation of urban trauma programs to maximize institutional volume. Further work is needed to identify differences in the process of care, the impact of individual surgeon volume, the role of fellowship training programs, trauma research activities and other factors that may be contributing to the observed outcome benefit at high volume trauma centers.

A Resource-Based Assessment of Trauma Care in the United States

To date, in some systems, implementation of regional trauma care has occurred through the incorporation of existing resources into system infrastructure. This method of system design may not be ideal in that an excess of designated trauma centers may prevent the accrual of volume and experience necessary to achieve optimal outcomes for the most critically injured patients, while in other areas their scarcity may increase the risk of overwhelming the resources that do exist. Additionally, in many regions the lack of accurate population-based data on injury incidence, severity, and resource utilization leaves the process of trauma systems planning up to little more than conjecture.

To better understand the resources needed for trauma care, we performed a population-based analysis of trauma discharge rates and resource utilization using a sample of 18 states, representative of all four major geographic regions of the country and comprising 62% of the United States population. We also examined how these trauma care needs were currently being met in these 18 states.

There were 523,870 trauma-related discharges in the 18 states upon which this analysis is based, representing a population of 127,116,384 persons, or 62% of the 1997 US population age >16 years. The discharge rate for all trauma was 412.1 per 100 000 person-years. At

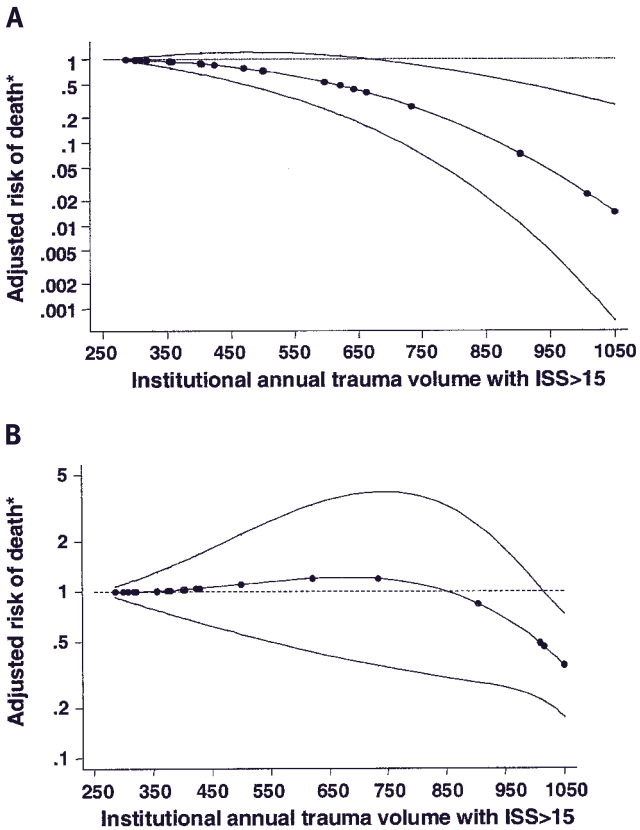

FIGURE 2: Relationship of the risk of death to trauma center volume in patients admitted in shock with penetrating abdominal injury (A) and in patients with coma and multisystem blunt trauma (B). Lines without • represent 95% confidence bands for the estimated odds ratio.
**Adjusted risk of death compared to the lowest volume institution.*

only 44.0 per 100,000 person-years, the incidence of major trauma was tenfold lower.

There were 2,317 institutions caring for patients with a principal diagnosis of trauma across these 18 states. Only 12.3% of these institutions were designated as a Level 1 or 2 trauma center. The large majority of institutions caring for trauma patients were not designated. Taken together, these non-designated centers cared for 56% of all trauma patients and over a third of patients with major trauma.

The institutions caring for trauma patients had a wide range of experience as assessed by the annual volume of major trauma patients discharged from these centers. Three-quarters of centers attended to 50 or fewer major trauma patients per annum. Centers in the lowest volume stratum cared for over half of all patients and 31.7% of patients with major trauma. The highest volume centers (>350 discharges per annum) comprised only 0.6% of all hospitals, but discharged a far higher proportion of all trauma patients and an even greater proportion of all major trauma patients.

To assess the availability and variability of resources available to care for the major trauma patient, we evaluated the number of Level 1 or 2 centers by state population and state size (land-area). Across all states, the median number of trauma centers designated to care for these patients was 1.7 per million population. There was a 20-fold variability across states from a low of 0.3 (AZ) to a high of 6.6 (IL) per million population. There was a 150-fold variability when the number of centers was evaluated on a land-area basis, with a median of 21.3 trauma centers per 100,000 square miles and a range of 0.9 (AZ) to 134.8 (NJ).

The variation in availability of designated centers affected the trauma experience of individual institutions. The mean trauma center volume (major trauma discharges per annum) dropped off significantly as the density of trauma centers per million population increased, from 348 discharges per annum in the least dense state to only 52 discharges per annum in the most dense (Figure 3). The difference in experience between the lowest and highest volume Level 1 and 2 trauma centers within each state ranged from 0 to 775 discharges per annum (mean+SD, 337(191)). A broad range of experience was evident in both low and high-density states.

This multi-state analysis depicting the trauma experience of over 60% of the US population provides an accurate representation of the status of trauma care in this country. This contemporary snapshot provides a reasonable estimate of anticipated resource requirements for regions without systems and for regions whose systems are in varying stages of evolution. The variability in trauma centers per capita (20-fold variation in rates) or per land-area (150-fold variation in rates) suggests that true regionalization of trauma care through the process of trauma center designation and trauma system design has not occurred. While some excess capacity may be desirable given the inevitable geographic or other barriers (e.g. traffic) to access, this degree of variation suggests that many states may have too many trauma centers, while others may have too few. Additionally, in spite of the relative abundance of designated trauma centers in some states, more than 1/3 of patients with major trauma are treated at non-designated centers or centers with very low volume. This analysis adds to our understanding of the current state of trauma care in the United States and serves to highlight the challenges facing lead agencies in their attempt to truly regionalize trauma care.

The Effect of Interfacility Transfer on Outcome in an Urban Trauma System

In urban environments where transport times are relatively short, there is little doubt that there is a survival benefit associated with the bypass of nearer non-designated centers in deference to the care provided at regional designated Level I centers. However, many hospitals in large metropolitan areas participate in inclusive trauma systems where they have been verified and designated at a lower level. The designation of these lower level centers serves a dual purpose. First, their presence limits the burden to regional Level I/II centers that would otherwise be inundated with large numbers

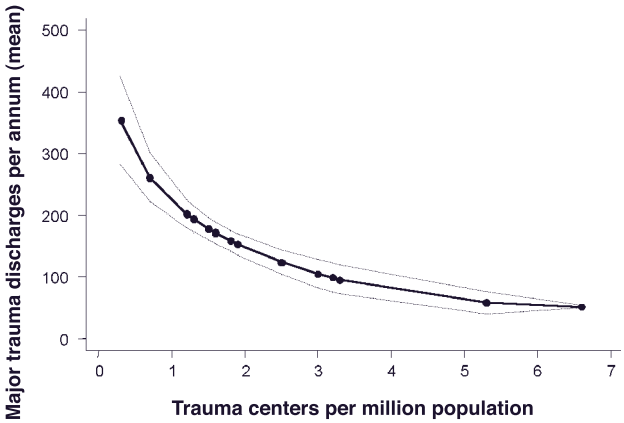

FIGURE 3: Mean major trauma discharges per annum for designated Level I and Level II trauma centers as a function of trauma center density. Point estimates (solid line) are demonstrated along with their 95% confidence bands (hatched line).

of patients with minimal injuries. These centers also serve to keep these individuals in their community while preserving a high standard of trauma care. The purpose of this retrospective cohort study was to determine if injured patients transported directly from the field to a Level I trauma center, have better outcomes than those who are transported from the field to one of 8 Level III or IV trauma centers in King County, and then transferred to the Level I center.

We demonstrated that among 10,030 persons injured in King County, 5,310 patients were first assessed at the Level III or IV centers, and 281 (5%) were ultimately transferred to the Level I facility. The remaining 4,439 patients were transported directly to the Level I. The relatively few patients undergoing inter-hospital transfer within the county is simply a reflection of the effectiveness of regional pre-hospital triage protocols. The mean time at the referring center prior to transfer was 3.1 hrs with a range of 11 min to 12.6 hrs.

The adjusted relative risk of death in transfer patients compared to those transported directly to the Level I facility suggested that there were no differences in outcome [RR of death: 1.05 (95% CI 0.61-1.80)]. Similarly, there was no difference in the length of stay in these two cohorts after adjusting for differences in injury severity. By contrast, the crude hospital charges incurred at the Level I facility were almost 40% greater (adjusted charges were 10% greater) in patients who were first assessed at a Level III or IV facility then transferred to the Level I center.

The initial assessment by a community trauma center (i.e. Level III/IV centers in this analysis) provides a second tier of triage such that regional facilities are not burdened by an excess of minimally injured patients who might best be cared for closer to home and who do not require the resources of a Level I center. However, the transfer patient appears to consume an inordinate amount of resources either because of their patterns of injury, or due to complications arising from the delays to definitive care.

Whatever the reason, these data suggest that the transfer patient represents a unique population of trauma patients characterized by intense resource utilization. This second triage tier provides an opportunity for Level III/IV centers to participate in the trauma system and prevents taxing the higher level centers with a large number of less severely injured patients. However, this study demonstrates that patients who required secondary triage and transfer to the regional facility expend significant resources, and therefore might have significant financial implications to regional facilities already burdened by large numbers of indigent patients. These economic realities must be recognized in trauma system design and call for the development of disproportionate reimbursement strategies to support regional referral centers to ensure their continued survival.

Randomized, Prospective Trial of Antioxidant Supplementation in Critically Ill Surgical Patients

Oxidant-mediated tissue injury induced by activated neutrophils or following ischemia-reperfusion injury is thought to be one of the key mechanisms leading to Acute Respiratory Distress Syndrome (ARDS) and multiple organ failure. This project was designed to evaluate the effectiveness of antioxidant supplementation in critically ill surgical patients admitted to the intensive care unit.

Patients (n=595) were randomized to receive either standard care or administration of alpha-tocopherol (3,000 units daily) and ascorbic acid (3 gms daily) for the duration of their ICU stay. The primary clinical endpoint was pulmonary morbidity, a composite of ARDS and pneumonia. A subset of these patients underwent bronchoalveolar lavage to assess the impact of antioxidant supplementation on markers of alveolar injury and inflammation.

The relative risk of pulmonary morbidity was 0.81 (95% CI: 0.60-1.1) in patients receiving antioxidant supplementation. Additionally, multiple organ failure was significantly less likely to occur in patients receiving antioxidants than in patients receiving standard care, with a relative risk of 0.43 (95% CI: 0.19-0.96). Patients randomized to antioxidant supplementation also had a shorter duration of mechanical ventilation and length of ICU stay. Bronchaolveolar lavage fluid F2αisoprostane levels, a measure of oxidative injury were lower in the patients receiving antioxidant supplementation. Associated with lower levels of oxidative stress in the antioxidant group was a tendency for an attenuated alveolar inflammatory response as demonstrated by a lower alveolar white blood cell and protein concentration as well as reduced concentrations of TNF-α, IL-1β, and IL-6.

This large, randomized prospective trial in a cohort of critically ill surgical patients suggests benefit from the routine early, prophylactic administration of α-tocopherol and ascorbate. The lack of adverse effects coupled with the minimal expense support that this combination is a reasonable therapeutic intervention in critically ill surgical patients.

RELATED PUBLICATIONS

1. Nathens AB; Jurkovich GJ; Cummings P; Rivara FP; Maier RV. The effect of organized systems of trauma care on motor-vehicle crash mortality. *JAMA,* 2000; 283:1990-1994.
2. Nathens AB; Jurkovich GJ; Rivara FP; Maier RV. Effectiveness of state trauma systems in reducing injury-related mortality: A national evaluation. *J Trauma,* 2000; 48:25-30.
3. Nathens AB; Jurkovich GJ; Maier RV; Grossman DC; MacKenzie EJ; Moore M; Rivara FP. Relationship between trauma center volume and outcomes. *JAMA,* 2001 Mar 7; 285(9):1164-71.
4. Nathens AB; Maier RV; Copass MK; Jurkovich GJ. Payer status: the unspoken triage criterion. *J Trauma,* 2001 May; 50(5):776-83.
5. Nathens AB; Neff MJ; Jurkovich GJ; Klotz P; Farver K; Ruzinski JT; Radella F; Garcia I; Maier RV. Randomized, prospective trial of antioxidant supplementation in critically ill surgical patients. *Ann Surg,* 2002 Dec; 236(6):814-22.
6. Arbabi S; Jurkovich GJ; Rivara FP; Nathens AB; Moore M; Demarest GB; Maier RV. Patient outcomes in academic medical centers: influence of fellowship programs and in-house on-call attending surgeon. *Arch Surg,* 2003 Jan; 138(1):47-51.
7. Nathens AB; Rivara FP; Jurkovich GJ; Maier RV; Johansen JM; Thompson DC. Management of the injured patient: Identification of research topics for systematic review using the Delphi technique. *J Trauma,* 2003 Mar; 54(3):595-601.
8. Nathens AB; Jurkovich GJ; MacKenze EJ; Rivara FP. A resource-based assessment of trauma care in the United States. *J Trauma,* 2004; 56:173.
9. Nathens AB; Brunet F; Maier RV. Development of trauma systems and impact on outcomes following injury. *Lancet,* 2004; 363:1794-1801.
10. Nathens AB; Maier RV; Brundage SI; Jurkovich GJ; Grossman DC. The effect of interfacility transfer on outcome in an urban trauma system. *J Trauma,* 2003; 55:444-449.

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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. In this report, we will demonstrate our findings regarding (1) the effect of genetic variations in the form of single nucleotide polymorphisms (SNPs) on cytokine production by whole blood leukocytes exposed to bacterial endotoxin, (2) relationships between SNPs and the severity of acute appendicitis and the associated cytokine production and, (3) the role of SNPs as markers for the development of severe sepsis and septic shock after trauma.

Polymorphisms in the Interleukin-6 (IL-6) Gene Promoter Affect Cytokine Production

Interleukin-6 (IL-6) plays a key role in the acute-phase response to infection and injury. A single nucleotide polymorphism guanine to cytosine substitution at -174 in the promoter region of the IL-6 gene has been associated with a lower circulating IL-6 concentration in healthy humans who carried the C-allele. However, there is controversy whether this polymorphism is associated with the IL-6 response to inflammatory stimuli. Thus, we sought to determine whether carriage of the less common C-allele was associated with a reduced production of IL-6 after stimulus using an *ex vivo* model.

We hypothesized that polymorphisms in genes involved in these defenses would be associated with clinical outcomes in local infections caused by the body's commensal microbial flora.

In this series of experiments, diluted whole blood from healthy volunteers was stimulated with bacterial endotoxin (lipopolysaccharide; LPS) for 24 hours and IL-6 production was measured in the supernatant fluid.

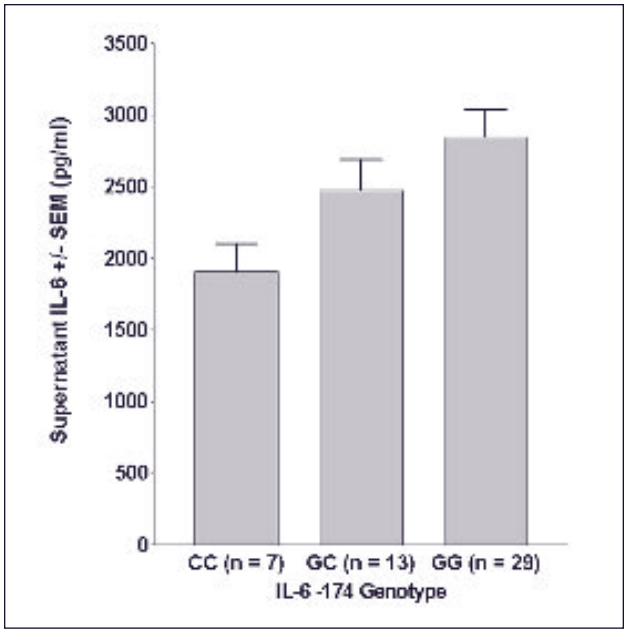


FIGURE 1

Individuals were genotyped for 3 polymorphisms in the promoter region of the IL-6 gene; each of which is considered to possibly influence IL-6 gene transcription. We observed that the polymorphism at the -174 position was associated with IL-6 cytokine production. The number of C-alleles that were carried was associated with a progressive decrease in the IL-6 response to LPS (results shown in figure 1).

While the *ex vivo* model that we use is meant to reflect the complex *in vivo* situation and avoid difficulties attendant with leukocyte isolation, it is not clear how these observations might translate into various, complex clinical circumstances. For example, a reduced capacity to produce IL-6 (or other cytokines) may be detrimental under some circumstances and beneficial in others. We therefore recognize that all our studies and observations made here must be re-examined in a range of clinical scenarios.

Evaluation of Single Nucleotide Polymorphisms in Five Innate Immunity Genes and the Severity of Acute Appendicitis

Innate immunity is the body's first line system for recognizing and destroying invading microbes. In the study summarized here, we hypothesized that polymorphisms in genes involved in these defenses would be associated with clinical outcomes in local infections caused by the body's commensal microbial flora. We tested this hypothesis by studying patients with acute infection-inflammation of the vermiform appendix, a localized infection that requires prompt surgical extirpation of the appendix to avoid complications. We looked for associations between the severity of acute appendicitis and allelic polymorphisms located within genes involved in recognizing bacterial molecules [CD14 (-159 C→T); TLR4 (896 A→G)] and in mounting the inflammatory response [IL-6 (-174 G→C), TNF-_, (-308 G→A), IL-1β (-31 C→T)].

We studied 134 patients with acute appendicitis. A total of 91 patients had uncomplicated disease and 43 had complicated appendicitis; which refers to the presence of microscopic evidence of gangrene, necrosis or perforation of the appendix. Polymorphisms in the IL-6 and TNF-α promoters were associated with a greater risk for complicated appendicitis; polymorphisms in the other genes were not. The results of our multivariate analysis are shown in the table below.

Interestingly, a "high-risk" genotype, defined by the presence of at least one A-allele at TNF-α -308 and GG-homozygosity at the IL-6 -174 position was associated with a 50% risk for complicated appendicitis. In contrast, a "low-risk" genotype, defined by the absence of the TNF-α -308 A-allele and at least one C-allele at IL-6 -174 position was associated with a 12% risk of complicated appendicitis. So, it seems that the severity of a common surgical disease, may in part be determined by genetic differences in at least two cytokine genes.

An additional advantage of appendicitis as a model of human inflammation is that it allows sampling of regional (peritoneal) and systemic (blood) compartments for cytokine measurements. We observed that both plasma and peritoneal fluid IL-6 concentrations were higher in the GG-homozygotes that the C-allele carriers (see data shown in figures 2 and 3). These observations are consistent with the findings in our *ex vivo* model of LPS stimulation of leukocytes.

RISK FACTOR	ODDS RATIO	95% CONFIDENCE INTERVAL	P-VALUE
Symptom duration	1.02	1.01–1.03	0.03
IL-6 -174 GG-homozygotes	5.2	1.5–18.5	0.01
TNF-α-308 A-allele	2.7	0.9–7.9	0.07

TABLE 1: Results of Multivariate Analysis

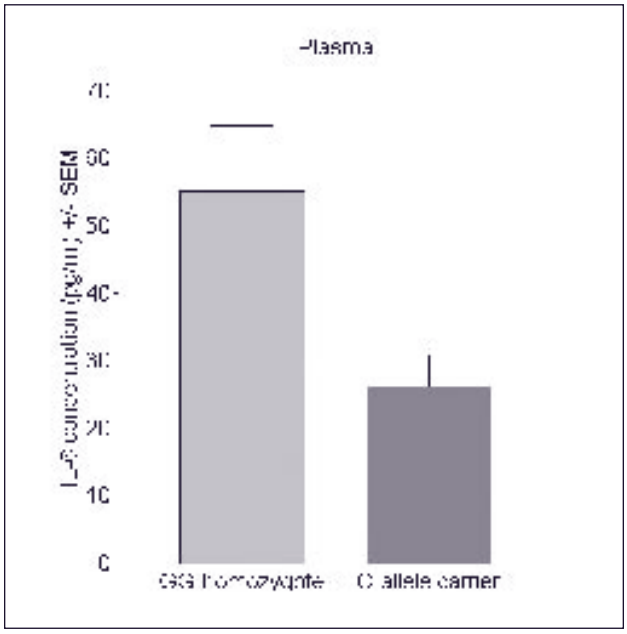


FIGURE 2: Plasma Concentrations

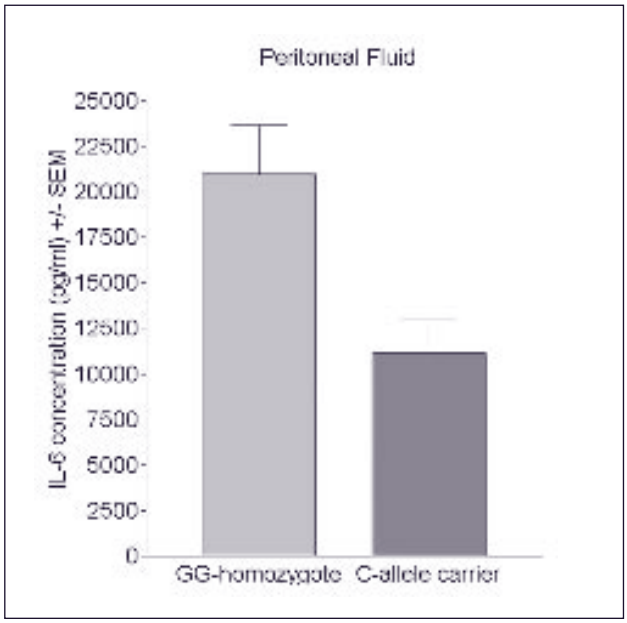


FIGURE 3: Peritoneal Concentrations

The G→A Single Nucleotide Polymorphism at the -308 Position in the TNF-α Promoter Increases the Risk for Severe Sepsis after Trauma

We have also conducted similar genetic evaluation in patients with severe injury, who are at risk for sepsis complicated by organ failure (severe sepsis) and septic shock — which are referred to here as "complicated sepsis." We have initially focused our efforts on the TNF-α promoter, in which a number of SNPs may affect transcriptional regulation of TNF-α production. A SNP at the -308 position (G→A substitution) was shown to alter TNF-α gene transcription in a transfection model. Carriage of the less common A-allele has been associated with increased risk of acquiring several infectious and inflammatory diseases and with adverse clinical outcomes in a number of clinical settings.

For example, increased risk for renal transplant rejection, death from meningococcal sepsis, and mortality from septic shock has been reported in A-allele carriers. However, others have found that the A-allele does not increase transcription rates in vitro and that carriage of this allele is not associated with increased risk for severe sepsis. Although less extensively studied than the -308 polymorphism, other TNF-α promoter SNPs may also be important in transcriptional regulation of TNF-α production. In the case of the -376 G→A transition, Knight and colleagues determined that carriage of the uncommon A-allele was associated with a higher incidence of cerebral malaria and that basal gene expression was significantly greater in monocytes transfected with the A-allele than those transfected with the G-allele.

In the study summarized here, we asked whether these naturally occurring genetic differences in the TNF-α promoter are markers for the development of complicated sepsis in severely injured patients. We hypothesized that carriage of the uncommon (A) allele at the -238, -308 or -376 position in the TNF-α promoter is associated with an increased risk for complicated sepsis.

Of the three SNPs in the TNF-α promoter that we studied, only the G→A transition at the -308 position was more frequent in patients with severe sepsis. The single patient who was homozygous for the A-allele

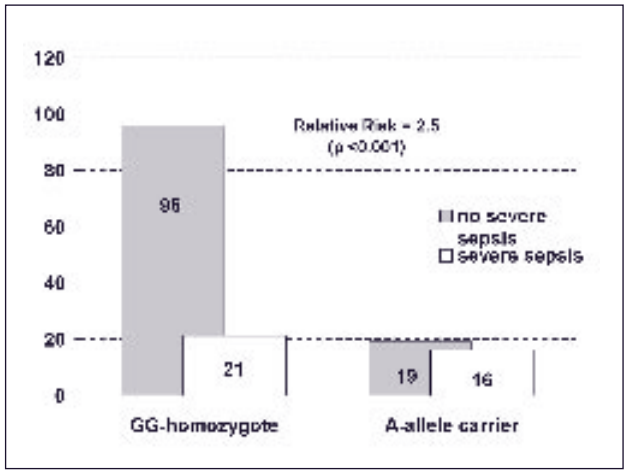


FIGURE 4: Sepsis Risk Factor

developed severe sepsis, as did 15 of the 34 (44%) heterozygotes. Therefore, carriage of the A-allele was associated with a 46% risk (16/35) of severe sepsis (See Figure 4). This is in contrast to the 18% risk (21/117) in patients homozygous for the G-allele (wild type).

The unadjusted relative risk for complicated sepsis associated with the A-allele was 2.5 (95% CI = 1.5–4.3). Carriage of the A-allele at either the -238 or -376 position was uncommon and was not associated with complicated sepsis. What could be considered as traditional clinical risk factors for complicated sepsis (age ≥ 55 years, early post-injury shock as indicated by an arterial base deficit of ≥ 6 meq/L from 6–24 hours after injury) were present to a similar extent in the -308 A-allele carriers and GG-homozygotes. After adjusting for these risk factors, carriage of the TNF-α -308 A-allele was associated with a 4.6-fold increased risk for severe sepsis or septic shock.

Summary

We have identified associations between gene polymorphisms and severe sepsis and septic shock after trauma. It will, however, be necessary to generate DNA databanks, linked to reliable detailed clinical data, in considerably larger cohorts of injury victims; this is one aim of our ongoing work. Appendicitis represents an interesting and potentially useful clinical model of inflammation. We will continue to study these patients in detail. Finally, *ex vivo* experimentation may provide data to identify important SNPs that can then be evaluated in more complex human models.

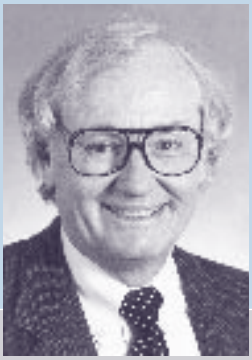
Our congruent observations regarding IL-6 production in patients with acute appendicitis and in healthy control subjects, suggest that this polymorphism does affect IL-6 production and potentially also the severity of local inflammation. Our research program will continue to address multiple SNPs for their effect on cytokine release and for the severity of acute inflammation. We will examine which SNPs are related to the most severe manifestations of sepsis — sepsis with organ failure and septic shock.

RELATED PUBLICATIONS

1. O'Keefe GE; Hunt J; Purdue G. A comprehensive evaluation of risk factors for mortality after burn trauma and the identification of gender-dependant differences in outcome. *Journal of the American College of Surgeons,* 2001; 192(2):153-160.
2. Cumming J; Hunt J; Purdue GF; O'Keefe GE. Objective estimates of the incidence and consequences of multiple organ dysfunction, organ failure and sepsis after burn trauma. *Journal of Trauma: Injury, Infection and Critical Care,* 2001; 50(2):510-515.
3. O'Keefe GE; Hybki DL; Munford RS. The G → A Single Nucleotide Polymorphism (SNP) at the -308 position in the TNF-α promoter increases the risk for severe sepsis after trauma. *Journal of Trauma: Injury, Infection and Critical Care,* 2002; 52:817-826.
4. Dahl B; Schiødt FV; Ott P; Wians F; Lee W; O'Keefe GE. Plasma concentration of Gc-globulin is associated with organ dysfunction and sepsis after injury. *Critical Care Medicine,* 2003; 31(1):152-156.
5. Fitzwater J; Purdue GF; Hunt JL; O'Keefe GE. The risk factors and time course of sepsis and organ dysfunction after burn trauma. *Journal of Trauma: Injury Infection and Critical Care,* 2003; 54:959-966.
6. Rivera-Chavez FA; Munford RS; Wheeler HT; Lindberg G; O'Keefe GE. The cytokine response to acute inflammation of the vermiform appendix. *Annals Surgery,* 2003; 237(3):408-416.
7. Peters DL; Barber RC; Flood E; Garner HR; O'Keefe GE. Genotyping methods and bias in studies of the TNF -308 SNP and bacterial sepsis. *Critical Care Medicine,* 2003; 31:1691-1696.
8. Barber RC; O'Keefe GE. Characterization of a SNP in the lipopolysaccharide binding protein locus and its association with sepsis. *American Journal of Respiratory and Critical Care Medicine,* 2003; 167:1316-1320.
9. Rothenbach P; Dahl B; Schwartz J; O'Keefe GE; Yamamoto M; Lee W; Horton J; Yin H; Turnage R. Recombinant plasma gelsolin infusion attenuates burn-induced pulmonary microvascular dysfunction. *Journal of Applied Physiology.* Published online May 2, 2003.
10. Kitchens RL; Thompson P; Munford RS; O'Keefe GE. Critical illness accelerates lipoprotein-mediated neutralization of bacterial lipopolysaccharide in both hypocholesterolemic and hypertriglyceridemic human serum. *Journal of Lipid Research* published electronically ahead of print in August 2003.
11. Rivera-Chavez FA; Peters-Hybki DL; Barber RC; Wheeler H; O'Keefe GE. Interleukin-6 promoter haplotypes and IL-6 cytokine responses. *Shock* 2003; 20(3):218-223.

Robert K. Winn, Ph.D.

- Ischemia-Reperfusion Injury
- Sepsis (Overwhelming Infection)
- Adult Respiratory Distress Syndrome



FUNDING
National Institutes of Health

The focus of our research is to better understand the cellular and molecular events that lead to organ dysfunction and organ failure in severely ill patients. Two causes of organ failure are severe infection or sepsis, and ischemia followed by reperfusion (i.e., following severe traumatic injury, hemorrhagic shock, myocardial infarction, stroke, organ transplantation, etc.). We are interested in understanding cellular and molecular events in those patients who suffer from these severe pathologic events.

It has been known for some time that these types of injuries result in an inflammatory response and that this response might contribute to organ dysfunction. More recently it has been observed that a portion of the cellular injury is the result of programmed cell death or apoptosis and this has led to increased research into this area. Our effort is directed toward understanding both inflammation and apoptosis as well as possible interactions or overlap of these cellular events. We are currently investigating the following areas:

Ischemia-Reperfusion Injury

We and others have shown that ischemia followed by reperfusion can result in a neutrophil (PMN) induced reperfusion injury, and that a portion of the injury can be ameliorated with monoclonal antibodies that recognize leukocyte adhesion molecules. These pre-clinical experiments led to a number of clinical trials using anti-adhesion therapy in an attempt to reduce injury following myocardial infarction, stroke and hemorrhagic shock following traumatic injury. Unfortunately these trials did not demonstrate the expected protective effect of anti-adhesion therapy in spite of the very strong pre-clinical data.

In an attempt to understand why the clinical trials failed, we examined the ischemic times in the experimental setting designed to model myocardial and cerebral ischemia-reperfusion and found them to be generally less than 1.5 hours with the majority of times being between 30 min and 1 hour. Since the time to treatment for myocardial infarction and stroke is considerable longer than1 to 1.5 hour, we questioned whether the duration of ischemia in the experimental setting was too short. In preliminary experiments, when skeletal muscle was made ischemic for 60 minutes or less, the injury was partially reduced by blocking a major PMN leukocyte adhesion molecule (CD18).

Additionally, preliminary results showed that the injury could no longer be reduced by blocking adhesion molecules if the ischemic time was increased to 90 minutes. The CD18 independent portion of the injury was associated with DNA strand breaks consistent with apoptosis but the earlier CD18 dependent portion of the injury had a reduced component of apoptosis. The extended ischemia that is independent of adhesion blockade was reduced by blocking apoptosis or by blocking the complement system, suggesting a mechanism for therapy. We are continuing investigations of these two potential mechanisms of injury with the hope of defining potential therapeutic agents.

Sepsis (Overwhelming Infection)

Sepsis or septic shock is a potentially lethal consequence of bacterial infection and is a significant complication in victims of traumatic injury. It is one of the leading causes of death in patients requiring intensive care. There are multiple bacterial products implicated as pathogenic molecules including bacterial lipoproteins, lipopolysaccharide, (LPS), lipoteichoic acid, peptidoglycans, cell wall products, etc. Sepsis was shown to activate the intrinsic cell "suicide" program leading to apoptosis of multiple cell types. Insights into the molecular basis of cellular activation/apoptosis in response to sepsis are under intense investigation in the hope of finding new approaches to therapy.

Signaling by bacterial products occurs through the recently described Toll-like receptors (TLRs) on the surface of cells. Intracellular pathways leading to activa-

tion proceed along similar pathways for TLR-2 and TLR-4 (the two receptors shown to respond to bacterial products). However, the apoptotic pathways have received less attention. We are examining sepsis-induced apoptosis and a novel activation pathway in cell culture as well as the effect of gene alterations that are expected to lead to decreased apoptosis in monocytes, lymphocytes and endothelial cells *in vivo.* These gene alteration experiments will help to identify cells that are critical in responding to invading organisms associated with sepsis.

Recent clinical investigations aimed at reducing the death rate in patients suffering from sepsis have been disappointing as a number of potential therapeutics have not shown any benefit in this disease. We hope to better define the process leading to organ dysfunction and organ failure in patients suffering from sepsis or sepsis syndrome. An understanding of the cellular and molecular events of this process is expected to provide information that will allow the development of therapeutics for the treatment of this devastating syndrome.

suggests that their survival in tissues can be regulated to some extent by local factors including adhesion, cytokines, and chemokines. Neutrophil persistence in the lung may be an important determinant of acute lung injury since the longer neutrophils are present in the lung tissue, the greater the possibility that they may provoke lung injury by release of proteases and reactive oxygen intermediates. While the resolution of acute lung inflammation ultimately depends upon the clearance of neutrophils, the mechanism(s) of clearance may also affect the duration and severity of lung inflammation.

Necrosis of neutrophils releases toxic products extracellularly, thereby perpetuating the inflammatory response and further damaging tissue. In contrast, apoptosis of neutrophils with their monocyte-derived macrophages may terminate the inflammatory reaction. We are investigating the role of neutrophil apoptosis in determining the severity and duration of acute lung inflammation. We hypothesize that factors promoting

Necrosis of neutrophils releases toxic products extracellularly, thereby perpetuating the inflammatory response and further damaging tissue.

Adult Respiratory Distress Syndrome

Adult respiratory distress syndrome (ARDS) is a major complication in patients who have suffered severe traumatic injuries and in patients with sepsis or sepsis syndrome. Patients suffering from ARDS have increased pulmonary edema resulting from endothelial and epithelial permeability that is thought to result from hyperactive leukocytes. Considerable progress has been made in inflammation; however, the factors regulating the fate of transmigrated neutrophils *in vivo* are not as well understood.

Neutrophils are thought to have an inherently limited lifespan in tissue (i.e., they undergo a constitutive programmed cell death), but recent evidence

neutrophil apoptosis and engulfment by macrophages will lead to more rapid resolution of lung inflammation, while those that prevent apoptosis will prolong the inflammatory response and increase the probability of acute lung injury.

It is hoped that these studies will yield new information on the molecular mechanisms involved in the resolution of acute lung inflammation and perhaps yield new approaches to the therapy of ARDS. In these studies we are particularly interested in understanding the role of neutrophil apoptosis in septic lung injury. The goal of these investigations is to identify potential molecular mechanisms that can provide protection from the development of ARDS.

RELATED PUBLICATIONS

1. Matute-Bello G; Winn RK; Jonas M; Chi EY; Martin TR; Liles WC. Fas (CD95) induces alveolar epithelial cell apoptosis *in vivo*: Implications for acute pulmonary inflammation. *Am J Pathol*, 2001; 158:153-161.
2. Becker K; Kindrick D; Relton J; Harlan J; Winn R. Antibody to the alpha4 integrin decreases infarct size in transient focal cerebral ischemia in rats. *Stroke*, 2001; 32:206-211.
3. Bannerman DD; Tupper JC; Ricketts WA; Bennett CF; Winn RK; Harlan JM. A constitutive cytoprotective pathway protects endothelial cells from lipopolysaccharide-induced apoptosis. *J Biol Chem*, 2001; 276:14924-14932.
4. Bannerman DD; Erwert RD; Winn RK; Harlan JM. TIRAP mediates endotoxin-induced NF-κB activation and apoptosis in endothelial cells. *Biochem Biophys Res Commun*, 2002; 295:157-162.
5. Bannerman DD; Tupper JC; Erwert RD; Winn RK; Harlan JM. Divergence of bacterial lipopolysaccharide pro-apoptotic signaling downstream of IRAK-1. *J Biol Chem*, 2002; 277:8048-8053.
6. Bannerman DD; Tupper JC; Kelly JD; Winn RK; Harlan JM. The Fas-associated death domain protein suppresses activation of NF-kappa B by LPS and IL-1 beta. *J Clin Invest*, 2002; 109:419-425.
7. Li X; Liu L; Tupper JC; Bannerman DD; Winn RK; Sebti SM; Hamilton AD; Harlan JM. Inhibition of protein geranylgeranylation and RhoA/RhoA kinase pathway induces apoptosis in human endothelial cells. *J Biol Chem*, 2002; 277:15309-15316.
8. Bannerman DD; Erwert RD; Winn RK; Harlan JM. TIRAP mediates endotoxin-induced NF-kappaB activation and apoptosis in endothelial cells. *Biochem Biophys Res Commun*, 2002; 295:157-162.
9. Erwert RD; Winn RK; Harlan JM; Bannerman DD. Shiga-like toxin inhibition of FLIP expression sensitizes endothelial cells to bacterial lipopolysaccharide-induced apoptosis. *J Biol,* 2002.
10. Iwata A; Harlan JM; Vedder NB; WinnRK. The caspase inhibitor z-VAD is more effective than CD18 adhesion blockade in reducing muscle ischemia-reperfusion injury: Implication for clinical trials. *Blood,* 2002; 100:2077-2080.

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PEDIATRIC SURGERY

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JOHN WALDHAUSEN, M.D.

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 from 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.

The biology of an individual neuroblastoma tumor varies, with advanced stage tumors manifesting very different molecular and genetic features from those with early stage disease.

RELATED PUBLICATIONS

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

OTHER CO-INVESTIGATORS

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• Surgical Treatment Review Improves Children's Healing Process

Pediatric surgery is in general a very clinically oriented field. At CHRMC most of our research activity has been oriented toward what we do in the operating room and on the hospital ward. Our goal is 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 for 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 and 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 adults and pediatrics based on its appeal to the patient or consumer rather than by any rigorous trial. In one attempt to correct this problem, several years ago an attempt was made at a national level with NIH funding to examine the efficacy of MIS in the pediatric oncology patient. The questions asked dealt with safety and accuracy in obtaining tissue for histologic diagnosis. Though this study never came to fruition at a national collaborative level, we examined our own results at CHRMC to determine whether both laparoscopy and thoracoscopy were useful, accurate ways to obtain tissue. We examined patient outcome and treatment of disease based on decisions made from tissues obtained by MIS techniques. MIS was found to be an excellent, accurate method with no adverse or inappropriate clinical decisions made based on the tissues obtained.

Many MIS procedures take special skills and advanced training in order to become proficient. Often these techniques are espoused to the surgical community with little regard as to what experience is needed to be able to reasonably perform the operation. In adult surgery there is generally ample opportunity to obtain MIS experience because of the frequency with which some procedures, such as cholecystectomy, are performed. 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

Other studies we have conducted have answered simple questions about every day clinical situations such as whether a period of water seal is needed to safely remove chest tubes in children.

experienced may be limited. One of our studies helped to establish a learning curve with laparoscopic splenectomy so that other surgeons learning how to do the operation might know what to expect in the early stages of learning the procedure.

Other studies we have conducted have answered simple questions about every day clinical situations such as whether a period of water seal is needed to safely remove chest tubes in children. We have evaluated our use of ERCP in children when symptoms or studies suggested common duct gallstones and tried to discern useful protocols or pathways to help determine when ERCP should be performed preoperatively rather than after cholecystectomy and intraoperative cholangiogram. Our goal was to avoid unnecessary ERCP and the general anesthetic needed to perform it in children. Ongoing collaborative efforts with colleagues in other divisions such as orthopedics have enabled us to expand the use of minimally invasive surgery for conditions such as pediatric scoliosis by doing thoracoscopic exposures as well as thoracoscopic anterior fusion and instrumentation. A joint effort with orthopedics and pulmonary medicine has allowed us to be part of a national collaborative study in 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 Jeunes syndrome or thoracic insufficiency from other congenital problems such as scoliosis, fused ribs or congenital diaphragmatic hernia.

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

RELATED PUBLICATIONS

1. Waldhausen JHT; Shaw DW; Hall DG; Sawin RS. Needle localization for thoracoscopic resection of small pulmonary nodules in children. *J Pediatr Surg,* 1997; 32:1624-1625.
2. Waldhausen JHT; Horgan S; Pellegrini C. Laparoscopic Heller myotomy and Dor fundoplication for achalasia in children. *Pediatric Endosurgery and Innovative Techniques,* 1999; 3:23-27.
3. Waldhausen JHT; Tapper D; Sawin RS. Minimally invasive surgery and clinical decision making for pediatric malignancy. *Surg Endosc,* 2000; 14:250-253.
4. Waldhausen JHT; Graham DD; Tapper D. Routine intraoperative cholangiography during laparoscopic cholecystectomy minimizes unneccessary endoscopic retrograde cholangiopancreatography in children. *J Pediatr Surg,* 2001; 36:881-884.
5. Cusick RA; Waldhausen JHT. The learning curve associated with pediatric laparoscopic splenectomy. *Am J Surg,* 2001; 181:393-397.
6. Kim SS; Waldhausen JHT; Weidner BCS; Grady R; Mitchell M; Sawin RS. Perineal reconstruction of female conjoined twins. *J Pediatr Surg,* 2002; 37:1740-1743.
7. Waldhausen JHT; Cusick RA; Graham DD; Pittinger TP; Sawin RS. Removal of chest tubes in children without water seal: a randomized prospective study. *J Am Coll Surg,* 2002; 194:411-415.
8. Kim SS; Lee SL; Waldhausen JHT; Ledbetter DL. Laparoscopic splenopexy for wandering spleen syndrome. *Pediatric Endosurgery and Innovative Techniques,* 2003; 7:237-241.
9. Schwarz A; Waldhausen JHT. Laparoscopic removal of giant ovarian cysts in children. *Pediatric Endosurgery and Innovative Techniques,* 2003; 7:309-313.
10. Weidner BCS; Waldhausen JHT. Swenson revisited, primary transanal pull-through for hirschsprung's disease. *J Pediatr Surg,* 2003; 38:1208-1211.

PLASTIC AND RECONSTRUCTIVE SURGERY

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- Hypertrophic Scarring
- UW Burn Injury Rehabilitation Model System

FUNDING
International Association of Firefighters
National Institute on Disability and Rehabilitation Research
• Department of Education
Washington State Council of Firefighters

Laboratory Topics

We have now 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 that we 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). In 2002, we received funding from the National Institute on Disability and Rehabilitation Research, Department of Education (NIDRR) and the Washington State Council of Fire Fighters Burn Foundation (WSCFFBF) to conduct further studies of these with three specific aims, which are summarized below. Drs. Zhu, Gibran and Isik are significantly involved in these activities.

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

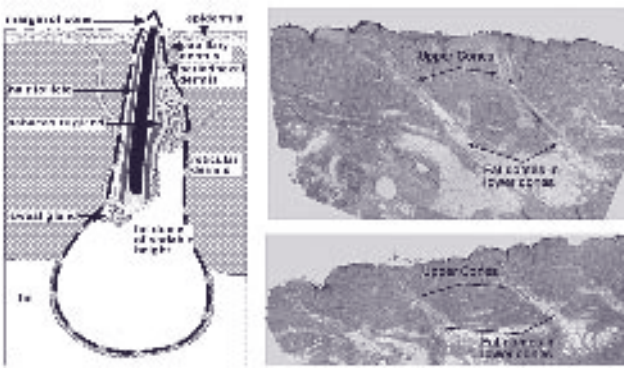

FIGURE 1: The Cones of Skin, Schematic and *in vivo*

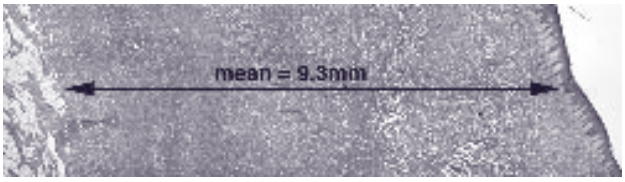

FIGURE 2: Thick Scar in Female, Red Duroc Pig

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 hyper-trophic 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 must be confirmed. We will do this by comparing the clinical appearance; histology; immunohistochemical localization of decorin, versican, transforming growth factor β1, and insulin-like growth factor one; in situ hybridization of insulin-like growth factor one; rtPCR of transforming growth factor β1 and insulin-like growth factor one, counts of mast cells and collagen nodules; and measurement of collagen fibril diameter in scar of the female, red Duroc pig to that reported in the literature for human, hypertrophic scar and to our library of human scar tissues.

The first several portions of this project have been completed and published verifying that the scar of the female, Duroc is quite similar to human hypertrophic scar tissue.3-6

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.

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

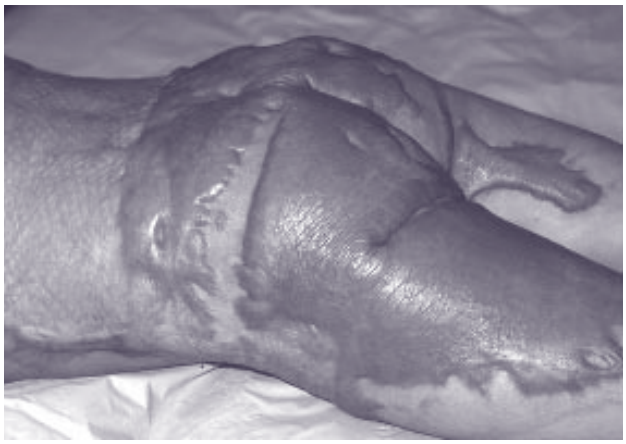

FIGURE 3: Hypertrophic Scar

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 mircoarray 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 have now

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.

piloted this project on two Duroc samples and found 128 significantly expressed genes to be up or down regulated four fold in the cones of deep Duroc scar. We will now proceed to do the full experiment on three female, Duroc pigs. Three Yorkshire pigs will be used as controls.

Model System Topics

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 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.

• *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 be 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.

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

Selected Clinical Topics Since Last Year's Report

Abstract 1: Impairment rating is regularly reported for trauma and other conditions but rarely for burns. The purposes of this study were 1) to report impairment

collected prospectively at our burn center, 2) to relate this impairment to measures of psychosocial and functional outcome, and 3) to compare these data to similar data from another burn center to verify that rating impairment is standardized and that the impairments are similar. We studied 139 patients from the University of Washington burn center and 100 patients from the University of Texas Southwestern burn center. The whole person impairment at the University of Washington was 17% and this correlated with total body surface area burned and days off work. It did not correlate with Brief Symptom Inventory, Functional Independence Measure, Short-Form 36-Item Health Survey, Satisfaction With Life Scale, and the Community Integration Questionnaire. Whole person impairment at UT Southwestern was similar at 19%. Several components of the impairment rating, however, differed at the two institutions. To minimize this variation, we recommend 1) use the skin impairment definitions of the 5th Edition of the Guides to the Evaluation of Permanent Impairment, and 2) include sensory impairment in healed burns and skin grafts in the skin impairment.

Abstract 2: Baxter described the use of 4cc/kg/%TBSA as a guideline for fluid resuscitation after burns. However, recent studies have shown that, at the present time, patients generally receive greater than the "Baxter" formula. Pruitt has called this phenomenon "fluid creep," and it has the potential for significant consequences including abdominal and extremity compartment syndromes and severe pulmonary insults. The purpose of this paper is to determine if this supra-Baxter resuscitation is a new phenomenon. We performed a retrospective chart review with two cohorts of patients. Group 1 consisted of 11 patients admitted between 1975 and 1978 to our burn center. Group 2 consisted of 11 patients admitted to our burn center in 2000 who were matched for age, sex and percentage total body surface area burned. Group 1 received 3.6 ± 1.1cc/kg/hour //wrong %TBSA of fluid in the first 24 hours. Group 2 received 8.0 ± 2.5cc/kg/hour //wrong %TBSA, which is 100% more than the Baxter formula. There was no difference in the median age, weight or 24-hour urine output between the two groups. Our data demonstrate that the "fluid creep" phenomenon is relatively new.

Abstract 3: Recent studies have shown that burn patients receive larger volumes of fluids than predicted by the Baxter formula and the reason for this is unclear. One potential reason is that increased analgesics are used which could blunt the response to fluid resuscitation. The purpose of this study was to compare the administration of opioid agonists in patients treated at a single burn center in the 1970s and in the year 2000. We performed a retrospective chart review comparing two matched cohorts. Group I consisted of 11 patients admitted between 1975 and 1978. Group II consisted of 11 patients admitted in 2000 matched for age, sex and %TBSA. Patients in Group II received a significantly higher mean opioid equivalent than those in Group I (26.5±12.3 vs. 3.9±2.2 in the first 24 hours, $p<0.001$). In addition, in Group II, a larger variety and combination of opioid agonists were used. This review demonstrates a significant increase from the 1970s to 2000 in the type, dose prescribed and dose delivered of opioid agonists. Along with "fluid creep," we have also increased our use of opioid agonists or "opioid creep." Higher doses of opioid agonists may have hemodynamic consequences, which may contribute to the increased fluid volumes.

RELATED PUBLICATIONS

1. Silverstein P; Goodwin MN, Jr; Raulston GL. Hypertrophic scarring, etiology and control. *Ann Res Progress Report of the US Army Institute of Surgical Research,* 30 June 1972; 1972:Section 37.
2. Silverstein P; Goodwin MN; Raulston GL; Pruitt B. Hypertrophic scar in the experimental animal. Longacre JJ, ed. *The ultrastructure of collagen,* Springfield, IL; Thomas, 1976.
3. Matsumura H; Engrav LH; Gibran NS; Yang TM, Grant JH, Yunusov MY, et al. Cones of skin occur where hypertrophic scar occurs. *Wound Repair Regen* 2001; 9:269-77.
4. Costa BA; Engrav LH; Holavanahalli R; Lezotte DC; Patterson DR; Kowalske KJ; Esselman PC. Impairment after burns: A two-center, prospective report. *Burns*, 2003; 29(7):671-675.
5. Zhu KQ; Engrav LH; Gibran NS; Cole JK; Matsumura H; Piepkorn M; et al. The female, red Duroc pig as an animal model of hypertrophic scarring and the potential role of the cones of skin. *Burns,* 2003; 29:649-664.
6. Friedrich JB; Sullivan SR; Engrav LH; Round KA; Blayney CB; Carrougher GJ; Heimbach DM; Honari S; Klein MB; Gibran NS. Is supra-baxter resuscitation in burn patients a new phenomenon? *Burns*, 2004; 30(5):464-466.
7. Liang Z; Engrav LH; Muangman P; Muffley LA; Zhu KQ; Carrougher GJ; et al. Nerve quantification in female red Duroc pig (FRDP) scar compared to human hypertrophic scar. *Burns* 2004; 30:57-64.
8. Sullivan SR; Friedrich JB; Engrav LH; Round KA; Heimbach DM; Heckbert SR; Carrougher GJ; Lezotte DC; Wiechman SA; Honari S; Klein MB; Gibran NS. "Opioid creep" is real and may be the cause of "fluid creep". *Burns*, 2004; 30(6):583-590.
9. Zhu KQ; Engrav LH; Tamura RN; Cole JK; Muangman PM; Carrougher GJ; et al. Further similarities between cutaneous scarring in the female, red Duroc pig and human hypertrophic scarring. *Burns* 2004; 30:518-350.
10. Zhu KQ; Engrav LH; Armendariz R; Muangman P; Klein MB; Carrougher GJ; et al. Changes in VEGF and nitric oxide after deep dermal injury in the female, red Duroc pig – Further similarities between female, Duroc scar and human hypertrophic scar. *Burns,* In press.

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

FUNDING
CHRMC Craniofacial Endowment Fund
KLS Martin Ltd.

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

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.

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

We have initiated a study to measure the metabolic rates of infants with cleft lip and/or cleft palate using indirect calorimetry, and to compare these with clinical measurements such as weight gain, growth, and diagnosis. The study 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 osteo-tomy, then to move the upper face forward and secure it in place with bone graft harvested from the child's ribs.

The limitations of this traditional Lefort III advancement are that some of the child's ribs need to be removed and, because of the tightness of the skin and

muscle overlying the upper facial skeleton, the face can usually only be moved forward around one centimeter. Repeat Lefort III operations, or inadequate advancements were therefore not uncommon in children with severe midface hypoplasia, or restricted growth.

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

Since midface distraction osteogenesis is a relatively new technique in evolution, we are actively researching ways to improve the process at the Craniofacial Center of Children's Hospital. A prospective Institutional Research Board (IRB) approved study is underway to examine the psychosocial impact of the three month long procedure on the patients and their families, and to suggest interventions to minimize the stress. Pre- and post-operative extensive sleep studies are being performed on all the children undergoing the procedure to examine the effect on quality of sleep. Sequential radiographic imaging is being used to learn how the facial bones adjust, remodel, and grow after they have been advanced such a large distance. Timing of how long it takes the new bone to form behind the advanced facial bones is also being studied to determine the optimum time to remove the distraction device.

Basic Science Research

Craniosynostosis

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

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

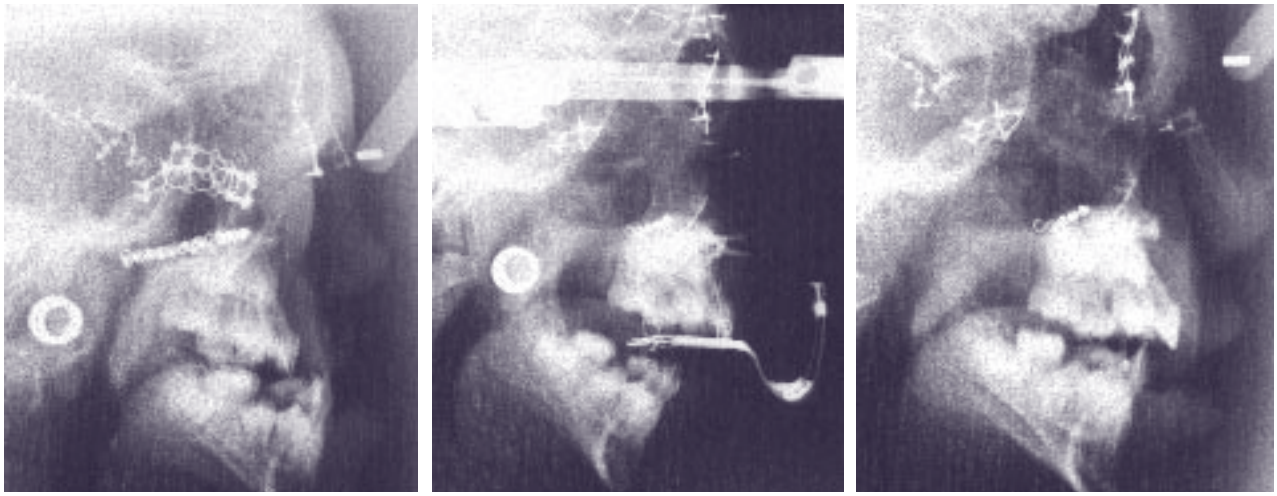

FIGURE 1: Lateral Cephalograms of a child undergoing midface distraction osteogenesis with an external device (Left) Before the operation, the child is having problems sleeping due to constriction of her nasopharynx, problems with dry eyes due to lack of cheek protection, and a problem chewing due to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

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 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.

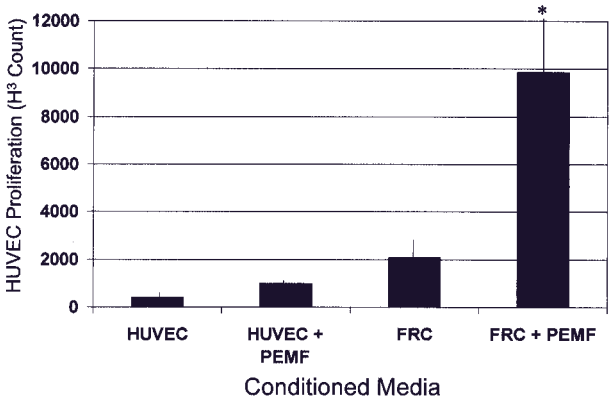

FIGURE 2: The effect of media from osteoblasts and endothelial cells stimulated with a pulsed electromagnetic field (PEMF) on endothelial cell proliferation. When human endothelial cells (HUVEC) were grown in media from fetal rat osteoblasts stimulated with PEMF (FRC+PEMF), their rate of proliferation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

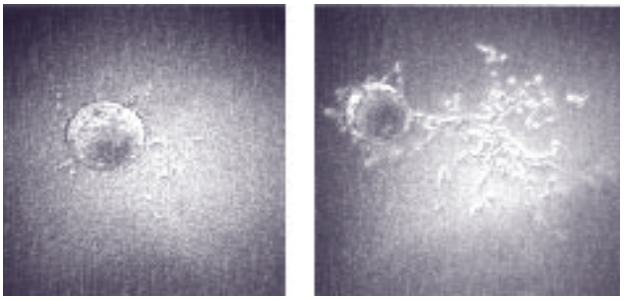

FIGURE 3: The effect of pulsed electromagnetic fields (PEMF) on endothelial cell tubulization. Endothelial cells attached to microspheres formed significantly more tubules when exposed to PEMF (left) than when not exposed the PEMF (right).

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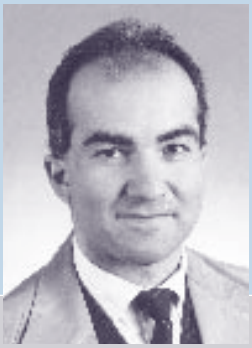
• Wound Healing



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.

lation 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

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.

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 popu-

reconstituting the dermis. We are currently investigating this unique role of bone marrow-derived cells in wound repair and are also interested in identifying the signaling pathway responsible for the differentiation of the progenitor cells in the wound.

Gene Expression Profiling of Normal Human Wound Healing

Response to acute cutaneous injury is dependent on the temporal activation and silencing of thousands of genes. Gene expression profiling using cDNA microarrays

allows for simultaneous comparison of thousands of genes. Using cDNA microarrays, we analyzed the gene expression profile of human skin during the first few hours following cutaneous wounding.

We observed significant up-regulation of gene expression at thirty minutes after wounding: expression of 334/4,000 genes was increased > 3 fold. Expression of genes involved in the inhibition of cell signaling including SOCS and the suppressor of ras-1 were up-regulated. In addition, expression of genes encoding regulators of the cell cycle (e.g. Rb) and proteases (e.g. uPA) were down-regulated. At 1 hour post wounding, 471/4,000 genes were increased > 3 fold. We observed down-regulation of transcriptional and signaling inhibitors, and up-regulation of multiple transcriptional activators. A searchable web site has been constructed to disseminate 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, identifying a cluster of genes encoding key components of the Wnt signaling pathway that are up-regulated in wound repair.

Developmental Genes Reintroduced in Adult Wound Healing: Wnt Genes

The Wnt signaling pathway plays an important role during embryonic development. Wnt signal transduction is involved in axis specification, 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.

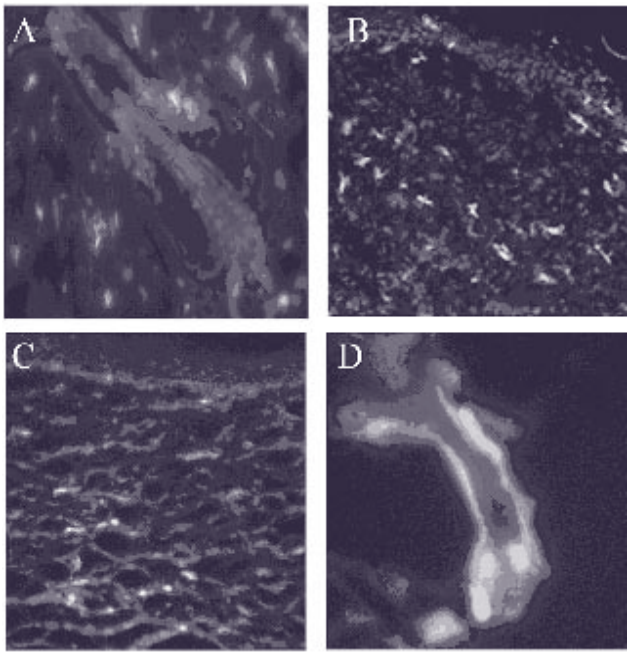


FIGURE 1A: Normal skin showing numerous green cells of bone marrow origin in normal mouse skin.

FIGURE 1B: 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).

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

The role of Wnt signal transduction during wound healing remains unexplored. However, it is clear that the Wnt signaling pathway can play an important role in the skin. Genes encoding Wnts and other components

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

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

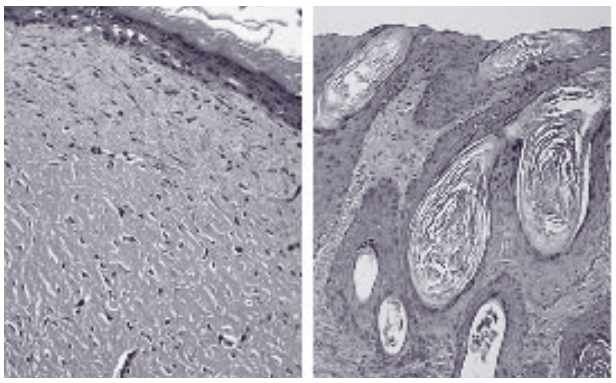


FIGURE 2: Figure on left shows a normal scar with simple stratified squamous epithelium over a collagen-rich dermal matrix. On right, a similar 40 day wound that overexpressed Wnt 5A retrovirus. Note the numerous epidermal cysts invaginating into the dermis, which later have structures resembling sebaceous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

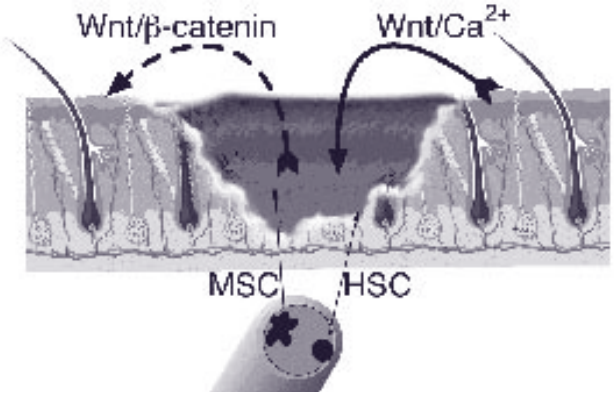


FIGURE 3: Graphic demonstration of our hypotheses. We think stem cells in the epidermis and stem cells from bone marrow can provide the missing cell types following loss due to injury. However, we think that the lack of morphogens in the wound may account for the lack of regeneration seen in wound repair.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

RELATED PUBLICATIONS

1. Arumugam S; Jang YC; Chen-Jensen C; Gibran NS; Isik FF. Temporal activity of plasminogen activators and matrix metalloproteinases during cutaneous wound repair. *Surgery,* 1999; 125(6): 587.
2. Jang Y-C; Arumugam S; Gibran NS; Isik FF. Role of αv integrins and angiogenesis during wound repair. *Wound Rep Reg*, 1999; 7:375.
3. Jang Y-C; Tsou R; Gibran NS; Isik FF. Vitronectin regulates wound fibrinolysis, microvascular angiogenesis and wound contraction in mice. *Surgery*, 2000; 127(6):696.
4. Tsou R; Cole JK; Nathens AB; Isik FF; Heimbach DM; Engrav LH; Gibran NS. Analysis of hypertrophic and normal scar gene expression using cDNA microarrays. *J Burn Care & Rehab*, 2001; 21:541.
5. Cole J; Tsou R; Wallace K; Gibran NS; Isik FF. Comparison of normal human skin gene expression using cDNA microarrays. *Wound Repair Regen,* 2001; 9(2):77.
6. Tsou R; Isik FF. Extracellular matrix and av integrins regulate FGF and VEGF growth factor receptors on human microvascular endothelial cells. *Mol Cell Biochem*, 2001; 224:81.
7. Cole J; Tsou R; Wallace K; Gibran NS; Isik FF. The early gene expression profile of human skin to injury using high-density cDNA microarrays. *Wound Rep Regen*, 2001; 9(5).
8. Wilson L; Fathke C; Isik FF. Tissue dispersion and flow cytometry for cellular analysis of wound healing by flow cytometry. *Biotechniques,* 2002; 32(3):548.
9. Cole J; Isik FF. Special Topic: Human genomics and microarrays: implications for the plastic surgeon. *Plast Recon Surg*, 2002; 110(3):849.
10. Tsou R; Fathke C; Wilson L; Wallace K; Isik FF. Retroviral delivery of dominant-negative vegfr2 to murine wounds inhibits wound angiogenesis. *Wound Rep Regen*, 2002; 10(4):222.
11. Fathke C; Wilson L; Hutter J; Kapoor V; Smith A; Hocking A; Isik F. Contribution of bone marrow-derived cells to skin: Collagen deposition and wound repair. *Stem Cells,* 2004; 22:812-822.

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TRANSPLANT SERVICE

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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.

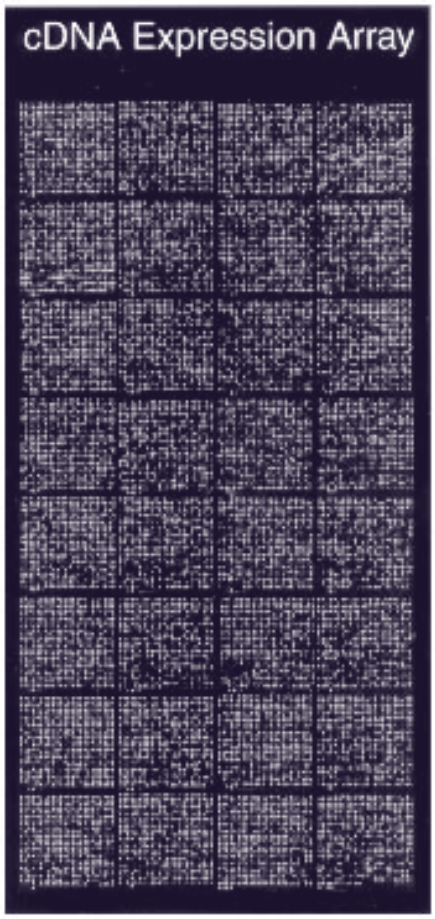

FIGURE 1

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,

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.

and currently have excellent renal function more than one year after renal transplantation (Fig. 2). We 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.

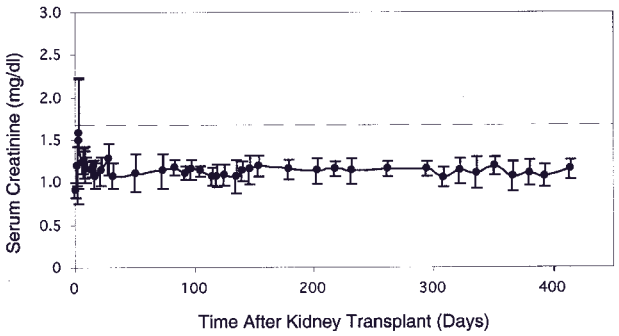

FIGURE 2

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 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.

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.

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 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.

RELATED PUBLICATIONS

1. Lu L; Li W; Fu F; Chambers FG; Qian S; Fung JJ; Thomson AW. Blockade of the CD40-CD40L pathway potentiates the capacity of donor- derived dendritic cell progenitors to induce long-term cardiac allografts survival. *Transplantation*, 1997; 64(12):1808-1815.
2. Li W; Fu F; Lu L; Narula SK; Fung JJ; Thomson AW; Qian S. Systemic administration of anti-IL-10 antibodies prolongs mouse allograft survival in normal and presensitized recipients. *Transplantation*, 1998; 66(12):1587-1596.
3. Li W; Fu F; Lu L; Narula SK; Fung JJ; Thomson AW; Qian S. Recipient pretreatment with cellular IL-10 prolongs cardiac allograft survival in mice. *Transplantation*, 1999; 68:1402.
4. Steptoe R; Li W; Fu F; O'Connell PJ; Thomson AW. Trafficking of APC from liver allografts of Flt3L-treated donors. *Transplant Immunology*, 1999; 7:51-57.
5. Lu L; Li W; Zhong C; Qian S; Fung JJ; Thomson AW; Starzl TE. Increased apoptosis of immunoreactive host cells and augmented leukocyte chimerism, not sustained inhibition of B7 molecule expression is associated with prolonged cardiac allograft survival in mice preconditioned with immature donor dendritic cells plus anti-CD40L mAb. *Transplantation,* 1999; 68:747-757.
6. Li W; Lu L; Wang Z; Wang L; Fung JJ; Thomson AW; Qian S. IL-12 antagonism enhances apoptotic death of T cells within hepatic allografts from Flt3-treated donors and promotes the restoration of transplant tolerance. *J Immunology,* 2001; 166:5619-5628.
7. Li W; Lu L; Wang Z; Wang L; Fung JJ; Thomson AW; Qian S. Costimulation blockade promotes the apoptotic death of graft infiltrating T cells and prolongs survival of hepatic allografts from Flt3L-treated donors. *Transplantation,* 2001; 72:1423-1432.
8. O'Connell PJ; Li W; Wang Z; Logar AJ; Thomson AW. Immature and mature CD8(+ dendritic cells prolong the survival of vascularized heart allografts. *J Immunology,* 2002; 168:143.
9. Li W; Chou S; Wang C; Kuhr C; Perkins J. The role of the liver in peripheral tolerance: induction through oral antigen feeding. *American Journal of Transplantation,* 2004; 4:1574-1582.
10. Li W; Zheng XX; Kuhr CS; Perkins JD. CTLA4 engagement is required for induction of mouse liver transplant spontaneous tolerance. *American Journal of Transplantation.* In press, 2004.

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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.")

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

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

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.

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 mechanism of NKT cell tolerance is unknown. Therefore, several mechanisms of peripheral tolerance may be active in the hepatic immune system.

Antigen given via a mucosal route favors the induction of peripheral tolerance. This type of induced peripheral tolerance is commonly called "oral tolerance." 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

Over the past year, we have used a murine transplant model to study the liver's role in inducing and maintaining peripheral tolerance induced via oral antigens (Fig. 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 7 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

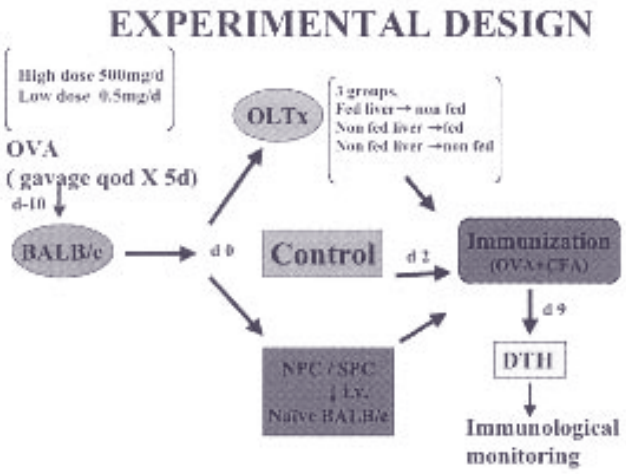

FIGURE 1: A model to study the liver's role in peripheral tolerance.

from mixed lymphocyte reaction (MLR) culture detected by ELISA.

How the Model Demonstrated Induction and Transfer of Tolerance

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

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

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 TH2 response, while that of the high dose fed group is not. Other reports have also indicated that IL-10 is enhanced in oral tolerance. Another mechanism involved with the immunologically diverse hepatic immune system is NKT cells. Our data 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.

RELATED PUBLICATIONS

1. Perkins JD; Wiesner RH; Banks PM; LaRusso NF; Ludwig J; Krom RAF. Immunohistologic labeling as an indicator of liver allograft rejection. *Transplantation,* 1987; 43(1):105-8.
2. Munn SR; Tominaga S; Perkins JD; Hayes DH; Wiesner RH; Krom RAF. Increasing peripheral T lymphocyte counts predict rejection in human orthotopic liver allografts. *Transplantation Proceedings,* 1988; 20:674-675 (suppl 1).
3. Perkins JD; Nelson DL; Rakela J; Grambsch PM; Krom RAF. Soluble interleukin 2 receptor level in liver allograft recipients: An indicator of rejection. *Transplantation Proceedings,* 1989; 21 (1 Pt 2):2275-2276.
4. Perkins JD; Rakela J; Sterioff S; Banks PM; Wiesner RH; Krom RAF. Immunohistologic pattern of the portal T-lymphocyte infiltration in hepatic allograft rejection. *Mayo Clinic Proceedings,* 1989; 64(5):565-569.
5. Lu L; Li W; Zhong C; Qian S; Fung JJ; Thomson AW; Starzl TE. Increased apoptosis of immunoreactive host cells and augmented donor leukocyte chimerism, not sustained inhibition of B7 molecule expression are associated with prolonged cardiac allograft survival in mice preconditioned with immature donor dendritic cells plus anti-CD40L mAb. *Transplantation,* 1999; 68(6):1.
6. Carithers R; Perkins JD. 4. Gastroenterology: XV – Liver and Pancreas Transplantation. In: *Scientific American Medicine,* Editors: Dale DC and Federman DO. New York: WebMD Corp., May 2001, 1-6.
7. Li W; Lu L; Wang Z; Wang L; Fung JJ; Thomson AW; Qian S. IL-12 antagonism enhances apoptotic death of T cells within hepatic allografts from Flt3 ligand-treated donors and promotes graft acceptance. *J Immunology,* 2001; 166:5619-5628.
8. Li W; Lu L; Wang Z; Wang L; Fung JJ; Thomson AW; Qian S. Costimulation blockade promotes the apoptotic death of graft-infiltrating T cells and prolongs survival of hepatic allografts from FLT3L-treated donors. *Transplantation,* 2001; 72:1423-1432.
9. O'Connell PJ; Li W; Wang Z; Specht SM; Logar AJ; Thomson AW. Immature and mature CD8alpha+ dendritic cells prolong the survival of vascularized heart allografts. *J Immunology,* 2002; 168:143-154.
10. Li W; Zheng XX; Kuhr CS; Perkins JD. CTLA4 engagement is required for induction of mouse liver transplant spontaneous tolerance. *American Journal of Transplantation.* 2004; In press.

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• The Breast Health Global Initiative (BHGI)

FUNDING
Amgen
AstraZeneca
Centers for Disease Control
Ethicon Endo-Surgery
International Atomic Energy Agency
• Division of Human Health
National Institutes of Health
• National Cancer Institute
The Susan G. Komen Breast Cancer Foundation
Pfizer, Inc.

BHGI Guideline Development

Background

Breast cancer is the most common cause of cancer related death for women worldwide. Breast cancer lethality is highest among economically disadvantaged countries. While major progress has been made to improve breast cancer outcomes in developed countries, resource constraints limit the application of early detection, diagnosis and treatment strategies in most of the world.

Resource-constrained countries lack the financial resources to implement optimal breast health care programs. As a result, breast care guidelines from economically privileged regions such as the U.S. and Western Europe have limited utility in low-level or mid-level resource countries. Instead, these countries need to implement breast cancer control programs that are appropriate for their country's resources and competing health care needs. Evidence-based guidelines can define strategies by which economically practical incremental improvements can be sequentially introduced within the context of resource constraints to create measurable improvements in health care administration and outcome.

Mission Statement

The Breast Health Global Initiative (BHGI) strives to develop evidence-based, economically feasible, and culturally appropriate guidelines for underdeveloped nations to improve breast health outcomes.

BHGI Guideline Principles

1. Resource-constrained countries have differing financial needs, resource limitations, social constraints and competing illness profiles that frame how national breast health care programs should be implemented.
2. Evidence-based guidelines from wealthy countries define optimal goals for resource-constrained countries, although these countries commonly lack the fundamental infrastructure that is required for direct implementation.
3. Health care systems cannot be transformed at once.
4. Improvements in early detection, diagnosis and treatment of breast cancer require organized, sequentially implemented steps to achieve improved outcome.
5. The development of international evidence-based breast health care guidelines oriented to developing countries of the world with low-level and medium-level financial resources is a crucial step toward improving breast health care and cancer treatment in these countries.
6. It is possible to define evidence-based "best practices with limited resources" for breast healthcare for use in countries where access to healthcare is challenged, breast cancer awareness is limited and cultural barriers need to be overcome.
7. Guidelines for countries of limited resources may require alternate strategies for care to those adopted in wealthy countries to allow sequential steps in improvement provided that those alternate strategies are not defining a "lowered" standard of care for that country.

While major progress has been made to improve breast cancer outcomes in developed countries, resource constraints limit the application of early detection, diagnosis and treatment strategies in most of the world.

Global Summit Guideline Development Conferences

Guideline Purposes

- Define principles in breast health care application in resource-constrained countries.
- Provide a structural framework for change and improvement in health-care delivery
- Cultivate international dialogue and an understanding based upon need and resources.
- Establish a benchmark for growth and improvement through outcomes analysis.

Global Summit 2002 (Seattle)

The BHGI held the first biennial Global Summit Consensus Conference on International Breast Health Care held in Seattle, Washington in October 2002 to establish breast health guidelines to address how care may best be provided in countries where significant gaps in health care resources exist. The guideline development followed consensus panel analysis of evidence-based breast care modeling. Based on definitions created by the World Health Organization (WHO) for national cancer programs, panels of breast cancer experts representing 17 countries and 9 world regions created guidelines to address early detection, diagnosis and treatment of breast cancer in countries with limited health care resources. The breast health care guidelines were published as a Breast Journal supplement in 2003 and have been made available in an unrestricted fashion on the Internet for worldwide access. 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)

With extended multilateral sponsorship of national and international collaborating organizations, the BHGI guidelines were re-examined, revised and extended at the biennial "Global Summit Consensus Conference on International Breast Health," January 12-15, 2005. The NCI Office of International Affairs in Bethesda, Maryland hosted this second iteration of this guideline process. Several national and international groups agreed to support the conference as collaborating organizations: WHO, the Pan American Health Organization (PAHO), the National Cancer Institute (NCI)/Office of International Affairs, the American Society for Breast Disease (ASBD), and the International Union Against Cancer (UICC). In order to implement these guidelines in practice, a separate but integrated effort needed to be made to establish a core resource for epidemiologic outcomes analysis to be developed in conjunction with guideline revision and expansion.

Global Summit Consensus Conference

Participants: Physicians, scientists, healthcare providers, and advocates who are representatives of world and regional health organizations, governmental health ministries, international organizations, countries and regions throughout the developing and developed world, with expertise in breast cancer screening/early detection, diagnosis and treatment, personal experience in health care delivery in countries with limited health care resources.

2005 Objectives: Approximately 50 international experts gathered from around the world in related fields of breast cancer screening, surgery, oncology, radiation therapy, pathology/cytology, and health economic analysis, to review and update the 2003 international breast health care and cancer treatment guidelines for developing countries. These breast care and cancer treatment experts convened for four days to develop consensus statements about breast care and cancer treatment oriented to developing regions in the world. Daily sessions consisted of specific topics within the topic of the four panel sections: 1) Early Detection and Access to Care, 2) Diagnosis and Pathology, 3) Cancer Treatment and Allocation of Resources, 4) Health Care Systems and Public Policy. The updated guidelines will be published in a second monograph in the Breast Journal in the September/October 2005 issue, will be made available on the internet, and that site will be linked to the WHO website, which receives 50 million hits per month.

The BHGI is a structure for linkages, research and pilot projects through interdisciplinary communication, cooperation and alliance building via the Global Summit Consensus Conference, on-going communications, website and research and demonstration projects between three core groups, which include the following:

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

Organizational Relationships

The Breast Health Global Initiative (BHGI) is an ongoing project based at the Fred Hutchinson Cancer Research Center to develop economically feasible, scientific and culturally appropriate guidelines for underdeveloped nations to improve breast health outcomes.

Sponsoring Organization

World renowned in cancer research and a global leader in the field with the mission of the elimination of cancer as a cause of human suffering and death, the Fred Hutchinson Cancer Research Center Public Health Sciences Division goal is to identify strategies that would ultimately reduce the incidence of and mortality from cancer and other diseases.

Co-Sponsoring Organization

The Susan G. Komen Breast Cancer Foundation is a global leader in the fight against breast cancer through its support of innovative research and community-based outreach programs. The Komen Foundation is fighting to eradicate breast cancer as a life-threatening disease by funding research grants and supporting education, screening and treatment projects in communities around the world.

Collaborating Organizations

- World Health Organization (WHO)
- National Cancer Institute (NIH/NCI)
- Middle East Cancer Consortium (MECC)
- World Society for Breast Health (WSBH)
- International Union Against Cancer (UICC)
- American Society for Breast Disease (ASBD)
- International Society of Breast Pathology (ISBP)
- International Society of Nurses in Cancer Care (ISNCC)
- Pan American Health Organization (PAHO/Division of WHO)
- International Atomic Energy Agency/Division of Human Health (IAEA)
- Alliance for Health Policy and Systems Research (AHPSR/Division of WHO)
- International Network for Cancer Treatment and Research (INCTR)

RELATED PUBLICATIONS

1. Parkin DM; Pisani P; Ferlay J. Global cancer statistics. *CA Cancer J Clin,* Jan-Feb 1999; 49(1):33-64.
2. Anderson BO. Global summit consensus conference on international breast health care: Guidelines for countries with limited resources. *Breast J,* May 2003; 9 Suppl 2:S40-41.
3. *Executive summary of the national cancer control programmes: Policies and managerial guidelines.* Geneva: World Health Organization; 2002.
4. Anderson BO; Braun S; Carlson RW; et al. Overview of breast health care guidelines for countries with limited resources. *Breast J,* May-Jun 2003; 9 Suppl 2:S42-50.
5. Anderson BO; Braun S; Lim S; Smith RA; Taplin S; Thomas DB. Early detection of breast cancer in countries with limited resources. *Breast J,* May-Jun 2003; 9 Suppl 2:S51-59.
6. Vargas HI; Anderson BO; Chopra R; et al. Diagnosis of breast cancer in countries with limited resources. *Breast J,* May-Jun 2003; 9 Suppl 2:S60-66.
7. Carlson RW; Anderson BO; Chopra R; et al. Treatment of breast cancer in countries with limited resources. *Breast J,* May-Jun 2003; 9 Suppl 2:S67-74.

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• Advancing the Clinical Science of Surgery Using Outcomes Research Tools

FUNDING
National Institutes of Health

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

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 should be considered the standard, who are the outliers (both good and bad) and what techniques work out of the research

Only by determining the full impact of procedures on individuals and society can we determine what should be done rather than simply what can be done for our patients.

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

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

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 appen-

dectomy 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. 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).

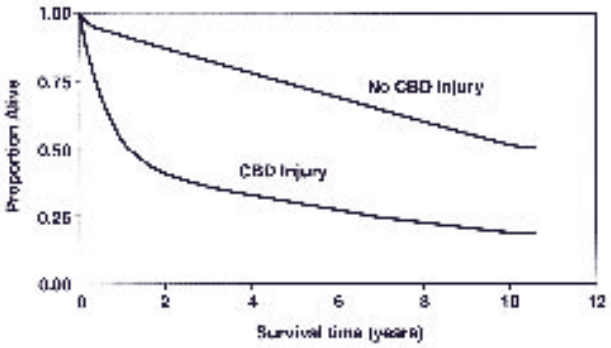

FIGURE 1: Survival after bile duct injury in Medicare beneficiaries (n=1.57 million).

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.

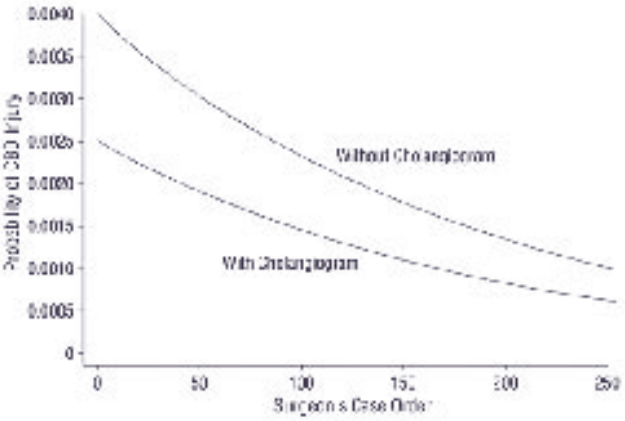

FIGURE 2: Probability of bile duct injury with and without cholangiogram, by case-order of surgeon (n=36,000).

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 inexperi-

ence 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).

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

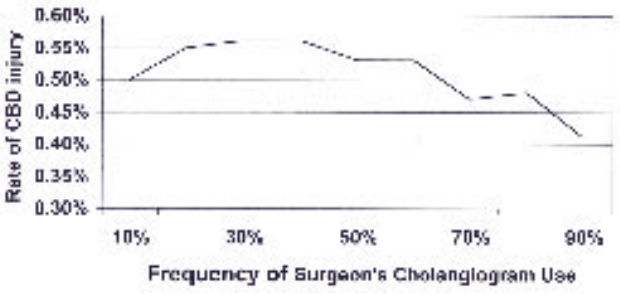

FIGURE 3: The effect of increasing the surgeon's frequency of cholangiogram use on the rate of common bile duct (CBD) injury.

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.

RELATED PUBLICATIONS

1. Flum DR; Koepsell T; Heagerty P; Sinanan M; Dellinger EP. Common bile duct injury during laparoscopic cholecystectomy and the use of intraoperative cholangiography: Adverse outcome or preventable error? *Arch Surg,* 2001; 136:1287-1292.
2. Flum DR; Morris A; Koepsell T; Dellinger EP. Has misdiagnosis of appendicitis decreased over time? A population-based analysis. *JAMA,* 2001; 286:1748-1753.
3. Flum DR; Flowers C; Veenstra D. A cost analysis of cholangiograms in the prevention of common bile duct injury. *J American College of Surgeons,* 2003; 196:385-396.
4. Flum DR; Cheadle A; Dellinger EP; Chan LC; Koepsell T. Intraoperative cholangiography and risk of bile duct injury during cholecystectomy. *JAMA,* 2003; 289:1639-1644.
5. Flum DR; Cheadle A; Prela C; Dellinger EP; Chan LC. Bile duct injury and survival in Medicare beneficiaries. *JAMA,* 2003; 290:2168-2173.

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Karen D. Horvath, M.D.

- 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 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.

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.

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 technologic 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 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 development of a laboratory-based, basic operating skills module for junior 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. This project parallels the efforts in our Center for Video Endoscopic Surgery to teach residents laparoscopic skills.

RELATED PUBLICATIONS

1. Horvath KD; Chabot J. An aggressive resectional approach to cystic neoplasms of the pancreas. *Am J Surg* 1999; 178(4):269-274.
2. Swanstrom LL; Jobe BA; Kinzie LR; Horvath KD. Esophageal motility and outcomes following laparoscopic paraesophageal hernia repair and fundoplication. *Am J Surg* 1999; 177:359-363.
3. Horvath KD; Swanstrom LL; Jobe BA. The short esophagus: pathophysiology, incidence, presentation and treatment in the era of laparoscopic anti-reflux surgery. *Ann Surg* 2000; 232(5):630-640.
4. Horvath KD; Kao LS; Ali A; Wherry KL; Pellegrini CA; Sinanan MN. Laparoscopic assisted percutaneous drainage of infected pancreatic necrosis. *Surg Endosc,* 2001; 15(7):677-82.
5. Horvath KD; Kao LS; Wherry KL; Pellegrini CA; Sinanan MN. A Technique for laparoscopic-assisted percutaneous drainage of infected pancreatic necrosis and pancreatic abscess. *Surg Endosc,* 2001; 15(10):1221-5.
6. Moskovitz AH; Bush WH; Horvath KD. Anaphylactoid reaction to intraoperative cholangiogram. Report of a case, review of literature and guidelines for prevention. *Surg Endosc,* 2001; DOI: 10.1007/s004640041033.
7. Flum D; Horvath KD; Koepsell T. Have outcomes of incisional hernia repair improved with time? A population based analysis. *Ann Surg,* 2003 Jan; 237(1):129-35.
8. Pavlin DJ; Horvath KD; Pavlin EG; Sima K. Preincisional treatment to prevent pain after ambulatory hernia surgery. *Anesth Analg,* 2003; 97:1627-32.
9. Kellogg T; Horvath KD. Minimal access approaches to complications of actue pancreatitis and benign neoplasms of the pancreas. *Surg Endosc,* 2003; 17:1692-1704.
10. Worrell SA; Horvath KD; Blakemore T; Flum D. Endorectal ultrasound detection of focal carcinoma within rectal adenomas. *Am J Surg,* 2004; 187:625-629.
11. Van Eaton EG; Horvath KD; Lober B; Pellegrini CA. Organizing the transfer of patient care: The development of a computerized resident signout system. *Surgery* 2004; 136:5-13.
12. Horvath KD; Coluccio G; Foy H; Pellegrini CA. A program for successful integration of international medical graduates (IMGs) into US surgical residency training. *Current Surgery* (in press).

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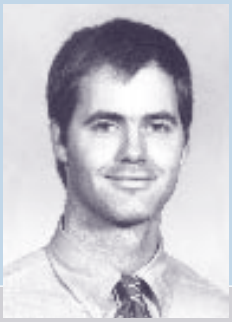
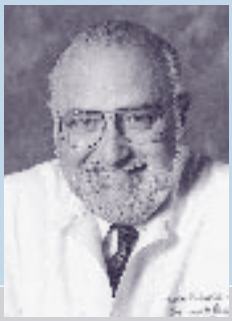
Carlos A. Pellegrini, M.D.

• The Swallowing Center at the University of Washington





AWARDS

Society for Surgery of the Alimentary Tract
- Ross Resident/Fellow Research Award

FUNDING

Cook Surgical, Inc.
Society of American Gastrointestinal and Endoscopic Surgeons
W.L. Gore, Inc.
Tyco, United States Surgical Corporation
Dennis and Mary Wise Esophageal Research Fund

Multichannel Intraluminal (Esophageal) Impedance

Multichannel Intraluminal Impedance (MII) is a new technology available for the detection of bolus presence within the esophageal lumen. This has potential applications for measuring esophageal motility (bolus moving from mouth to stomach) and reflux (bolus moving from the stomach retrograde up the esophagus). Based on ionic flow current, it has the capability of detecting both the bolus presence characteristics (liquid, gas or mixed) as well. The catheter has multiple pairs of sensors distributed along the esophagus (Figure 1), and 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 patient swallow a viscous material that theoretically "tests" the motility of the esophagus more than water. We've found in 278 water swallows, 5% having 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% that had abnormal manometry and complete bolus clearance. These results coincide with the ones obtained by other investigators, in the fact that patients with incomplete bolus clearance can have "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.

We performed simultaneous MII, manometry, and pH monitoring in 10 asymptomatic subjects, 22 con-

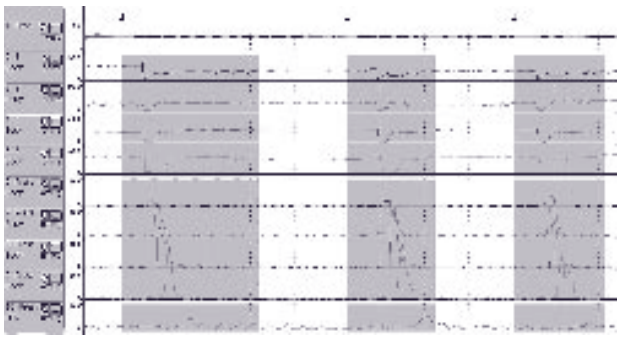


FIGURE 1: Impedance and manometry tracing showing complete bolus transit (MII) with a normal manometric tracing.

	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)*

secutive 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 four (non-acid reflux), 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's 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 to wonder if some patients with microaspiration may go undetected by pharyngeal pH testing. Pharyngeal reflux detection with MII is being carried out in our department. Our results in normal subject 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)

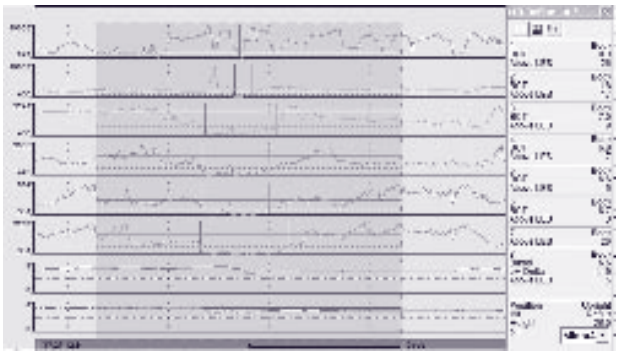


FIGURE 2: Pharyngeal reflux episode detected by impedance.

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

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.

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.

Of the 288 patients, 51 (18%) had preoperative diagnosis of Barrett's esophagus. 11 patients (22%) had complete regression of Barrett's after surgery. 2 patients developed high grade dysplasia after LARS. Of the 237 patients that had no Barrett's preop, only one patient developed Barrett's after LARS (0.02% per patient year). 12 patients had a redo surgery, two for acute complications, 8 for recurrent GERD and two patients 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.

Response of Respiratory Symptoms to Surgery

A strong link exists between gastroesophageal reflux disease (GERD) and airway disease. Medical anti-acid therapy may not provide long-term relief for patients with airway manifestations of GERD; laparoscopic anti-reflux surgery (LARS) is often considered for these patients. Surgery is more apt to provide relief since it not only prevents acid reflux, but also prevents regurgitation and thus potential aspiration. We sought to determine the long-term results of LARS for multiple airway manifestations of GERD.

We evaluated 136 patients who experienced cough, hoarseness, or wheeze more than once per week; sore throat and dyspnea were frequent concomitant symptoms with a median follow-up of 53 months (range 19-110 months).

Our data suggest that LARS provides a durable, long-term relief of airway symptoms of GERD in 65-74% patients for whom aggressive medical therapy has been inadequate. LARS also provides excellent relief of typical esophageal symptoms of GERD in these patients (88-92%). As a result, this is the first clear evidence that LARS should be a considered for patients with airway manifestations, especially those with inadequate response to medical therapy.

Success of Laparoscopic Re-operative Fundoplications

Though laparoscopic antireflux surgery has established

Symptom	Number of Patients	% Pts Improved	Pre-Op Freq Score* (Mean ± SD)	Post-Op Freq Score* (Mean ± SD)	*p*-value
Heartburn	125	87	3.45 ± 0.86	0.84 1.23	0.001
Regurgitation	104	93	2.95 ± 0.96	0.56 1.07	0.001
Dysphagia	71	75	2.73 ± 1.04	0.56 1.07	0.001
Cough	108	74	3.51 ± 0.65	1.58 ± 1.62	0.001
Hoarseness	82	66	2.93 ± 1.09	1.45 ± 1.54	0.001
Wheezing	37	69	2.86 ± 1.13	1.46 ±1.52	0.001
Sore Throat	41	70	2.56 ± 1.10	1.10 ± 1.36	0.001
Dyspnea	31	65	2.84 ± 1.00	1.97 ± 1.66	0.01

** Data given as mean +standard deviation, Frequency 1-once a month, 2-once a week, 3-once a day, 4 several times per day.*

itself as an effective treatment for gastroesophageal reflux disease (GERD), in a small subset of patients, the initial operation fails, typically resulting in recurrent reflux or severe dysphagia. Although redo fundoplications can be performed laparoscopically, few studies have examined their long-term efficacy. To that end, we identified from a prospective database all patients undergoing redo laparoscopic fundoplications at the University of Washington between 1996 and 2001, and for the purpose of this study, contacted them for long-term follow-up. Forty-one patients were contacted with a median length of follow-up of 50 months (range 20-95).

Symptoms Questionnaire Results

Symptoms	Resolved (%)	Improved† (%)	Frequency Score Pre-op*	Freq Score Post-op*	*p*-value
Heartburn	45	61	3.0 +1.2	1.4± 1.6	‹0.001
Regurgitation	41	69	2.7± 1.0	1.4± 1.5	‹0.001
Dysphagia	38	74	3.0 ±1.2	1.5 ±1.6	‹0.001

† *Improved = (Resolved+Improved)*
* *Data given as mean + standard deviation, Frequency 1-once a month, 2-once a week, 3-once a day, 4 several times per day.*

After redo fundoplication, there was a significant reduction in frequency of presenting complaints, with the majority of patients having substantial improvement or complete resolution of symptoms (see Table above). The most common side effect was diarrhea (26 patients), in only six patients (23%) this was a new symptom after surgery. Overall, 68% rated the success of the procedure as either "excellent" or "good," and when asked whether they were happy they chose to have the redo procedure, 78% said "yes."

This is the largest study of redo fundoplications with long-term follow-up to date. Although not nearly as successful as primary fundoplications, the majority of patients with reflux or dysphagia following antireflux surgery can expect a durable improvement in symptoms with a laparoscopic redo fundoplication.

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, University of California at San Francisco, and Washington University. This trial is now underway, and should answer whether this product has a positive impact on this difficult disease.

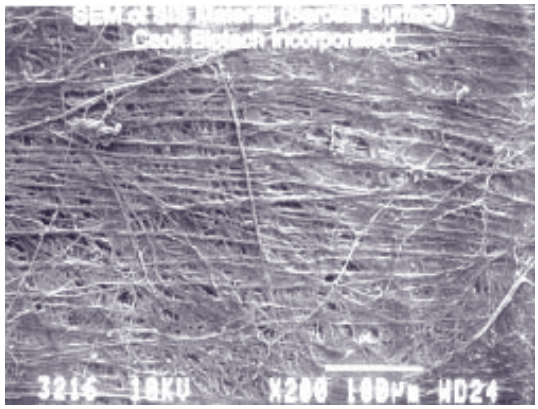


FIGURE 3: Small intestinal submucosa (SIS).

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

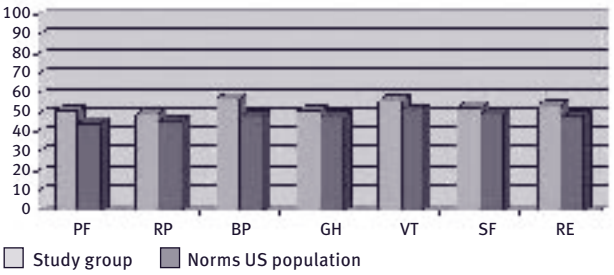
Morbid obesity is strongly associated with GERD, and both have an independent association with motility disorders. The importance of this is that impaired esophageal function can play a role in the development of dysphagia after fundoplications and bariatric procedures.

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 on 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 from 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 (see Table below).

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.



RELATED PUBLICATIONS

1. Barreca M; Oelschlager BK; Pellegrini CA. Outcomes of laparoscopic nissen fundoplication in patients with the "hypercontractile esophagus". *Arch Surg,* 2002; 137(6):724-728.
2. Oelschlager BK; Eubanks TR; Maronian N; Hillel A; Oleynikov D; Pope C; Pellegrini CA. Laryngoscopy and pharyngeal pH are complementary in the diagnosis of gastroesophageal-laryngeal reflux. *J Gastrointest Surg,* 2002; 6(2):189-194.
3. Oelschlager BK; Eubanks TR; Oleynikov D; Pellegrini CA. Symptomatic and physiologic outcomes after operative treatment for extraesophageal reflux. *Surg Endosc,* 2002; 16:1032-1036.
4. Oelschlager BK; Chan MM; Eubanks TR; Pope CE; Pellegrini CA. Effective treatment of rumination with nissen fundoplication. *J Gastrointest Surg,* 2002; 6:638-644.
5. Oleynikov D; Eubanks TR; Oelschlager BK; Pellegrini CA. Total fundoplication is the operation of choice for patients with gastroesophageal reflux and defective peristalsis. *Surg Endosc,* 2002; 16:909-913.
6. Chang L; Oelschlager BK; Barreca M; Pellegrini CA. Improving accuracy in the identification of the gastroesophageal junction during laparoscopic antireflux surgery. *Surg Endosc,* 2003; 17(3):390-393.
7. Oelschlager BK; Chang L; Pellegrini CA. Improved outcome after extended gastric myotomy for achalasia. *Arch Surg,* 2003; 138(5):490-497.
8. Oelschlager BK; Barreca M; Chang L; Oleynikov D; Pellegrini CA. Clinical and pathologic response of Barrett's esophagus to laparoscopic antireflux surgery. *Ann Surg,* 2003; 238:458-466.
9. Oelschlager BK; Barreca M; Chang L; Pellegrini CA. The use of small intestinal submucosa (SIS) in the repair of paraesophageal hernias: Initial observations of a new technique. *Am J Surg,* 2003; 186:4-8.

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Richard Satava, M.D.

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

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

Objective Assessment of Surgical Skills

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

The conceptual change is to train residents (in the future) not for a given time, but rather to a given criterion level, a level which reduces errors to the absolute minimum and provides maximum quality, especially for patient safety. The above will be implemented by using the Minimally Invasive Surgery Trainer — Virtual Reality (MIST-VR) and the Xitact 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

Recent introduction of robotic systems into clinical surgery indicates a fundamental new direction for surgeons.

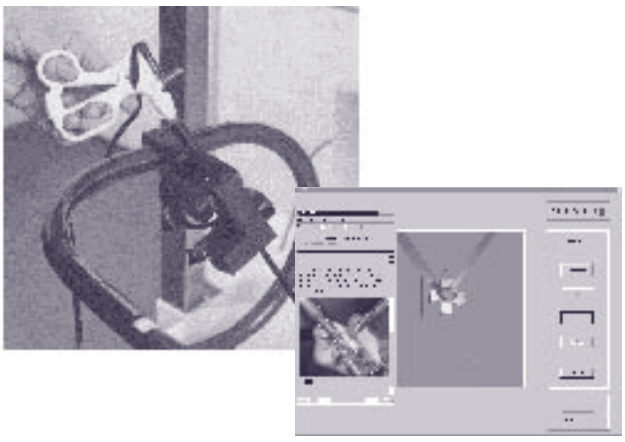

FIGURE 1: MIST-VR basic surgical skills simulator illustrating the image on the simulator screen, and the input handles for tracking motion.

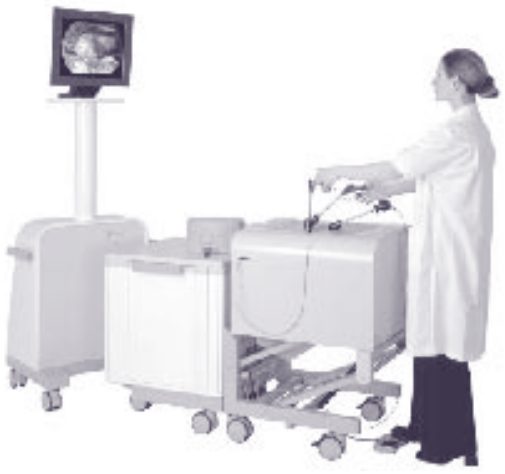

FIGURE 2: Xitact Laparoscopic Cholecystectomy simulator illustrating the portable system and video image.

FIGURE 3: Zeus surgical robotic system.

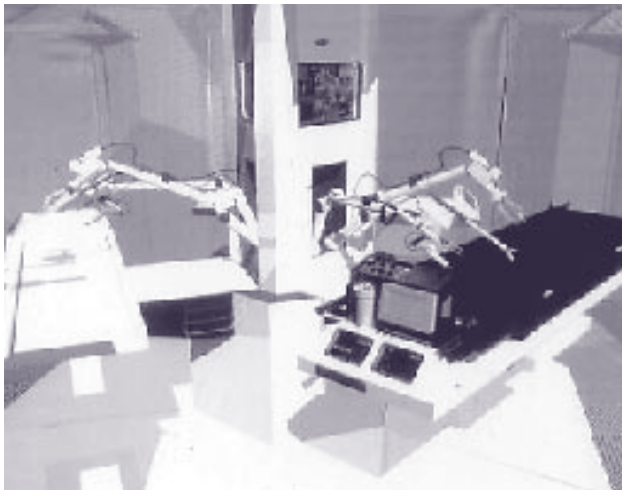

FIGURE 4: OR of the future – concept drawing from Integrated Medical Systems.

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.

RELATED PUBLICATIONS

1. Satava RM; Jones SB. Preparing surgeons for the 21st century, implications of advanced technologies. *Surgical Clinics of North America, Minimal Access Surgery, Part 1,* 2000 Aug; vol 80, no 4, pp. 1353 - 1365.
2. Pearson AM; Gallagher AG; Rosser JC; Satava RM. Evaluation of structured and quantitative training methods for teaching intracorporeal knot tying. *Surgical Endoscopy,* 2001 Nov; vol 16, pp. 130-137.
3. Satava RM. Accomplishment and challenges of surgical simulation: Dawnng of the next-generation surgical education (Review article). *Surgical Endoscopy,* 2001 Mar; vol 15, No. 3, pp.232-241.
4. Satava RM; Bowersox JC; Mack; Krummel TM. Symposium: Robotic surgery state of the art and future trends. *Contemporary Surgery*, 2001 Oct; vol 57, no. 10.
5. Satava RM. Surgical robotics: The early chronicles a personal historical perspective. *Surg Laparoscopy Endoscopy & Percutaneous Techniques,* 2002 Feb; vol 12:1, pp. 6-16.
6. Satava R; Cuschieri A; Jakimowicz J; Deane S; Hamdorf J; Darzi A; Hanna G; Gallagher A; Buess G; Fried G; Regher R; Krummel T; Hasson H; Narwold D; Dunn M. Metrics for objective assessment: Preliminary summary of the surgical skills workshop. *Surgical Endoscopy,* 2003 Feb; 17(2):220-6.
7. Satava RM; Fried MP. A methodology for objective assessment of errors: An example using an endoscopic sinus surgery simulator (ES3). *Otolaryngol Clin North Am,* 2002 Dec; 35(6):1289-301.
8. Seymour NE; Gallagher AG; Roman SA; O'Brien MK; Bansal VI; Andersen DK; Satava RM. Virtual reality training improves operating room performance: results of a randomized, double-blinded study. *Ann Surg,* 2002 Oct; 236(4):458-63.

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• 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 training systems, for example the anesthesia simulator or interventional cardiology simulator

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.

(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 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 has allocated premium space on the first floor of the Surgery Pavilion, between the new 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.

Timeline

ISIS training will commence in the spring of 2005. Planning and renovation of space will proceed concurrently.

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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.

The focus of our research is to understand how tumors come about as a result of TSC1 and TSC2 inactivation.

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.

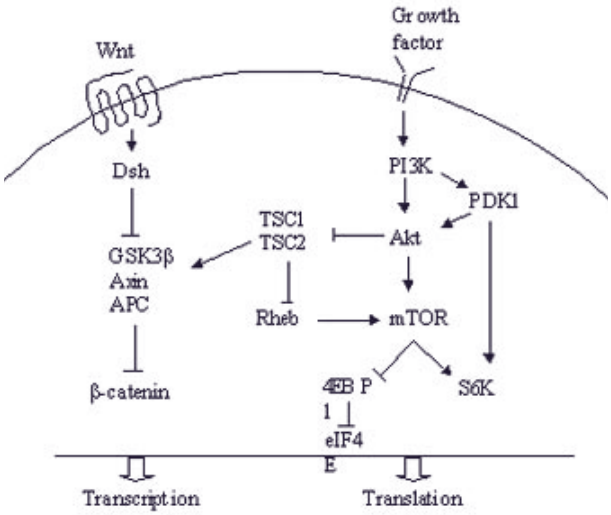

FIGURE 1: Model of TSC1/TSC2 pathway.

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/β-catenin pathway. The latter has been implicated in the regulation of cell proliferation, differentiation, and migration. The Wnt family of secreted growth factors, acts on multiple signaling cascades among which the β-catenin canonical pathway is best understood for its role in various human cancers (e.g., colon, skin, liver). β-Catenin is a highly conserved 95-kD protein involved in cell-cell adhesion and intracellular signaling. In its latter role, β-catenin shuttles from the cytosol to the nucleus upon Wnt stimulation where it binds the LEF/Tcf family of transcription factors to activate downstream target genes such as cyclin D1 (Figure 1).

Our observations showed that renal tumors derived from our TSC animal model expressed high levels of β-catenin and cyclin D1. In 293T renal epithelial cells, expression of TSC1 and TSC2 reduced β-catenin levels by promoting its degradation. Correspondingly, TSC1/TSC2 inhibited β-catenin dependent activity of the LEF/Tcf transcription factors. Evidence suggested that TSC1 and TSC2 act at the level of the β-catenin degradation complex by associating with its components (i.e., GSK3, Axin) in a Wnt-dependent manner. Collectively, the TSC proteins likely function in multiple pathways giving rise to the diverse manifestations of the pathology resulting from their inactivation (Figure 1). Efforts to demonstrate in vivo participation of these pathways and their relative contribution to the disease phenotype are currently our focus of investigation.

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.

RELATED PUBLICATIONS

1. Aicher LD; Campbell JS; Yeung RS. Tuberin phosphorylation regulates its interaction with hamartin: Two proteins involved in tuberous sclerosis. *J Biol Chem,* 2001; 276:21017-21021.
2. Yeung RS; Gu H; Lee M; Dundon T. Genetic identification of a locus, Mot1, that affects renal tumor size in the rat. *Genomics,* 2001; 78:108-112.
3. Yamamoto Y; Jones KA; Mak BC; Yeung RS. Multi-compartmental distribution of the tuberous sclerosis gene products, hamartin and tuberin. *Arch Biochem Biophys,* 2002; 404:210-217.
4. Goncharova EA; Goncharov DA; Eszterhas A; Hunter DS; Glassberg MK; Yeung RS; Walker CL; Noonan D; Kwiatkowski DJ; Chou MM; Panettieri RA; Krymskaya VP. Tuberin regulates activity of p70S6 kinase and phosphorylation of ribosomal protein S6: A functional link between pulmonary lymphangioleiomyomatosis and the TSC2 tumor suppressor gene. *J Biol Chem,* 2002; 277:30958-30967.
5. Gao X; Zhang Y; Arrazola P; Hino O; Kobayashi T; Yeung RS; Ru B; Pan D. Tsc tumor suppressor proteins antagonize amino-acid-TOR signaling. *Nat Cell Biol,* 2002; 4:699-704.
6. Dan HC; Sun M; Yang L; Feldman RI; Sui XM; Yeung RS; Halley DJ; Nicosia SV; Pledger WJ; Cheng JQ. PI3K/AKT pathway regulates TSC tumor suppressor complex by phosphorylation of tuberin. *J Biol Chem,* 2002; 277:35364-35370.
7. Kenerson HL; Aicher LD; True LD; Yeung RS. Activated mTOR pathway in the pathogenesis of tuberous sclerosis complex renal tumors. *Cancer Res,* 2002; 62:5645-5650.
8. Li Y; Inoki K; Yeung R; Guan KL. Regulation of TSC2 by 14-3-3 binding. *J Biol Chem,* 2002; 277:44593-44596.
9. Mak BC; Takemaru KI; Kenerson HL; Moon RT; Yeung RS. The tuberin-hamartin complex negatively regulates b-catenin signaling activity. *J Biol Chem,* 2003; 278:5947-5951.

VAPSHCS/GENERAL SURGERY

LORRIE LANGDALE, M.D.

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- 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 in 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

Ironically, cytokines generally accepted as pro-inflammatory (and thus potentially harmful in nature), have also been shown to confer protection under clinically relevant conditions.

injury. 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

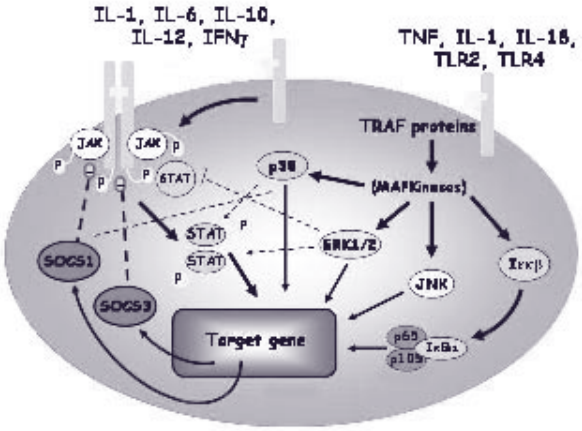

FIGURE 1: Cell Signaling Pathways of Inflammation

transcription proteins (STATs), which are initiated when cytokines such as IL-1, IL-6, IL-12 and IFNγ 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.

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

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

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 perinatal 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.

RELATED PUBLICATIONS

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

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• Heparin, Platelets, and Vascular Cells

FUNDING
National Institutes of Health
VA Merit Review Grant

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

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.

Heparin Interactions with von Willebrand Factor

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

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

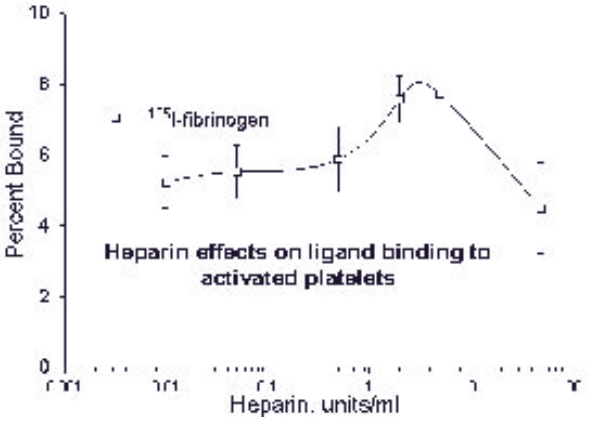

FIGURE 1: 125I-Fibrinogen binding to thrombin-activated platelets was measured over a range of heparin concentrations. At concentrations of 2 and 5 units/ml heparin, fibrinogen binding was significantly increased.

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 ($\alpha_{IIb}\beta_3$), the K562 cell line was transfected with different integrins, and the effects of heparin on integrin-mediated cell adhesion were studied. Surprisingly, the effect of heparin on integrin function depended on the integrin subunit. A stimulatory effect was observed in all β_3 containing integrins ($\alpha_{IIb}\beta_3$, $\alpha_v\beta_3$) but the type of α subunit did not seem to be as important. The effect of heparin was structure specific, as other glycosaminoglycans and low molecular weight heparins showed no enhancement of adhesion[6]. Because integrins are such ubiquitous receptors in vascular cells, a detailed understanding of precisely how heparin modulates these receptors may lead to novel drugs to modulate thrombosis and vascular healing.

Heparin Modulation of Endothelial Cell Migration and Proliferation

Matrix proteins and growth factors (and their respective cellular receptors — integrins and receptor tyrosine kinases) are key actors in angiogenesis and vascular healing. Integrins and growth factor receptors work together to enhance the extracellular signals from each pathway, leading to increased endothelial cell proliferation and migration. Vascular Endothelial Growth Factor (VEGF) and fibronectin appear to have a unique complementary relationship. In a recent publication, VEGF was shown to preferentially bind to fibronectin over other matrix proteins[7]. Platelets actually release pre-formed VEGF/fibronectin complexes, and these complexes have significantly more potent mitogenic effects than VEGF or fibronectin alone on endothelial cells. Heparin further supports the synergistic biological effects of VEGF/fibronectin. Once again, heparin (and cell-surface heparan sulfate proteoglycans) may be playing a key role in modulating the extracellular assembly of specific ligands on their cellular receptors.

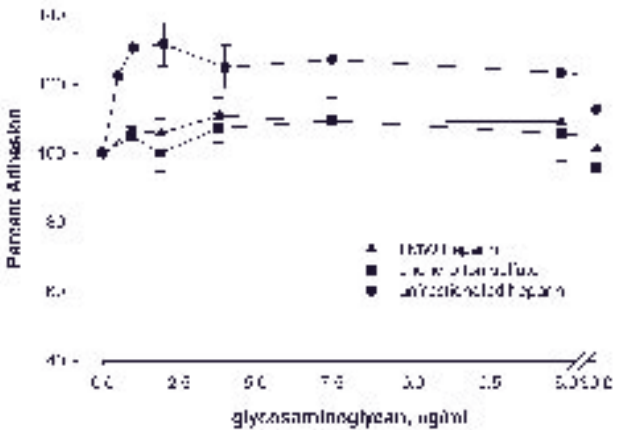

FIGURE 2: Thrombin activated platelets.

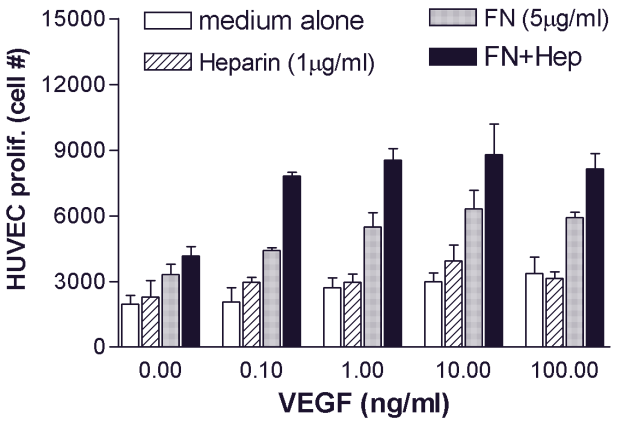

FIGURE 3: Adhesion of K562 $\alpha_v\beta_3$ cells to vitronectin. Unfractionated heparin enhances integrin-mediated adhesion, but other glycosaminoglycans do not.

RELATED PUBLICATIONS

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

VASCULAR SURGERY

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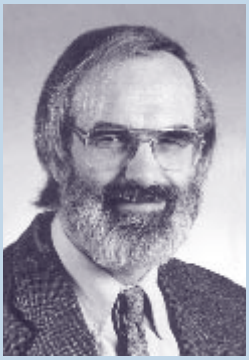


- Tissue Pulsatility Imaging for the Evaluation of Tissue Perfusion
- Tissue Pulsatility Imaging of Muscle
- Intracranial Tissue Pulsatility Imaging (TPI)
- Arteriolar and Venular Plethysmograpic Imaging
- TPI and Doppler Waveforms
- Real-Time Vibrometry

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.

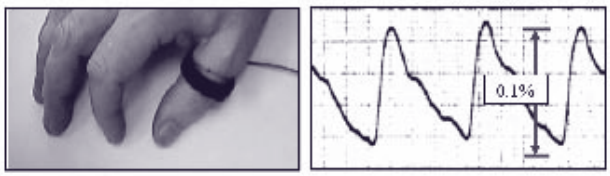


FIGURE 1: (Left) Mercury strain gauge plethysmograph around the thumb; (Right) three cardiac cycles of the plethysmographic waveform from the thumb.

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

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 wave-forms from 40,000 voxels from a single 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. We call the results "Tissue Pulsatility Imaging" (TPI).

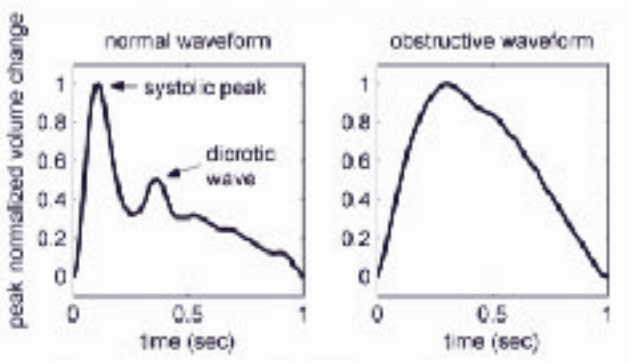


FIGURE 2: Normal and obstructive (oxygen deficit) plethysmographic waveforms shown for a single cardiac cycle.

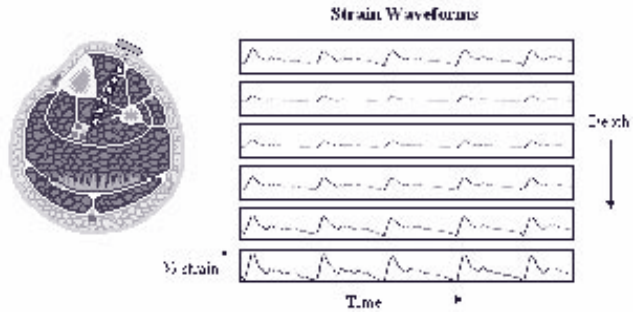


FIGURE 3: Tissue expansion (strain) measured at multiple depths in the anterior tibial muscle (schematic at left). The strain waveforms are from sample volumes separated by approximately 1 mm in depth. Notice the similarity of the waveshapes to the normal waveshape in Figure 2.

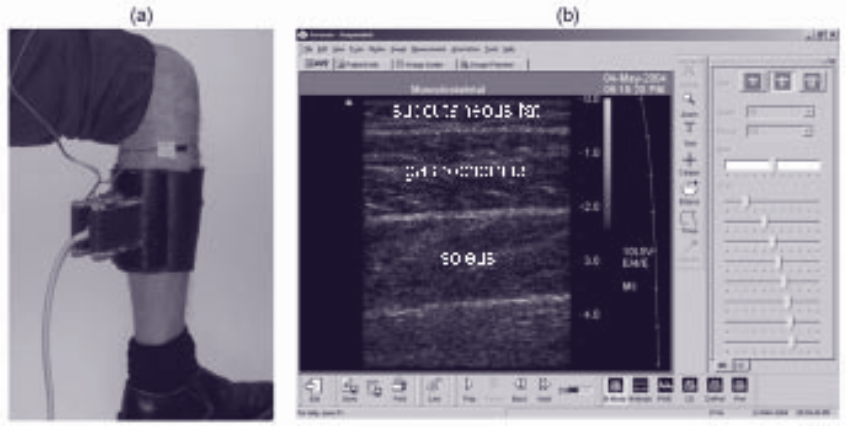


FIGURE 4: (a) Photograph of the ultrasound transducer and strain gauge around the leg. (b) Screen shot taken from the ultrasound scanner showing the muscle anatomy. Notice that the ultrasound scanhead must be held in a stable position during the 10 second periods of data acquisition.

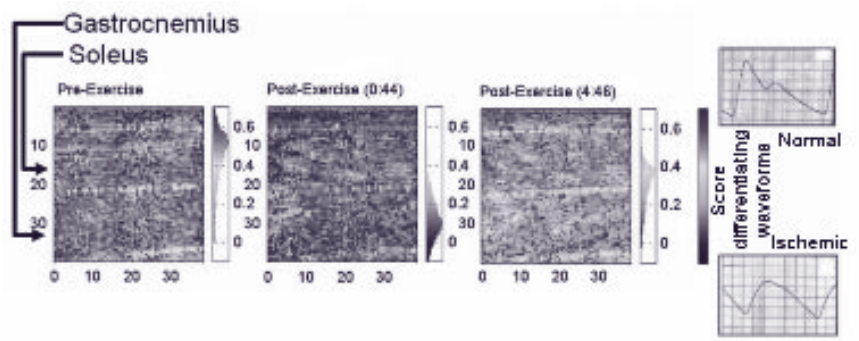


FIGURE 5: Results From a 2D Pulsatility Exercise Study. The histogram to the right of each 2-dimensional image shows the distribution of "more normal" and "more ischemic" voxels in the image. (Left) pre-exercise; (Center) < 1 minute post-exercise; (Right) ~5 minute post-exercise.

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, left). 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, center). After 5 minutes of rest, the muscles are still in the process of recovering (Figure 5, right). We plan to use this method to identify normal and ischemic tissue.

Intracranial Tissue Pulsatility Imaging (TPI)

TPI is able to study arterial and venous perfusion effects with a time resolution of 10 seconds (to span both the cardiac cycle and respiratory cycle for venular effects) and spatial resolution of millimeters in most regions of the body. MRI is an alternative way to study these effects, however, the cost of MR is high and MRI systems are not portable. We are developing applications of TPI in the brain to study intracranial pressure effects (Figure 6) and other phenomona.

In a test, measuring pulse arrival time in the brain tissues of a volunteer on a tilt table, the pulse arrives in the brain earlier when the head is tilted down and later when the head is tilted up. This may be caused by the increased speed of pulse propagation when carotid artery pressures are higher with the head down and the decreased speed of pulse propagation when the carotid artery pressures are lower with the head tilted up.

The primary regulation of blood flow in the brain is the level of carbon dioxide (CO_2). With elevated CO_2, the blood flow to the brain increases. That increased flow appears as increased pulse amplitude (Figure 7) in the central regions of the brain.

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 3 sources of data from the Soleus muscle (Figure 8).

Independent Component Analysis accepts a series

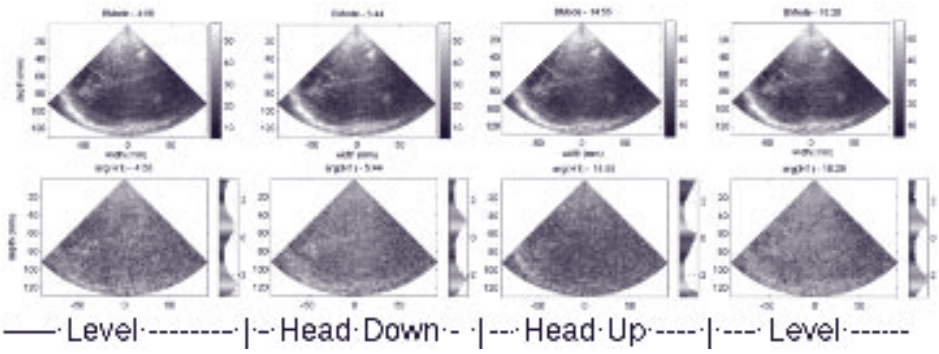


FIGURE 6: Effect of Increased Intracranial Pressure on Pulsatile Waveforms.
UPPER: B-mode images of the brain through the temporal window of the skull.
LOWER: 2-dimensional images of the pulse timing.
LOWER: Histograms showing timing of the pulse waveform with respect to the ECG.
The volunteer was on a tilt table, and tilted sequentially level, head down, head up, level . Although the gray scale does not show the changes in the pulse timing in the 2-dimesional images, the histograms on the right of each 2-D image show a systematic shift. With head down, the pulse arrives (on the average) 42 milliseconds earlier than when the volunteer is level, with the head tilted up, the pulse arrives (on the average) 15 milliseconds later than when the volunteer is level.

of input signals (in this case, from a series of voxels in the soleus contained in the black box, Figure 6) and automatically identifies the component signals that are combined in different ways in each of the voxels. In this example, ICA was not given the wave shapes of the source signals. Two of the signals resemble expected signals, the arteriolar pulse and the venular (respiratory associated) cycle. David Sumner (co-author with Strandness of Hemodynamics for Surgeons) has identified the remaining signal as representing the autonomic reactivity of the vessels.

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 this model, 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

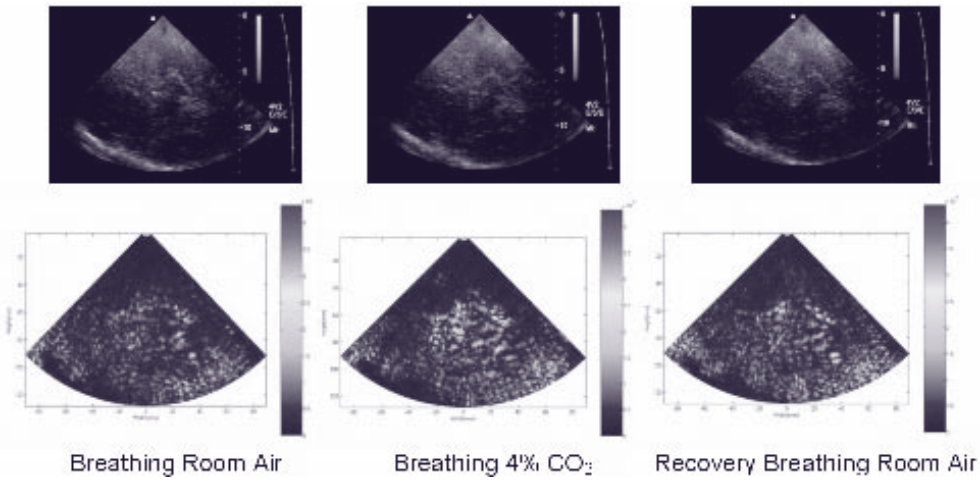


FIGURE 7: Brain Pulse Amplitude Associated with Vasodilatation Due to CO_2 Exposure.
UPPER: B-mode image
LOWER: Pulse Amplitude Image showing increased pulse amplitude during CO_2 exposure. (Left) The highest pulse amplitude = 0.2%; (Right) The highest pulse amplitude = 0.25%; (Center) The highest pulse amplitude is 0.43%.

Arterial bleeding causes tissue vibrations in the audible frequency range, but below the audible intensity threshold of a stethoscope. We are now able to include the display of vibration frequency and amplitude in ultrasound images to permit the identification localization of bleeding sites and other sources of bruits and murmurs.

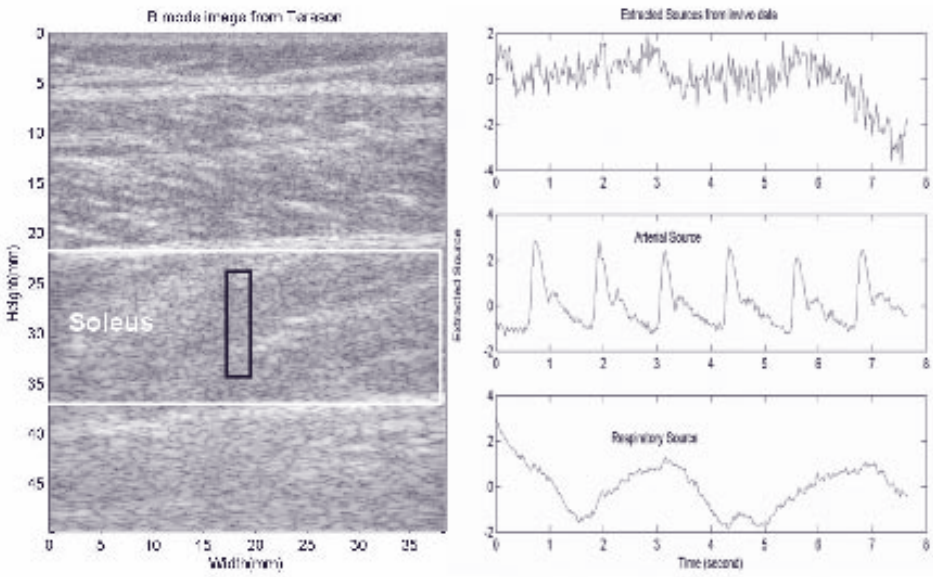
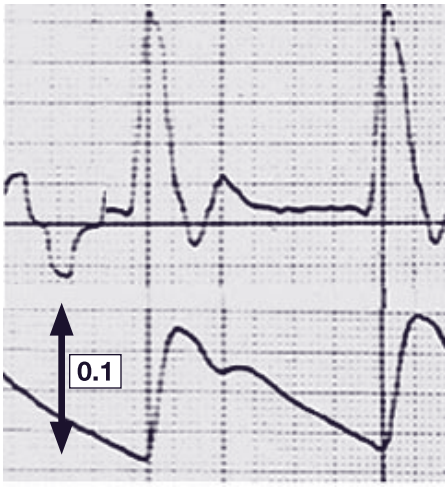


FIGURE 8: Independent Component Analysis applied to the Soleus Muscle. Extracted components including the arterial waveform (Right Middle), venous waveform (Right Lower) and an additional component (Right Upper).



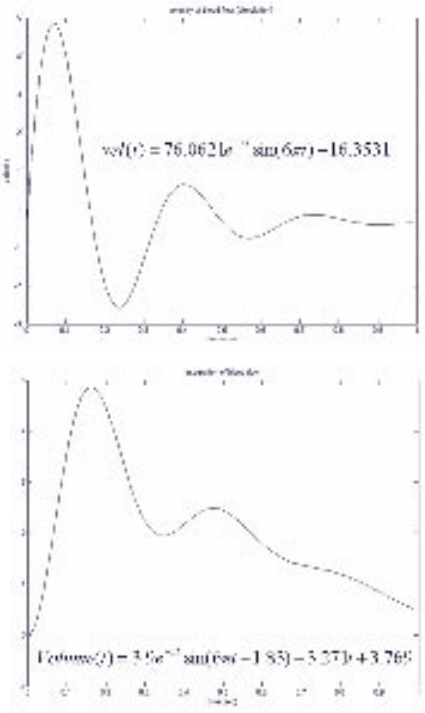


FIGURE 9: Damped Oscillator Parametric Model of Arterial Flow and TPI Waveform.

LEFT UPPER: Arterial Doppler waveform from the brachial artery (10 cm/s calibration)

LEFT LOWER: Time registered strain gauge plethysmographic waveform from finger. Notice plethysmographic waveform is 80 milliseconds delayed from the arterial waveform because of the time required for the pulse to propagate along the artery.

RIGHT UPPER: Windkessel model blood flow waveform for use in analysis.

RIGHT LOWER: Mathematical integration of the model velocity waveform to match with plethysmographic wave from each voxel in tissue.

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

The detection of the source occult internal bleeding allows therapy to be rapidly instituted. Arterial bleeding causes tissue vibrations in the audible frequency range, but below the audible intensity threshold of a stethoscope. We are now able to include the display of vibration frequency and amplitude in ultrasound images to permit the identification localization of bleeding sites and other sources of bruits and murmurs.

By using the vibration frequency and the amplitude, a mathematical formula based on the Strouhal number allows the computation of the rate of theoretical blood loss.

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 1955. 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 many arteries, but their effectiveness in the carotid arteries is still under investigation. The Ultrasound Reading Center 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.

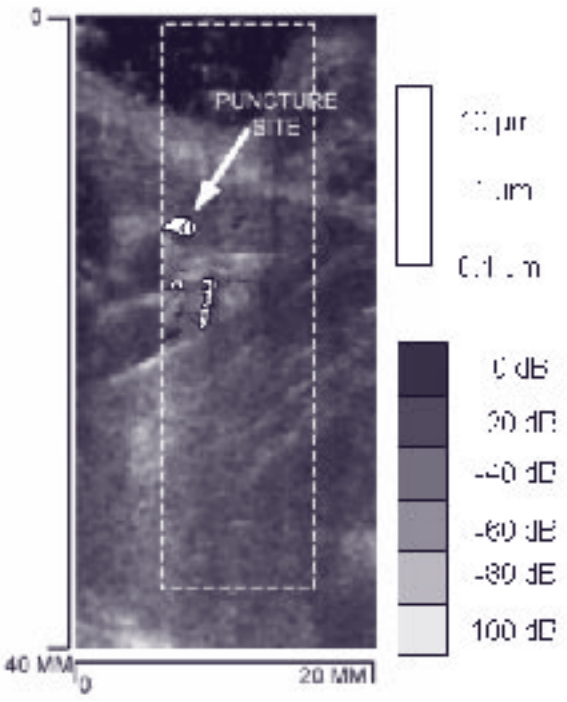


FIGURE 10: Vibration Caused by Arterial Bleeding. The amplitude of the vibrations caused by this arterial bleed are about 1 micrometer. Vibrations are present at the bleeding site (13 mm deep) and at nearby locations (17-19 mm deep). The vibrating regions are shown as white with the vibration amplitudes shown as contour lines.

RELATED PUBLICATIONS

1. Kucewicz JC; Huang L; Beach KW. Plethysmographic arterial waveform strain discrimination by Fisher's method. *Ultrasound Med Biol,* 2004 Jun; 30(6):773-82.
2. Bruce M; Averkiou M; Tiemann K; Lohmaier S; Powers J; Beach K. Vascular flow and perfusion imaging with ultrasound contrast agents. *Ultrasound Med Biol,* 2004 Jun; 30(6):735-43.

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• 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 (see figure 1). Further surgery is required since no pharmacology is available to inhibit this process. The primary objective of our laboratory is to understand the factors that stimulate and inhibit the growth of smooth muscle cells, and to develop new strategies for the pharmacological control of intimal hyperplasia.

Regulation of intimal hyperplasia in damaged arteries: We use the rat carotid artery stripped of its endothelium by the passage of a balloon embolectomy catheter as a simplified model of vascular repair after endarterectomy or angioplasty. As in human arteries, the response to injury in rat carotid arteries involves a series of events leading to intimal hyperplasia. Medial smooth muscle cells start proliferating at 24-48 hours. They begin to migrate into the intima at four days, and they continue to proliferate and to synthesize matrix for several weeks before resuming the resting state. The net result is a substantial increase in wall mass.

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

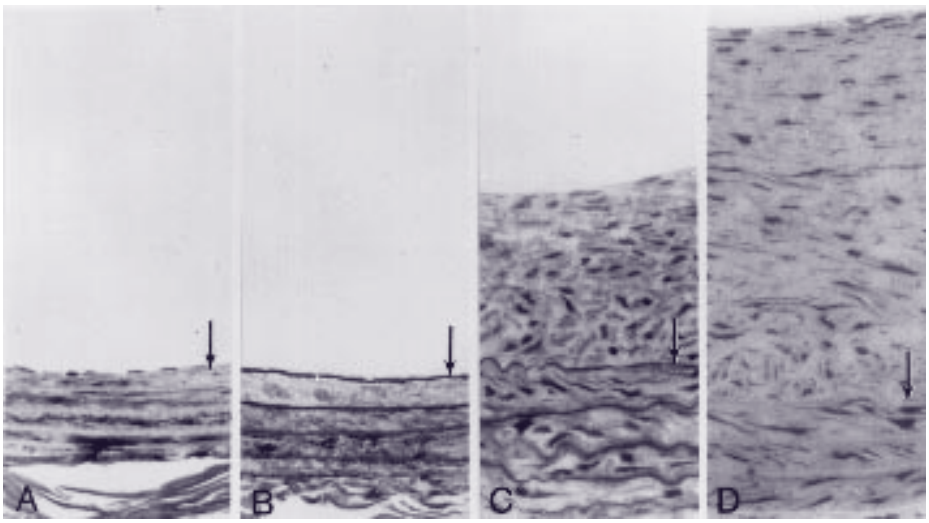

FIGURE 1: This series of photographs shows how a normal rat carotid artery (panel A-histologic cross-section) responds to injury. Angioplasty of the artery removes the surface endothelium (panel B). By two weeks (panel C), smooth muscle cells have migrated from the media into the intima (region above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

Recent studies in the laboratory have defined a novel pathway of smooth muscle cell activation which depends on these receptors. Thrombin and Factor Xa can induce cell growth by interacting with its G-protein coupled receptor. 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. We have found that the FGF receptor, instead of the EGF receptor, mediates the cellular stimulus induced by PDGF, thrombin, and Factor Xa in human vascular smooth muscle cells. We are currently pursuing experiments designed to understand "cross-talk" 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 phosphrotein (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: We have found that smooth muscle cell proliferation and neointimal hyperplasia in primate PTFE grafts are exquisitely regulated by changes in blood flow. 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.

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.

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.

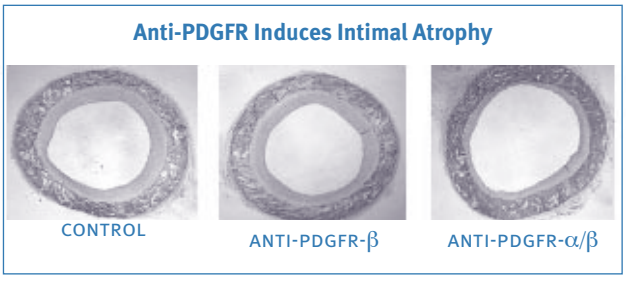

FIGURE 2: Histological cross-sections of normal flow PTFE grafts at 2 weeks following initiation of treatment with vehicle control, blocking antibodies to PDGFR-β, or blocking antibodies to both PDGFR-α and PDGFR-β. (H&E staining, 16X).

Blockade of both PDGF receptors may be necessary. When we block both PDGFR-α and PDGFR-β, we not only suppress intimal thickening but we induce ca 50% intimal atrophy (Figure 2) by 2 weeks. This novel finding indicates to us that restenosis might be a pharmacologically reversible process.

RELATED PUBLICATIONS

1. Mattsson EJR; Kohler TR; Vergel SM; Clowes AW. Increased blood flow induces regression of intimal hyperplasia. *Arterioscler Thromb Vasc Biol*, 1997; 17:2245-2249.
2. Kalmes A; Vesti BR; Daum G; Abraham JA; Clowes AW. Heparin blockade of thrombin-induced smooth muscle cell migration involves inhibition of epidermal growth factor (EGF) receptor transactivation by heparin-binding EGF-like growth factor. *Circ Res*, 2000; 87:92-98.
3. Kenagy RD; Fischer JW; Davies MG; Berceli SA; Hawkins SM; Wight TN; Clowes AW. Increased plasmin and serine proteinase activity during flow-induced intimal atrophy in baboon PTFE grafts. *Arterioscler Thromb Vasc Biol*, 2002; 22:400-404.
4. Berceli SA; Davies MG; Kenagy RD; Clowes AW. Flow-induced neointimal regression in baboon polytetra fluoroethylene grafts is associated with decreased cell proliferation and increased apoptosis. *J Vasc Surg*, 2002; 36:1248-1255.
5. Chan AK; Kalmes A; Hawkins S; Daum G; Clowes AW. Blockade of the epidermal growth factor receptor decreases intimal hyperplasia in balloon-injured rat carotid artery. *J Vasc Surg*, 2003; 37(3):644-649.
6. Serruys PW; Heyndrickx GR; Patel J; Cummins PA; Kleijne JA; Clowes AW. Effect of an anti-PDGF-beta-receptor-blocking antibody on restenosis in patients undergoing elective stent placement. *Int J Cardiovasc Intervent*, 2003; 5:214-222.
7. Englesbe MJ; Hawkins SM; Hsieh PC; Daum G; Kenagy RD; Clowes AW. Concomitant blockade of platelet-derived growth factor receptors alpha and beta induces intimal atrophy in baboon PTFE grafts. *J Vasc Surg*, 2004; 39:440-446.
8. Chitaley K; Chen L; Galler A; Walter U; Daum G; Clowes AW. Vasodilator-stimulated phosphoprotein is a substrate for protein kinase C. *FEBS Lett*, 2004; 556:211-215.
9. Rauch BH; Millette E; Kenagy RD; Daum G; Clowes AW. Thrombin- and factor Xa-induced DNA synthesis is mediated by transactivation of fibroblast growth factor receptor-1 in human vascular smooth muscle cells. *Circ Res*, 2004; 94:340-345.
10. Chen L; Daum G; Chitaley K; Coats SA; Bowen-Pope DF; Eigenthaler M; Thumati NR; Walter U; Clowes AW. Vasodilator-stimulated phosphoprotein regulates proliferation and growth inhibition by nitric oxide in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol*, 2004; 24:1403-1408.

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- 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.

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.

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 4 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 1 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

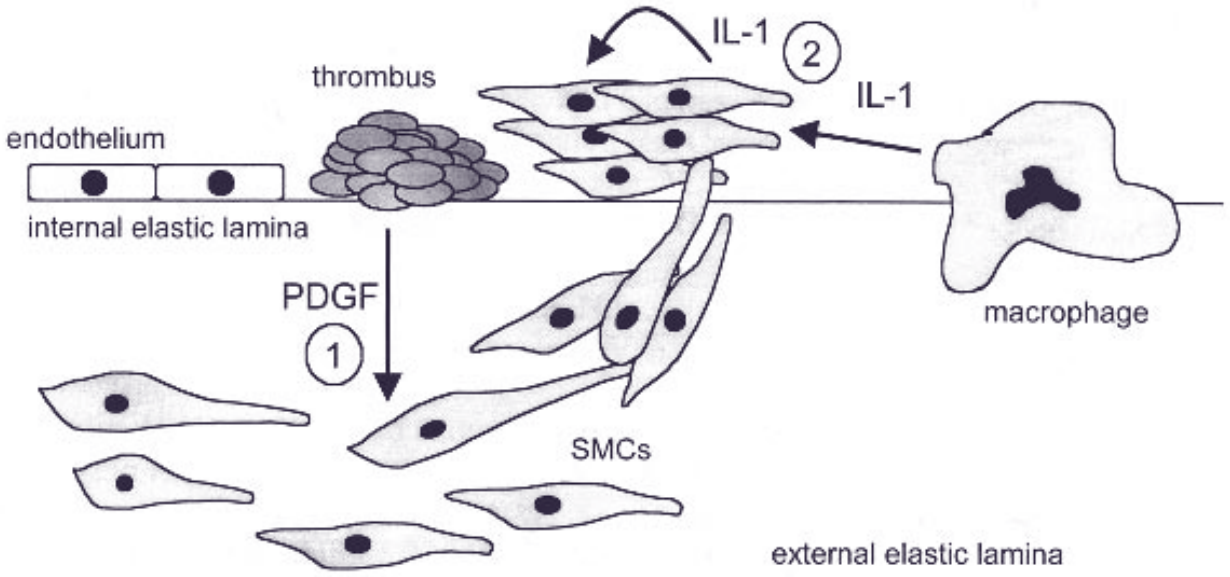

FIGURE 1: Potential Cross-Talk Between IL-1 and PDGF. 1) Following arterial injury, platelets deposit and release PDGF, which functions as a chemoattractant for SMCs. 2) IL-1 is released by the contracting thrombus and by adhering macrophages. 3) IL-1 promotes proliferation of intimal SMCs. Sources of 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.

(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 inhibiting 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.

RELATED PUBLICATIONS

1. Liao DF; Duff JL; Daum G; Pelech SL; Berk BC. Angiotensin II stimulates MAP kinase kinase kinase activity in vascular smooth muscle cells: Role of raf. *Circ Res,* 1996; 79:1007-1014.
2. Daum G; Hedin U; Wang T; Clowes AW. Diverse effects of heparin on mitogen-activated protein kinase dependent signal transduction in vascular smooth muscle cells. *Circ Res,* 1997; 81:17-23.
3. Hedin U; Daum G; Clowes AW. Disruption of intergrin a5b1-signaling does not impair PDGF-BB-mediated stimulation of the extracellular signal-regulated kinase pathway in smooth muscle cells. *J Cell Physiol,* 1997; 172:109-116.
4. Lille S; Daum G; Clowes MM; Clowes AW. The regulation of p42/p44 mitogen-activated protein kinases in the injured rat carotid artery. *J Surgical Res,* 1997; 70:178-186.
5. Daum G; Kalmes A; Levkau B; Wang Y; Davies MG; Clowes AW. Pervanadate inhibits mitogen-activated protein kinase kinase-1 in a $p38^{MAPK}$ dependent manner. *FEBS Lett,* 1998; 427:271-274.
6. Daum G; Levkau B; Chamberlain NL; Wang Y; Clowes AW. The mitogen-activated protein kinase pathway contributes to vanadate toxicity in vascular smooth muscle cells. *Mol Cell Biochem,* 1998; 183:97-103.
7. Hedin U; Daum G; Clowes AW. Heparin inhibits thrombin-induced mitogen-activated protein kinase signaling in baboon arterial smooth muscle cells. *J Vasc Res,* 1998; 27:512-520.
8. Kalmes A; Deou J; Clowes AW; Daum G. Raf-1 is activated by the p38 mitogen-activated protein kinase inhibitor SB203580". *FEBS Lett,* 1999; 444:71-74.

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- Magnetic Resonance Imaging of the High-Risk Atherosclerotic Plaque

FUNDING
National Institutes of Health
Astra-Zeneca Pharmaceutical
Pfizer Pharmaceutical

Introduction

Complications of atherosclerosis are the leading cause of morbidity and mortality in many countries, and a major contributor to the rising cost of health care. Although much has been learned from studies in animal models of atherosclerosis, translation of basic science findings into improvements in the diagnosis and treatment of human vascular disease has been difficult. Efforts to better understand the pathophysiology of human atherosclerosis has been hampered by the inability to accurately assess the diseased arterial wall in a serial, non-invasive fashion. The development of imaging techniques to accurately characterize the morphology and composition of the vessel wall is critical for progress to be made in understanding the development of human vascular disease and its response to therapy. Improved methods for detecting those at increased risk for vascular disease progression will permit initiation of preventative and therapeutic measures at an earlier, sub-clinical stage. Better stratification of individuals into those likely to respond versus those likely to fail therapy will permit more cost-effective post-procedure surveillance in the subgroup at greatest risk.

Magnetic resonance imaging (MRI) has developed into a highly promising tool for quantifying atherosclerosis plaque burden and for characterizing the morphology and composition of the diseased vessel wall in vivo. MRI is ideally suited for serial examination of disease progression, as it is non-invasive. It has superior capability for distinguishing tissue types, compared to other imaging modalities. This article will briefly review work performed in this laboratory validating the MRI technique, using detailed histological examination of corresponding excised carotid endarterectomy specimens as a gold standard.

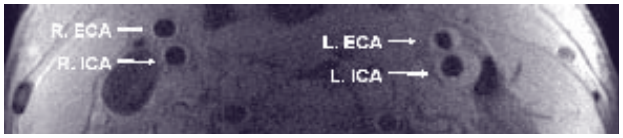

FIGURE 1: MRI of right and left internal and external carotid arteries demonstrating good suppression of flow artifact and clear delineation of the lumen and outer boundary of normal (right) and diseased (left) carotid arteries. Note evidence of compensatory (expansive) enlargement on the left side. 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.

Methodology

Significant improvements in MR image quality have been made possible by a combination of hardware development, in the form of custom-made surface coils that increase signal-to-noise, and novel image acquisition sequences that reduce artifact and image acquisition time (Figure 1). With approval of the University of Washington Institutional Review Board and following informed consent, subjects who are scheduled for carotid endarterectomy undergo pre-operative *in vivo* MRI examination of the carotid arteries. The endarterectomy specimen is excised with minimal handling and disruption of the plaque to preserve the overall morphology and lumen surface characteristics (Figure 2). Ten-μm thick sections are obtained every 0.5 to 1.0 mm throughout the length of the surgical

Magnetic resonance imaging (MRI) has developed into a highly promising tool for quantifying atherosclerosis plaque burden and for characterizing the morphology and composition of the diseased vessel wall in vivo.

specimen, and corresponding MRI cross-sections are identified using the common carotid bifurcation and overall shape of the lumen as internal fiducial markers. Analysis of the findings are performed by independent readers who are blinded to the corresponding histology or MRI results.

Results

Plaque Burden: In a study comparing in vivo carotid MRI to ex vivo MRI of fresh excised endarterectomy specimens, there was a high level of agreement in MRI measurements of the disease arterial wall. Paired in vivo and ex vivo maximal wall area measurements demonstrated a mean difference of 13 mm^2 and the SD of the difference of only 6.5 mm^2. This area difference (13.1 mm^2) corresponds to a thickness of 0.4 mm, which is consistent with the thickness of the adventitia and residual media left behind following endarterectomy. Intra- and inter-observer variability was small: intra-observer intraclass correlation coefficient (ICC) was 0.95 (lower bound of 95% CI = 0.87) and inter-observer ICC was 0.96 (lower bound of 95% CI = 0.91). Findings from this study demonstrated that this MRI technique is highly accurate for in vivo measurement of maximal wall area in atherosclerotic carotid artery lesions and has low intra- and inter-observer variability. A follow-up study examining the reproducibility for measuring atherosclerotic carotid wall volume demonstrated that in vivo MRI is capable of detecting a 10% change in volume with 95% confidence.

Fibrous Cap Status: Subjects scheduled for carotid endarterectomy underwent an MRI scan using a 3-D multiple overlapping thin slab angiography (MOTSA) protocol. The fibrous cap was categorized as thick, thin, or ruptured on pre-operative MRI and compared to gross and histological examination of the excised carotid endarterectomy specimen. Thick fibrous caps were defined as greater than 0.25 mm on histological examination. On the 3D-MOTSA image, thick caps appear as a juxtaluminal band of low signal. In plaques with thin fibrous caps, this dark juxtaluminal band is absent. In plaques with fibrous cap rupture, the dark band is absent and there is a region of hyperintense signal adjacent to the lumen (Figure 3).

There was a high level of agreement between MRI and histological findings: 89% agreement, Kappa (95% confidence interval) = 0.83 (0.67 – 1.0), weighted Kappa = 0.87. Spearman's correlation coefficient was 0.88, significant to the 0.01 level. Based on these findings, we concluded that high-resolution MRI, using a 3D-MOTSA protocol, is capable of distinguishing intact fibrous caps measuring > 0.25 mm in thickness from intact thin (< 0.25 mm) and disrupted caps in atherosclerotic human carotid arteries in vivo. Blinded comparison demonstrated a sensitivity of 81% (± 6%)

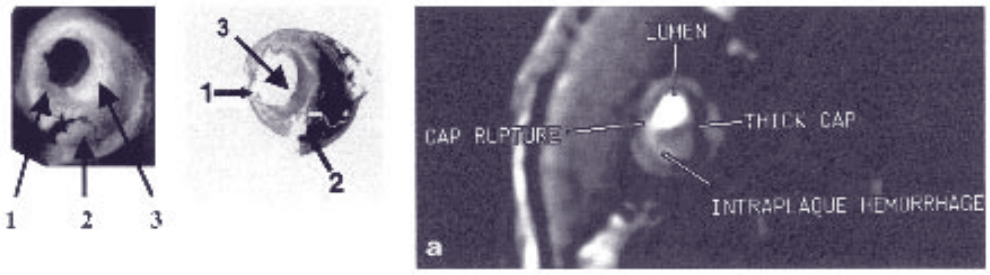

FIGURE 3: Example of a common carotid plaque with fibrous cap rupture and intraplaque hemorrhage. Photo of gross section of common carotid artery (left panel), trichrome stained histological section (middle panel), and corresponding TOF MR image (right panel). Arrow 1 indicates an area of cap rupture,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

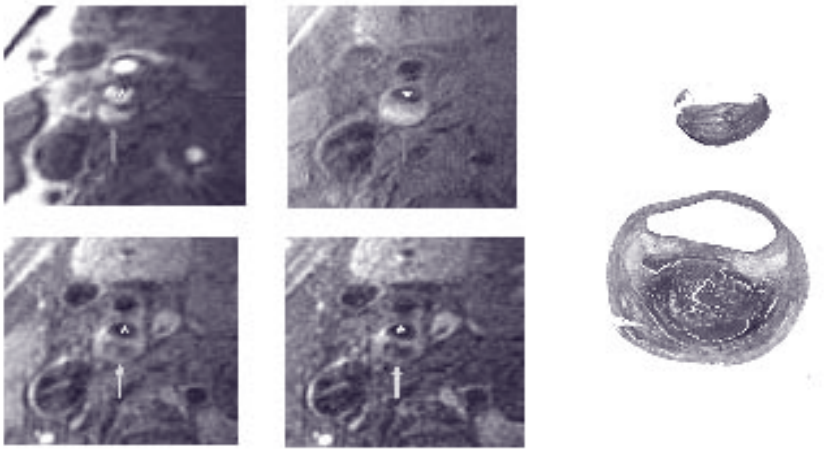

FIGURE 4: Example of an AHA Type VI (complicated) lesion with acute intraplaque hemorrhage. The asterisks indicate the lumen of the internal carotid artery. Early intraplaque hemorrhage, seen on the corresponding histological cross-section on the right, is identified by a hyperintense (bright) signal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

and specificity of 90% (± 2%) for distinguishing a thick cap from a thin or ruptured cap.

American Heart Association (AHA) Lesion Types: A standardized classification scheme is critical for describing the characteristics of the atherosclerotic lesions at baseline and during follow-up in longitudinal studies examining atherosclerosis progression or regression. In a recently published study, 60 patients scheduled for carotid endarterectomy were studied to determine the accuracy of multi-contrast weighted MRI for categorizing carotid plaque characteristics using a modified AHA histological classification scheme. Categories were defined as summarized in Table I. Figure 4 demonstrates the appearance of an AHA Type VI (complicated) lesion, with intraplaque hemorrhage. Overall, there was very good agreement between MRI and histology for classifying atherosclerosis lesion types, with Cohen's Kappa (95% CI) of 0.74 (0.67 to 0.82) and weighted Kappa of 0.79. The sensitivity and specificity of MRI for identifying type III – VII lesions ranged from 80-88% and 90-98%, respectively. The numbers of type I, II, and VIII lesions were too small to obtain good estimates of sensitivity and specificity. This study demonstrated that MRI is capable of accurately describing carotid plaque characteristics in vivo, using standardized, histology-based criteria.

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.

Gadolinium Contrast Enhancement and Neovasculature: Studies suggest that the presence and density of neovasculature within the plaque may be a marker for more active lesions that are potentially unstable. As early as the 1930s, Winternitz reported that neovasculature may be involved in the pathogenesis of atherosclerosis. More recently, O'Brien noted that neovasculature within plaques may represent a pathway for the recruitment of macrophage infiltration, and that the endothelial cells lining these microvessels are a site of inflammatory activation. Work by Galis, Libby and Nikkari have shown that macrophages, typically found in the shoulder regions adjacent to the fibrous cap, express matrix metalloproteinases that can result in weakening of the fibrous cap. Furthermore, studies by McCarthy and Mofidi demonstrated a statistically significant association between the density of neovasculature in carotid endarterectomy specimens and the patients' pre-operative history of TIA or stroke.

In a recent study, we demonstrated that dynamic contrast-enhanced MRI can be used to identify and quantify plaque neovasculature (Figure 5). Sixteen patients scheduled for carotid endarterectomy were imaged with a dynamic contrast enhanced (DCE) MRI protocol. Images were obtained at 15 sec intervals and a gadolinium contrast agent was injected coincident with the second of 10 images in the sequence. The resulting image intensity within the plaque was tracked over time

and analyzed using the Patlak model. From the model, the fractional blood volume within the plaque tissue was computed. Following endarterectomy, sections that matched the MRI were identified and stained (Ulex and CD-31 antibody) to highlight neovessels, and their total area was computed as a fraction of the plaque area. The fractional blood volume measured by DCE MRI and the fractional vascular area of neovessels on histology were highly correlated, with a correlation coefficient of 0.76 ($p < .001$; Figure 6). Kerwin concluded that DCE MRI provides a quantitative indication of the extent of neovasculature within carotid atherosclerotic plaque, and that DCE MRI provides a means for prospectively studying the link between neovasculature and plaque vulnerability.

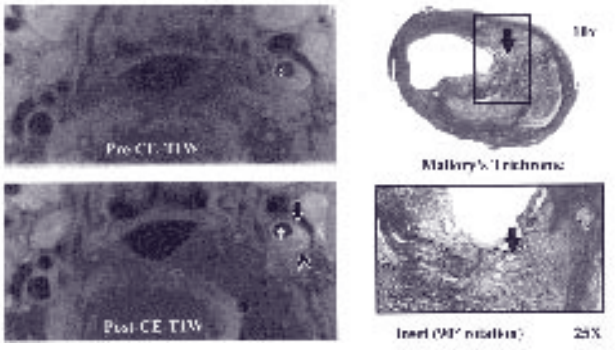

FIGURE 5: Pre-gadolinium contrast enhanced T1-weighted image of common carotid artery in left upper panel, post-contrast enhanced T1W image in left lower panel, and corresponding 10X and 25X trichrome stained histological sections. Note the enhancement seen in the shoulder region (arrow) in the post-contrast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

Clinical Studies

Cap Status and History of TIA or Stroke: We have shown that fibrous cap status, as identified by MRI, is strongly correlated with the clinical status of the patient. In this study, pre-operative carotid artery MRI was performed on 53 consecutive patients and the fibrous cap was categorized as thick, thin or ruptured. Twenty-eight subjects had a history of TIA or stroke on the side appropriate to the index carotid lesion within 90 days prior to the MRI, and 25 were asymptomatic. There was a strong and highly significant association between the *in vivo* state of the fibrous cap and patient symptoms, with a higher percentage of symptomatic patients with thin and ruptured caps compared to a thick cap ($p = 0.001$ Mann-Whitney test for cap status vs. symptoms). 70% of the patients with ruptured fibrous caps had a history of recent TIA or stroke, compared to only 9% of those with thick fibrous caps on MRI. Findings from this study provide further support for prospective studies to assess the predictive value of a thin or ruptured cap, as identified by MRI, for subsequent ischemic events.

Intensive Lipid Lowering and MRI Plaque Characteristics: In a case control study of patients undergoing aggressive lipid-lowering therapy and matched controls, we compared the carotid plaque characteristics of the two groups using MRI. The treatment group had undergone triple therapy for 10 years using niacin, cholestipol, and lovastatin. Baseline LDL prior to MRI was 84 mg/dl in the treated group and 158 mg/dl in the controls. HDL levels were 51 mg/dl and 37 mg/dl respectively. Total plaque area was similar in the two groups (58 and 64 mm^2 in treated and control groups). However, the proportion of the plaque with MRI signal characteristics for lipid was significantly lower amongst those undergoing intensive lipid-lowering therapy (0.7 mm^2 or 1% of total plaque area in the treated group vs. 10.2 mm^2 or 17% of total plaque area in controls). Findings from this study demonstrated the potential of MRI for longitudinally assessing changes not only in the size, but importantly, the composition of human atherosclerosis in response to treatment, *in vivo*.

Conclusions

The development of accurate, high-resolution imaging techniques will be critical for longitudinal studies examining the pathophysiology of human atherosclerosis. 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

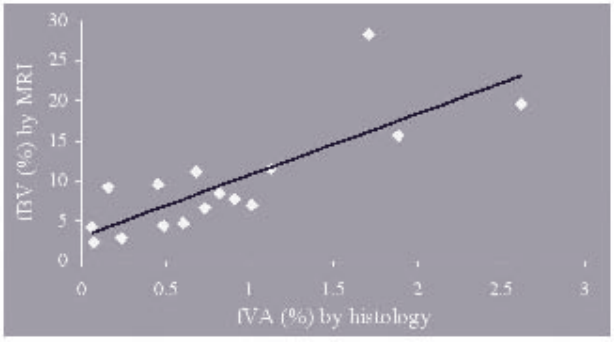

FIGURE 6: Scatter plot demonstrating high correlation ($R = 0.80$, $p < 0.001$) between fractional blood volume in plaque wall on contrast-enhanced MRI and size/concentration of plaque neovasculature (fractional vascular area) in corresponding histological cross-sections.

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 associated risk factors will permit identification of high-risk individuals and more selective, appropriate intervention, and potentially lead to the development of novel methods for therapeutic intervention.

RELATED PUBLICATIONS

1. Nelson JA; Yuan C; Hatsukami TS. MR cardiovascular imaging. *Nature Medicine,* 1995; 1:996-997.
2. Hatsukami TS; Ferguson M; Beach KW; Gordon D; Detmer P; Burns D; Alpers C; Strandness DE, Jr. Carotid plaque morphology and clinical events. *Stroke*, 1997; 28:95-100.
3. Yuan C; Beach KW; Smith HL; Hatsukami TS. Measurement of atherosclerotic plaque size in vivo using high-resolution magnetic resonance imaging. *Circulation,* 1998; 98(24):2666-2671.
4. Hatsukami TS; Ross R; Yuan C. Visualization of fibrous cap thickness and rupture in human atherosclerotic carotid plaque *in-vivo* with high resolution magnetic resonance imaging. *Circulation,* 2000; 102:959-964.
5. Yuan C; Mitsumori LM; Ferguson MS; Polissar NL; Echelard D; Ortiz G; Small R; Davis JW; Hatsukami TS. The *in vivo* accuracy of multispectral MR imaging for identifying lipid-rich necrotic cores and intraplaque hemorrhage in advanced human carotid plaques. *Circulation,* 2001; 104:2051-6.
6. Yuan CY; Zhang S; Polissar N; Echelard D; Ortiz J; Davis JW; Ellington E; Hatsukami TS. Identification of fibrous cap rupture with magnetic resonance imaging is highly associated with recent TIA or stroke. *Circulation,* 2002; 105(2):181-185.
7. Cai JM; Hatsukami TS; Ferguson MS; Small R; Polissar NL; Yuan C. Classification of human carotid atherosclerotic lesions with *in vivo* multi-contrast MR imaging. *Circulation,* 2002; 106:1368-1373.
8. Yuan C; Miller ZE; Cai JM; Hatsukami T. Carotid atherosclerotic wall imaging by MRI. In: Fayad ZA ed. *Neuroimaging Clinics of North America*, 2002; 12(3):391-401, vi. Philadelphia: W.B. Saunders Company, 2002.
9. Kerwin W; Hooker A; Spilker M; Vicini P; Ferguson M; Hatsukami T; Yuan C. Quantitative MRI analysis of neovasculature volume in carotid atherosclerotic plaque. *Circulation,* 2003; 107:851-856.
10. Hatsukami TS; Kerwin W; Cai JM; Kang XJ; Polissar N; Ferguson M; Small R; Zhao XQ; Yuan C. High-resolution magnetic resonance imaging of carotid atherosclerosis. *International Congress Series,* 2004; 1262C:95-98.

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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

FUNDING
National Institutes of Health
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.

Intimal hyperplasia causes failure of almost one-third of all vascular reconstructions… Our laboratory is studying the effects of altered blood flow on intimal hyperplasia, and is evaluating new vascular devices to reduce restenosis.

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,

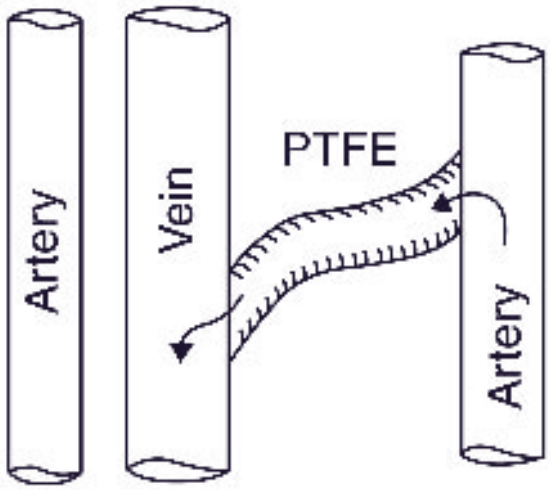
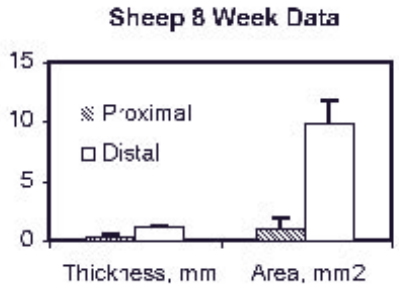


FIGURE 1: Fistula created between artery and vein to provide high-flow conduit

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.

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



GRAPH 1: Sheep Eight Week Data

fistula beyond the graft. We have also found that special coating of the graft surface with phospholipids can stop this thickening process.

The three principle components of graft healing and lumen narrowing are endothelial ingrowth, smooth muscle cell proliferation, and thrombosis. These are evaluated using scanning electron microscopy, morphometry, and 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.

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.

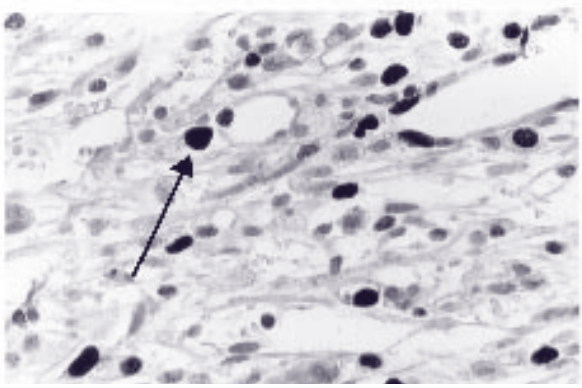


FIGURE 2: PCNA-positive nucleus

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.

We will compare standard PTFE grafts with grafts that are more porous. Increased porosity allows in-growth 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.

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

RELATED PUBLICATIONS

1. Mattsson E; Kohler TR; Vergel S; Liao JK; Clowes AW. Increased blood flow induces regression of intimal hyperplasia. *Arterio Thromb Vasc Biol,* 1997; 17:2245-2249.
2. Kohler TR; Kirkman TR. Central venous catheter failure is induced by injury and can be prevented by stabilizing the catheter tip. *J Vasc Surg,* 1998; 28:59-66.
3. Kohler TR; Kirkman TR. Dialysis access failure: A sheep model of rapid stenosis. *J Vasc Surg,* 1999; 30:744-51.
4. Gibson KD; Caps MT; Kohler TR; Hatsukami TS; Gillen DL; Aldassy M; Sherrard DJ; Stehman-Brenn CO. Assessment of a policy to reduce placement of prosthetic hemodialysis access. *Kid Int,* 2001; 59:2335-45.
5. Fontaine AB; Nicholls S; Borsa JJ; Hoffer E; Bloch RD; Kohler TR. Seat belt aorta: Endovascular management with a stent-graft. *J Endovasc Ther,* 2001; 8:83-86.
6. Leotta DF; Paun M; Beach KW; Kohler TR; Zierler RE; Strandness DE, Jr. Measurement of abdominal aortic aneurysms using three-dimensional ultrasound imaging: Preliminary report. *J Vasc Surg,* 2001; 33:700-7.
7. Gibson KD; Stehman-Brenn CO; Kohler TR. Use of the vascular diagnostic laboratory in improving the success of angioaccess procedures. *Sem Vasc Surg,* 2001; 14:222-26.
8. Gibson KD; Gillen DL; Caps MT; Kohler TR; Sherrard DJ; Stehman-Breen CO. Vascular access and incidence of revisions: A comparison of prosthetic grafts, simple autogenous and venous transposition fistulas from the United States Renal Data System Dialysis Morbidity and Mortality Study (USRDS DMMS. *J Vasc Surg,* 2001; 34:694-700.
9. Yutan E; Glickerman DJ; Caps MT; Hatsukami T; Harley JD; Kohler TR; Davies MG. Percutaneous transluminal revascularization for renal artery stenosis: VA Puget Sound Health Care experience. *J Vasc Surg,* 2001; 34:685-93.
10. Leotta DF; Paun M; Beach KW; Kohler TR; Zierler RE; Strandness DE, Jr. Measurement of abdominal aortic aneurysms using three-dimensional ultrasound imaging: Preliminary report. *J Vasc Surg,* 2001; 33:700-7.

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Daniel F. Leotta, Ph.D.

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

FUNDING
National Institutes of Health

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 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

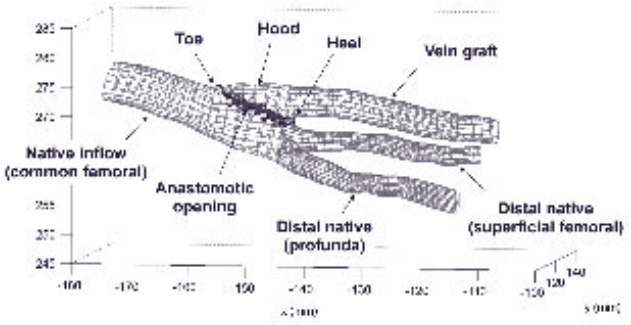

FIGURE 1: Components of a vein graft proximal anastomosis that are used for analysis of the 3D geometry. The anastomosis geometry is quantified by 1) the cross-sectional areas of the graft, native inflow artery, and the combined native/hood segment, 2) the cross-sectional area and shape of the anastomotic 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.

A recent development in abdominal aortic aneurysms 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.

distinguish normal remodeling from pathologic changes that threaten vein graft patency.

We are also able to track changes following endovascular interventions. Figure 1 shows a 3D reconstruction of the distal anastomosis of a bypass graft with a stent placed distal to the graft to treat a stenosis. Sequential follow up studies show progressive narrowing of the vessel within the stent (Figure 2). Enhanced displays of the 3D computer reconstructions are also being developed for more intuitive presentation of vessel anatomy. Figure 3 shows the proximal anastomosis of a jump graft with a calibrated '3D tape measure' placed along the center axis of the graft. Its fixed width of 2 mm provides a reference for vessel diameter, and grid marks are placed at 1-mm and 1-cm intervals to provide distance references. In addition, the skin surface is displayed along the track of the ultrasound transducer to indicate the depth of the graft.

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 for diagnosis and in 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

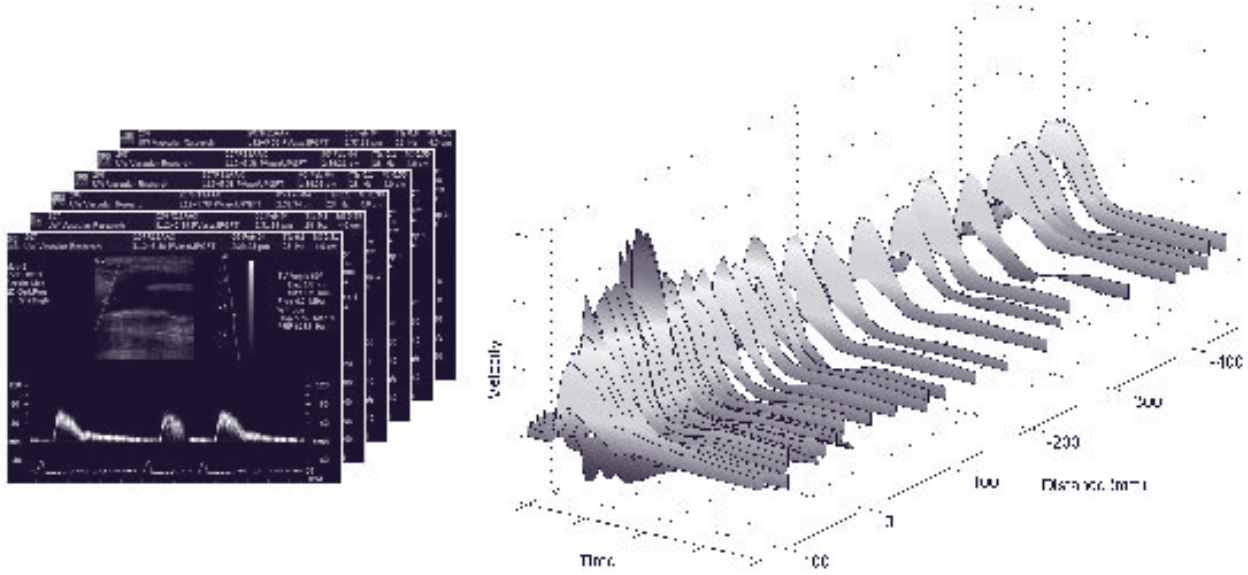

FIGURE 2: Doppler spectral waveforms for a single cardiac cycle are outlined on a series of 2D duplex ultrasound images of the vein graft (left). The waveform outlines are then spatially registered along the length of the vein graft (right). The registered waveforms show the velocity as a function of 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.

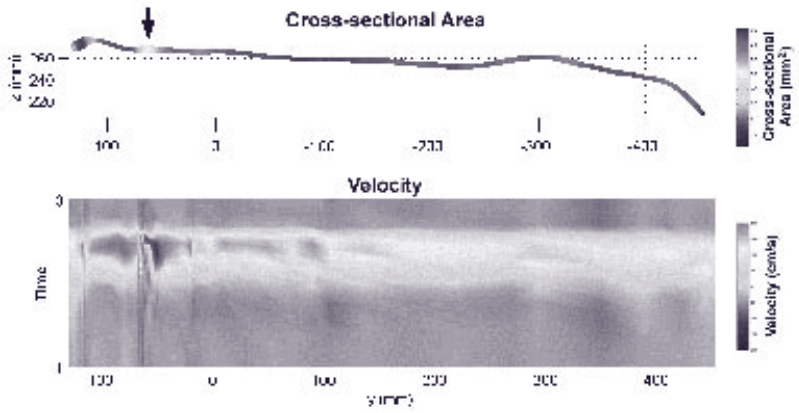

FIGURE 3: The 3D waveform plot from Figure 2 is interpolated and displayed as a 2D projection, showing velocity (represented by the gray scale intensity) as a function of time and space for the entire length of the vein graft. Time zero corresponds to the ECG R-wave. A registered 3D surface reconstruction 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.

monitoring is generally required to ensure that the endograft is stable and that there are no leaks. While decrease in aneurysm 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 4 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.

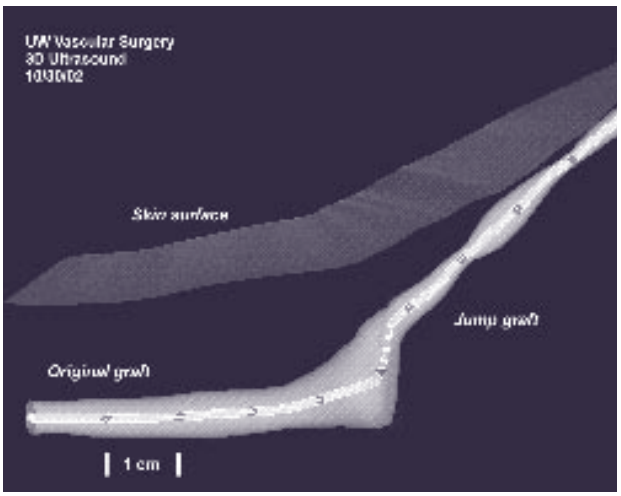

FIGURE 4: Three-dimensional surface display of the proximal anastomosis of a jump graft. A surface strip constructed along the center axis serves as a 3D tape measure to provide references for vessel width and length. The tape measure surface is 2 mm wide, with 1-mm grid marks and diamond markers at 1-cm intervals. The skin surface is displayed as a sheet to indicate the depth of the vessel.

Muscle Blood Flow Assessment with 3D Ultrasound Imaging

A National Space Biomedical Research Institute project under Dr. Martin Kushmerick in the Department of Radiology is investigating the use of 3D ultrasound to measure blood flow changes in muscle in response to exercise. Three-dimensional scans of the anterior tibial muscle were performed after several minutes at rest, and during the 15-minute period of recovery following one minute of exercise (foot dorsiflexion/plantarflexion). At each time point, 120 images were captured over an approximately 5-cm length of the muscle. The position

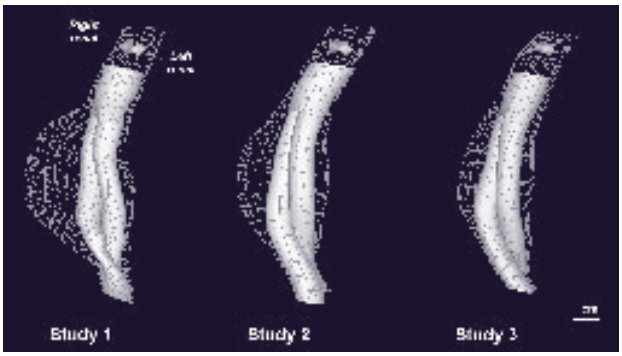

FIGURE 5: Serial study of an AAA repaired by an endovascular graft. The 3D reconstructions show both the aneurysm (outer mesh) and the graft (inner surface). The studies were performed 1) 2 weeks, 2) 6-months, and 3) 1 year after graft placement. Diamond markers show the origins of the renal arteries.

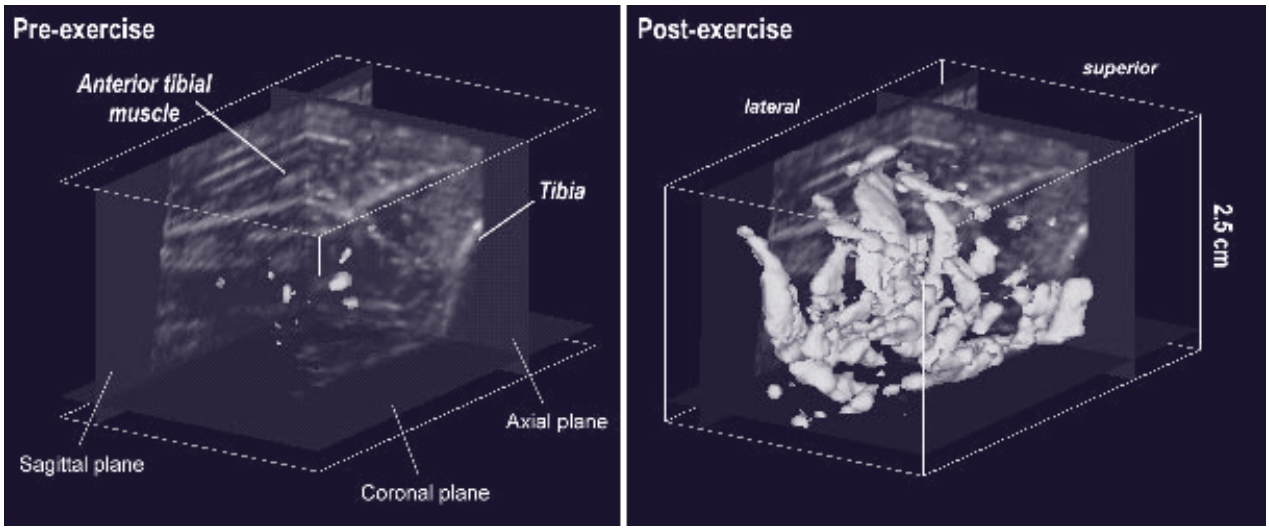

FIGURE 6: 3D volume reconstructions of the anterior tibial muscle before exercise (top) and immediately after exercise (bottom). Gray-scale and color data, representing tissue and blood flow respectively, were reconstructed separately and re-combined in a single display as orthogonal slices (anatomy) and surface rendering (blood flow). Voxel size = 0.5 mm.

and orientation of the ultrasound scanhead was recorded during image acquisition by the magnetic tracking system described above.

Within the scanned volume the 3D reconstructions showed a large vasodilation after exercise (Figure 5) which returned to baseline (Figure 6). The time course of these changes could be fitted to an exponential decay. In nine subjects, the mean recovery time constant was 2.3 ± 1.1 minutes. The initial response and time to recovery may provide objective measures of the efficacy of countermeasures designed to reduce muscle atrophy during extended spaceflight.

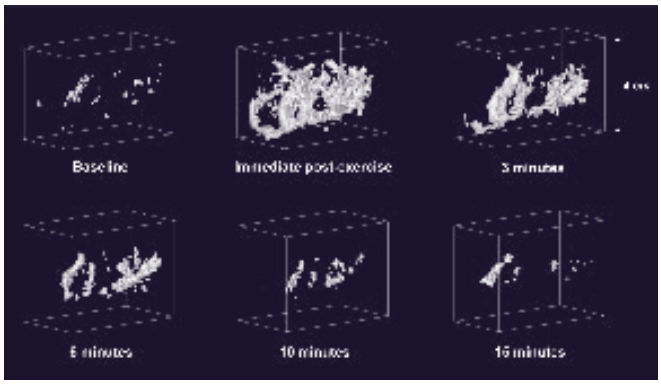

FIGURE 7: Series of volume reconstructions for 3D ultrasound scans acquired during a single exercise experiment. A baseline scan is obtained after several minutes of rest. Scans are then acquired immediately after exercise and at 3, 6, 10 and 15 minutes after exercise. The color voxels, representing blood flow, are segmented from the gray scale background and displayed as surfaces. Voxel size = 0.75 mm.

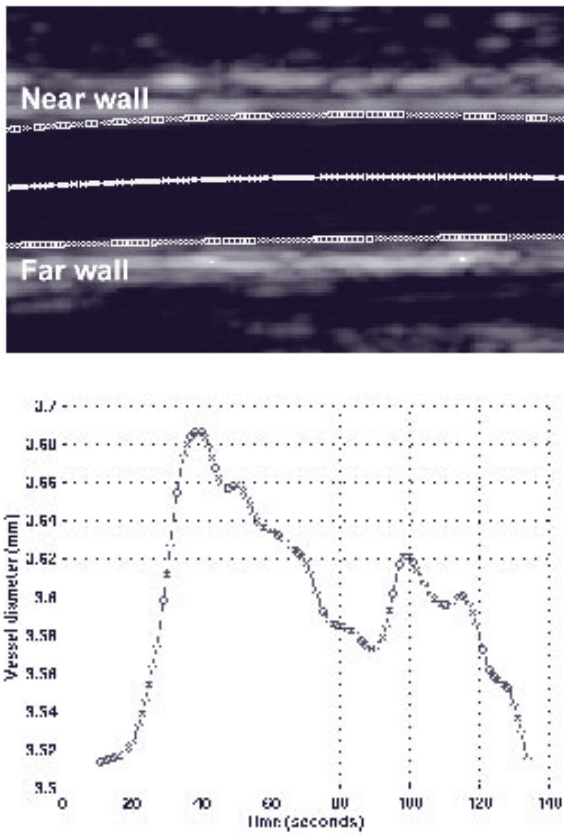

FIGURE 8: A longitudinal image of the brachial artery is shown at the top, with the locations of the vessel walls superimposed as detected by automated image processing. The diameter is measured along lines perpendicular to the vessel center axis; the measurements are averaged over the length of the vessel segment shown. The flow-mediated response of the brachial artery is plotted below as a function of time after release of a blood pressure cuff (time = 0).

Automated 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 a research study of preeclampsia conducted by Dr. Darcy Carr in the Department of Obstetrics and Gynecology, we have developed an automated image 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, and the automated edge detection algorithm reduces observer variability associated with manual measurement.

RELATED PUBLICATIONS

1. Leotta DF; Detmer PR; Martin RW. Performance of a miniature magnetic sensor for three-dimensional ultrasound imaging. *Ultrasound Med Biol,* 1997; 23:597-609.
2. Leotta DF; Munt B; Bolson EL; Martin RW; Kraft C; Otto CM; Sheehan FH. Quantitative three-dimensional echocardiography by rapid imaging from multiple transthoracic windows: in vitro validation and initial in vivo studies. *J Am Soc Echocardiography,* 1997; 10:830-839.
3. Leotta DF; Martin RW. Three-dimensional spatial compounding of ultrasound scans with weighting by incidence angle. *Ultrasonic Imaging,* 2000; 22:1-19.
4. Leotta DF; Martin RW. Three-dimensional ultrasound imaging of the rotator cuff: spatial compounding and tendon thickness measurement. *Ultrasound Med Biol,* 2000; 26:509-525.
5. Leotta DF; Primozich JF; Beach KW; Bergelin RO; Strandness DE, Jr. Serial measurement of cross-sectional area in peripheral vein grafts using three-dimensional ultrasound. *Ultrasound Med Biol*, 2001; 27:61-68.
6. Leotta DF; Paun M; Beach KW; Kohler TR; Zierler RE; Strandness DE, Jr. Measurement of abdominal aortic aneurysms using three-dimensional ultrasound imaging: preliminary report. *J Vasc Surg*, 2001; 33: 700-707.
7. Leotta DF; Primozich JF; Beach KW; Bergelin RO; Zierler RE; Strandness DE, Jr. Remodeling in peripheral vein graft revisions: Serial study by three-dimensional ultrasound imaging. *J Vasc Surg*, 2003; 37:798-807.
8. Leotta DF. An efficient calibration method for freehand 3-D ultrasound imaging systems. *Ultrasound Med Biol*, 2004; 30:999-1008.

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