



# SURGERY RESEARCH

DEPARTMENT OF SURGERY 2004 REPORT

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

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**2 0 0 4   R E P O R T**

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# Research Converts Dreams into Reality

We have always accepted the premise that research has been the key to progress. In fact, as we look back in history, the most notable advances in medicine were the result of arduous research. What is different today is the pace at which research leads to applications that have a direct and measurable effect on our lives. What appeared to be impossible just a few years back, is suddenly a reality that impacts us every day. Think, for example, of the Internet. If you are like a growing number of people, you probably use it on a daily basis. This truly remarkable tool has revolutionized the way we live our lives. Yet, only 25 years ago — how many people do you suppose had even dreamed of such a thing?

In medicine, many fields have seen similar staggering advances in recent years. Consider for a moment laparoscopic surgery. In the early 1990's it was considered to be fairly innovative — yet it offered an extremely limited viewing field. Training sessions were challenging, most structures were “imagined” as we could not really see them as well as we did with open surgery, the instruments were crude copies of those we used in open surgery and so on. However, technological advances that occurred in just a decade have improved the equipment to a level not previously thought possible. Extremely accurate cameras; miniaturized, light-weight tools; and high-resolution, wide-screen monitors to just name a few, have allowed the entire operating team to observe and follow the procedure. Moreover, these advancements have allowed us to effectively teach this technique to our residents. The recent introduction of robotics to the field of minimally invasive surgery promises to take this field to the next level. Yet it was because of somebody's research that these innovative ideas became possible, and even routine.

While there is no single way to determine the quality of the research being conducted at an academic institution, we generally accept the level of National Institutes of Health (NIH) funding as a proxy in assessing both the quantity and quality of research. Many of our faculty are, in addition to excellent

clinicians, investigators funded by NIH grants. In fact, at the close of this past fiscal year (FY03), our investigators held a total of 38 active NIH grants — up from 29 grants in FY02. We received a total of \$8.5 million in NIH funding, which is a full 50% increase over the previous year and places us at the top of academic departments of surgery in the nation. While our NIH funded grants increased 50%, total research funding increased 29% overall. Other funding sources include the Centers for Disease Control, U.S. Army & Navy, and other federal and private peer-reviewed sources.

Other prestigious organizations also recognized the research conducted by several of our faculty investigators and their students this past year. I invite you to take a few moments to read some examples of their activities in the sidebar that accompanies this letter.

It is incredible how quickly a new idea or invention can change society. In this book, you will read about some of the exciting ways our department is involved in those changes. If you would like to be a partner with us in transforming today's dreams into tools that can help save and improve lives, please contact Megin Flaherty Edwards, Associate Director of Development, at (206) 221-2847, email: [megin@u.washington.edu](mailto:megin@u.washington.edu).

Our top goal is to continue to provide you, your family, and your friends with the very best in surgical care. To do that, we need to continue to discover new tools and new ways to meet the challenges of disease.

A handwritten signature in blue ink, reading "Carlos A. Pellegrini". The signature is stylized with a large, looped "C" and a long, sweeping underline.

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

## RESEARCH HONORS & AWARDS

**DANIEL ANAYA, M.D., GENERAL SURGERY RESIDENT:** awarded first prize in the residents' category at the annual meeting of the Surgical Infection Society for paper entitled, "Risk factors for severe sepsis in peritonitis."

**EILEEN BULGER, M.D., ASSISTANT PROFESSOR:** awarded a four-year, \$1.6 million NIH: Heart, Lung, & Trauma Institute grant for work entitled, "The effect of hypertonic resuscitation for blunt trauma."

**ALBERT CHONG, M.D., GENERAL SURGERY RESIDENT:** won the Samson Resident Prize Essay at the annual meeting of the Western Thoracic Surgical Association during the scientific program for his paper entitled, "Toll-like receptor 4 mediates ischemia/reperfusion injury." One of the most prestigious competitions in thoracic surgery, Dr. Chong joins **DRS. BAIYA KRISHNADASAN & EDWARD BOYLE** as previous winners from our department in this distinct honor. (**DR. EDWARD D. VERRIER**, mentor)

**DAVID R. FLUM, M.D., MPH, ASSISTANT PROFESSOR:** awarded a five-year, \$1.6 million NIH grant to create a Bariatric Surgery Clinical Research Consortium. The grant is designed to help the NIH set the agenda for clinical research in obesity surgery and address several important issues in the application of this procedure. It will also help in the development of the UW Surgical Center for Outcomes Research.

**JONATHAN HUTTER, M.D., PLASTIC SURGERY RESIDENT:** won the *Best Paper* award for work presented by a resident at the Plastic Surgery Research Council Meeting for his work addressing bone marrow contribution to cutaneous wound healing. (**DR. FRANK ISIK**, mentor) UW residents have won this prestigious award six times within the past 10 years.

**F. FRANK ISIK, M.D., ASSOCIATE PROFESSOR:** awarded a four-year, \$1.3 million NIH grant for project entitled, "Surgical injury and the Wnt pathway".

**RONALD V. MAIER M.D., HMC SURGEON-IN-CHIEF:** invited by the Director of the NIH to participate in a two-day workshop to develop a ten-year plan to re-engineer NIH funding of the clinical research program. This proposal will support the NIH in its restructuring to more adequately speak to the challenges of addressing the enormous increase in basic science understanding and translating this knowledge to the patient's bedside, and also allowing for the identification of unique observations and empiric understanding from the bedside to be translated back to collaborative efforts in developments in the basic bench arena. The group of 27 internationally and nationally recognized investigators was drawn from a national pool, with Dr. Maier being the sole representative of the surgical arena.

**AVERY NATHENS, M.D., PH.D., ASSISTANT PROFESSOR:**

- awarded a three-year, \$652,000 grant from the National Institute of General Medical Sciences for project entitled, "Effect of leukoreduced blood transfusions on infection risk in trauma."
- heading a project as part of a five-year, \$15.7 million NIH/NHLBI grant with **DR. THOMAS R. MARTIN**, Department of Medicine, as overall program PI. This Specialized Center of Clinical Research (SCCOR) grant will investigate novel aspects of the pathogenesis and treatment of acute lung injury in humans. Dr. Nathens' project entitled, "Acute lung injury clinical trials incubator unit", will perform pilot projects of new treatments with the aim of taking forward positive results to larger clinical trials.



# 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

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

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*Our results suggest that cardiotomy suction should be eliminated whenever possible and challenge long-held precepts that adverse outcomes possibly associated with thrombin generation, inflammation and platelet activation are inevitable whenever CPB is used.*

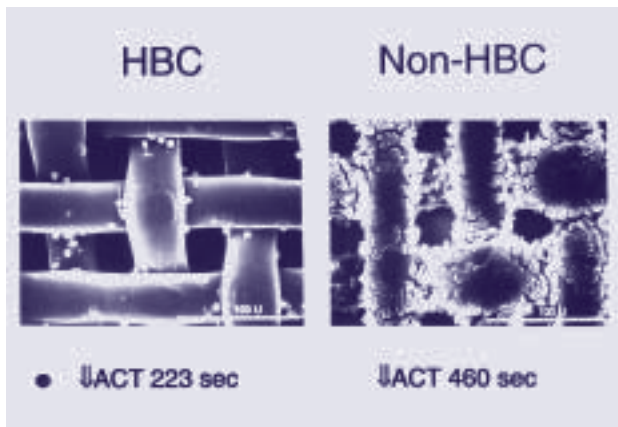
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into contact with the large surface area of the CPB circuit. Despite anticoagulation with heparin, this interaction results in extensive activation of platelets, neutrophils, complement, cytokines and the fibrinolytic system, producing a complex and 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

incidence of perioperative MI to less than 1%, neurological deficits to less than 1%, and pulmonary complications to 1.5%. Compared to previous reports, the incidence of neurological and persistent neuropsychological deficits following CABG were markedly reduced to near baseline.

Figure 1 shows a representative scanning EM at 200-fold magnification of the arterial filter (the last barrier to debris before the blood from the CPB circuit reaches the systemic circulation). This comparison demonstrates dramatic reduction (quantified in 60

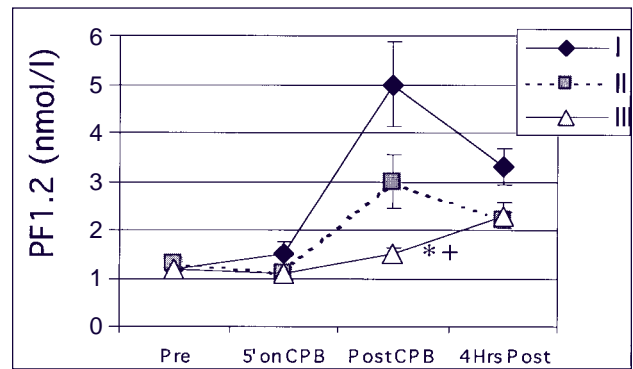


**FIGURE 1:** Scanning electron micrographs at 200 fold magnification of arterial filter. Lowest activating times (ACT) in seconds are noted. HBC= heparin-bonded circuits. Non-HBC- control non-heparin-bonded circuits.

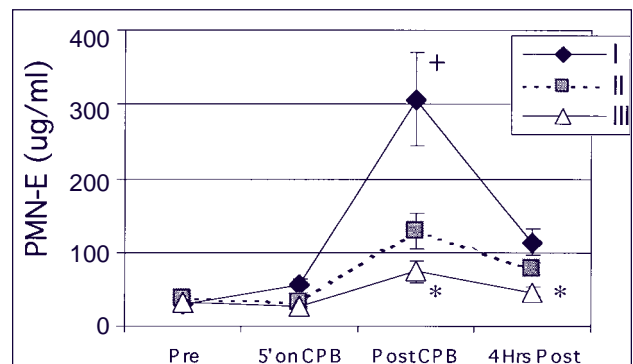
patients to be >80% reduction) in debris and inflammation resulting from the use of biocompatible heparin-bonded circuits with reduced anticoagulation protocol (HBC) compared with conventional non-biocompatible circuits with full anti-coagulation.

We are involved in several ongoing clinical investigations to study ways to disassociate the contribution of biocompatible circuits from the specific surgical techniques (the effects of cardiomy suction vs. use of cell saver technology) on markers of hemostasis, inflammation, neurological and neuropsychological deficits. Although both result in blood conservation, one (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.

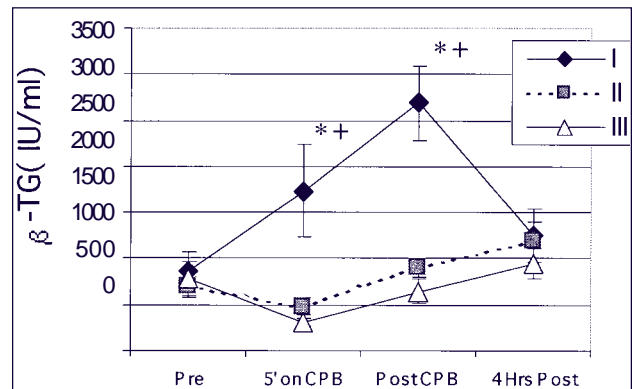
Heparin bonded circuits (HBC) have been proven to be effective in several research groups, including our own, in preserving platelet function and decreasing inflammation during CPB. However, markers of thrombin generation (PF1.2), inflammation (IL-6, IL-8, elastase, complement), platelet function ( $\beta$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



**FIGURE 2:** PF1.2 for thrombin generation



**FIGURE 3:** PMN-E for elastase



**FIGURE 4:**  $\beta$  Thromboglobulin for platelet activation

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 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 used 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.

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#### DEPARTMENT CO-INVESTIGATORS

**Michael Mulligan, M.D. / Mathew Rosengart, M.D. / Louise Soltow, B.S. / Edward D. Verrier, M.D. / Craig Vocolka, C.C.P.**

#### OTHER CO-INVESTIGATORS

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

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# Riyad Karmy-Jones, M.D.



- INDICATIONS AND OUTCOME FOLLOWING THORACOTOMY
- TRAUMATIC AORTIC RUPTURE
- LUNG INFLAMMATION

#### AWARDS

Resident Teaching Award, 1999

#### 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

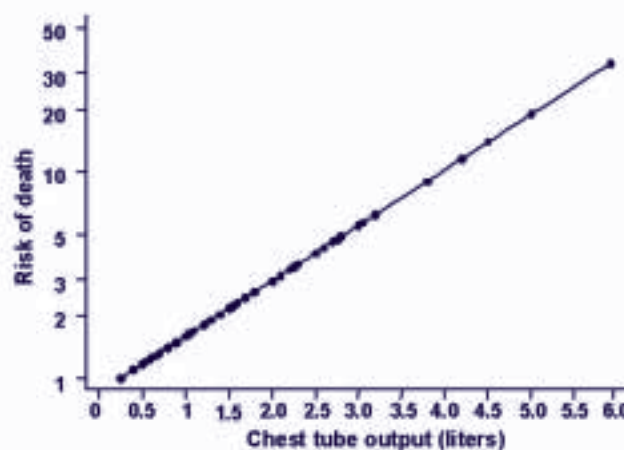


FIGURE 1: Risk of death and volume chest tube output

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*One such approach for patients who may not be operative candidates is blood pressure control with B-blockers. We are currently leading a multi-center prospective study of non-operative management of aortic trauma.*

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problematically 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).

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,

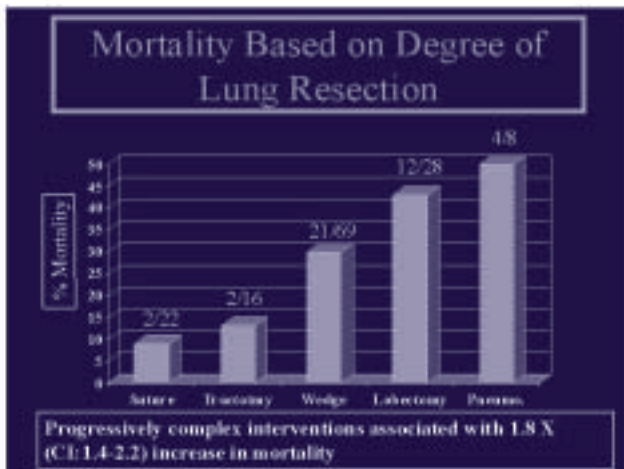


FIGURE 2: Mortality based on degree of lung resection

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.

### 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 a significant independent predictor 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 psuedoaneurysms 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

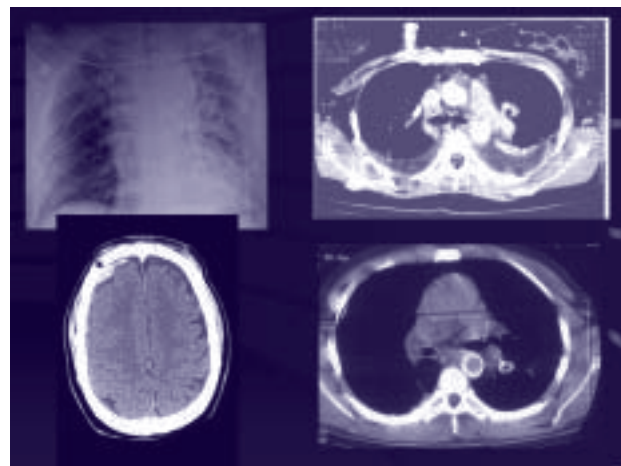


FIGURE 3: 18 year old female involved with MVC. Patient sustained closed head injury with elevated ICP, severe pulmonary contusion and required splenectomy. Her pelvis was unstable and angiographic embolisation needed for arterial bleeding. EVSG was utilized to control her TRA.



lesser curve to the superior aspect of the tear was 5.8 mm, aortic diameter 19 mm and mean degree of curvature 270. The majority of aortic tears will require the origin of the subclavian to be covered. We have 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-extendors"; 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, under the leadership of Dr. Mattos, 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

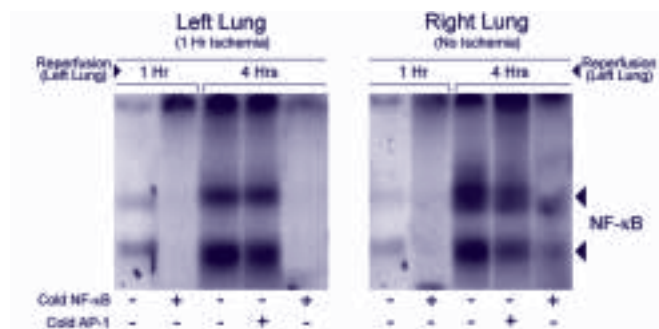


FIGURE 4: Left lung with ischemia compared to right lung with no ischemia

occlusion to demonstrate that ischemia-reperfusion to one lung results in an indirect injury to the contralateral lung with release of NFκB (Figure 4).

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

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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<sub>3</sub>C, and C. The CC chemokines or the  $\beta$  chemokines have two adjacent cysteine residues, and function primarily as monocyte and lymphocyte chemotactic agents. Members of this family include MCP-1, RANTES and MIP-1 $\alpha$ , MIP-1 $\beta$ , to name just a few. The second group, the CXC chemokines, are also

referred to as the  $\alpha$  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<sub>3</sub>C families. These chemokines are chemotactic for T lymphocytes and monocytes and include lymphotactin (C) and fractalkine, also known as neurotactin (CX<sub>3</sub>C).

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

Likewise three C-C chemokines, MCP-1, MIP-1 $\alpha$ , and RANTES, have been shown to play roles in the development of several of these models, but only IL-8 has been investigated for any potential role in lung ischemia reperfusion injury. MIP-1 $\alpha$  is upregulated *in vitro* following hypoxic stress and increased MIP1 $\alpha$  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-1 $\alpha$  in reperfusion injury has been suggested by preliminary findings. Unmanipulated control lungs and those from animals undergoing ischemia plus 0.5, 1, 2, 3 and 4 hours of reperfusion were extracted for MIP-1 $\alpha$  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  $0.09 \pm 0.05$ . 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  $0.75 \pm 0.01$  ( $p < .001$  compared to controls). In contrast, animals treated with blocking antibody to MIP 1 $\alpha$ , 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.

chemokine blockade at numerous points in the inflammatory cascade.

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

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

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*These studies should reveal the maximal effectiveness of chemokine blockade at numerous points in the inflammatory cascade.*

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Increased tissue neutrophil content is detectable after two hours of reperfusion, is significant by three hours and is marked by four hours. In contrast, lungs from animals treated with anti-MIP-1 $\alpha$  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

OB has gained acceptance. This technique, originally described in mice and now adapted to rats, produces an experimental OB that is histologically indistinguishable from human OB. We have used this model to investigate the potential role of beta chemokines in the development of experimental OB.

In addition to a variety of other mediators, two of the  $\beta$ - 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-I 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-I 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-I 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-I and RANTES mRNA were also increased in allograft tracheas but not in isografts. These data suggest that MCP-I and RANTES play important regulatory roles in the development of experimental OB.

A heterotopic rat heart transplant model is also being used to determine the role of CC chemokines in

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

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

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# Edward D. Verrier, M.D.



## • 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 (2)

#### 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.

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.

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

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*Ischemia/reperfusion injury contributes significantly to morbidity and mortality in surgical patients.*

*It is the principal pathogenetic event in stroke, complications of peripheral vascular disease, hemorrhagic shock, and early transplant graft dysfunction.*

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**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

molecular mechanisms of I/R injury into applications for clinical practice.

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- $\kappa$ B, that regulates transcription and expression of the genes that encode these proteins.

NF- $\kappa$ B is composed of subunits from the NF- $\kappa$ B/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- $\kappa$ B 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- $\kappa$ B activation during ischemia and reperfusion; (2) determine the role of NF- $\kappa$ B-mediated gene transcription in regional and systemic I/R injuries; and (3) identify novel therapies that block NF- $\kappa$ B 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).

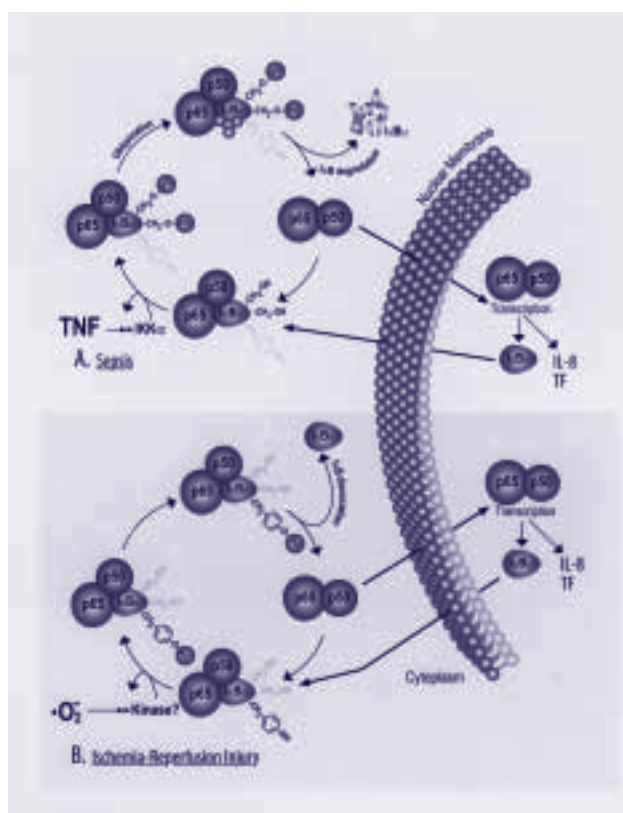
**Experimental techniques:** We utilize cultured cells to examine molecular mechanisms that are involved in the response to I/R injury. Although cell culture is a highly artificial system, it allows us to examine in precisely controlled conditions specific questions about the effects of hypoxia and reoxygenation 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- $\kappa$ B, 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 IκBα, dissociates from NF-κB without degradation. NF-κB 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.

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# Eileen Bulger, M.D.



- 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-Ayer Research Scholarship Award, 2001
- John H. Davis Research Scholarship Award, 2002

### American College of Surgeons

- Committee on Trauma Competition, Best Basic Science Paper 1995 & 1999, Finalist 1996
- Washington State Chapter Henry N. Harkins Resident Paper Competition, 2nd place (1994)
- Helen and John Schilling Resident Research Symposium, First Place (2000)
- Seattle Surgical Society Award

### Shock Society

- Young Investigator Award, Finalist (1996)

## 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 intracranial pressure for brain-injured patients and modulation of the inflammatory response at the time of reperfusion, thus lessening the subsequent development of inflammatory organ injury such as ARDS. With the support of the National Heart Lung and Blood Institute of the NIH, we 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.



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

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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.

#### **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 Dept 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 appropriate 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 been 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.

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# Nicole Gibran, M.D.



- 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 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

the enzyme neutral endopeptidase in skin and wounds from diabetic mice. We have shown that increased glucose and fatty acids increases neutral endopeptidase levels in cultured endothelial cells. Most interestingly, this increase can be inhibited with antioxidant treatment.

Our lab is focused on determining the endothelial cell derived signals that govern nerve cell differentiation. Sensory nerve-derived neuropeptides stimulate endothelial cells following injury to round up, proliferate and synthesize adhesion molecules and cytokines. These studies are currently focussed on intracellular signaling pathways that mediate substance P mediated changes to the endothelial cell.

Activated endothelial cells stimulate reinnervation of the injury site. We have defined this process to be a neuro-endothelial axis and believe that it may contribute to the pathophysiology of hypertrophic scar formation. Our latest effort has been to determine the mechanism

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*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.*

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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

by which substance P upregulates an inflammatory response. We have evidence that change in substance P-induced cell shape with the accompanying reorganization of the cytoskeleton may be an intermediary step. Most recently we have focused on the role of nitric oxide synthase and the EGFr as means of mediating substance P activity. These studies have been funded by the NIH.

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# Gregory J. Jurkovich, M.D.



- 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

### American Trauma Society

- Trauma Prevention Research

### Centers for Disease Control and Prevention

- National Center for Injury Prevention and Control

### National Highway Traffic Safety Administration

### National Institute of Mental Health

### Nestle Clinical Nutrition, USA

### University of Washington Clinical Research Unit

## 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. In order to guide us in developing a study which would be well regarded by the trauma, rehabilitation, and hospital community, we developed a National Advisory Committee of leading experts from around the country. We have held three meetings with them, as well as a number of conference calls. They have proved invaluable in their advice.

We have sought to develop and conduct a study that would provide us with nationally representative data. 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 the using the UB-92 standard format. To supplement the CDC funds for this project, we wrote a grant and were funded by the National Institute on Aging to obtain Medicare data on the study patients aged 65 and older. We obtained data from MarketScan to determine national data on professional fee costs for trauma.

We anticipate that all interviews will be completed by September 30, 2003 and all chart abstraction to be completed by December 31, 2003.

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.

### 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.

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

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*The purpose of the three-year, \$4.8 million, direct-cost grant is to examine variations in and outcomes from trauma care, in designated trauma centers compared to non-trauma centers across the United States.*

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#### **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, pediatrics, and psychiatry on the assessment and treatment of Posttraumatic stress disorder (PTSD) in trauma patients. PTSD occurs in 20-40% of patients over the course of the year after physical injury. Youth admitted to the hospital for physical injury are at increased risk for recurrent traumatic life events; identifiable risk factors appear to be assault injury and history of injury prior to inpatient admission. 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.

development of screening and intervention protocols for patients with psychiatric and substance abuse disorders in the acute care setting a tier I 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 ROI funded randomized intervention trial targeting PTSD and posttraumatic functional impairment among injured adolescents.



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**Doug Zatzick, M.D.**; Department of Psychiatry, University of Washington

# Ronald V. Maier, M.D.



- HARBORVIEW INJURY PREVENTION AND RESEARCH CENTER
- CLINICAL TRIALS IN THE SURGICAL INTENSIVE CARE UNIT AT HARBORVIEW MEDICAL CENTER
- 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

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

### National Science Foundation

**T**rauma 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.

per year, and these benefits were only evident in patients at the highest risk for adverse outcomes and not in the vast majority of lesser-injured patients.

### Clinical Trials in the Surgical Intensive Care Unit 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

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*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.*

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### 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 3I 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

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 3 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 bio-materials 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.

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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)

## FUNDING

American Trauma Society

Atlantic Philanthropies

Medic One Foundation

National Highway Traffic Safety Administration (NHTSA)

United States Agency for International Development (USAID)



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 underway in today's less developed countries. In middle income countries, as in East Asia and Latin America, injury has become a leading cause of years of life lost. In low income countries, such as in South Asia and Africa, infectious diseases continue to predominate because of their 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:

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 and at the Harborview Injury Prevention and Research Center has also represented a valuable resource for my work.

## **Strengthening Trauma Systems in Developing Countries** **Surveillance and research on the basic epidemiology of injuries in developing countries**

In developed countries, the usual sources of data on the incidence and consequences of injury include vital 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 which 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, prehospital care, emergency room care, or other aspects of hospital based care. Hence, there is a need to know where in a nation's trauma system the greatest mortality 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 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.

### Prehospital care

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

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

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

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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 are scheduled to provide similar "roll out" training to over 500 other doctors and nurses throughout that country.

### **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 seven 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 two years. This has entailed mostly long distance communication. In addition, there was a meeting of the two groups in Geneva in June 2004 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 is currently In Press at the WHO. This publication is intended to serve as:

1. Part science, in that it includes a list of the items of trauma care that a panel of experts has evaluated as the most cost effective.
2. Part planning guide to assist clinicians, hospital administrators, and planners in ministries of health globally in their efforts to strengthen trauma care in their own countries.
3. Part advocacy tool, in that the Guidelines contain a delineation of the trauma care services that the

WHO and the International Society of Surgery have endorsed as “Essential” and which can realistically be assured to virtually every injured person worldwide, even in the poorest countries.

A sample of some of the elements contained in the Guidelines is shown in a sample table (at end of text).

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 Guidelines are being finalized and are due out in early 2004.

Following this, 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 preliminary progress is underway, even before the Guidelines have been formally published. 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 Mexican Association for the Medicine and Surgery of Trauma has officially endorsed the Guidelines for use in Mexico.
3. 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.

### 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 2 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 which includes six other trauma centers nationwide. At each center, persons injured in motor vehicle crashes are identified, and a team of investigators examines the involved vehicles for crash deformation patterns. 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.

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.

On preliminary analysis, 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.

	BASIC #	GP #	SPECIALIST #	TERTIARY #
<b>Airway Skills</b>				
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
<b>Airway Equipment</b>				
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
<p># <b>Basic:</b> Outpatient clinic, often non-doctor staffed.  <b>GP:</b> General Practitioner staffed hospitals.  <b>Specialist:</b> Specialist staffed hospital, usually having a general surgeon and possibly other specialities.  <b>Tertiary:</b> Tertiary care hospitals, often university hospitals; wide range of specialists.  E: Essential; D: Desirable</p>				

**FIGURE 1. Sample 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 are under development for other aspects of trauma care.

*NB: This is an example only – the final recommendations are still in development.*

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

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# Avery B. Nathens, M.D., Ph.D., MPH



- 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 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

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.

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*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.*

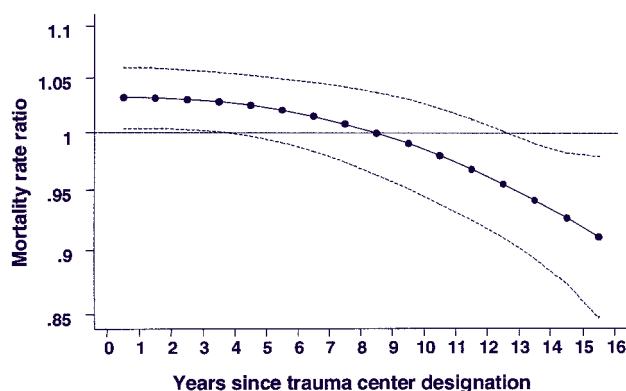
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organized systems of trauma care. After controlling for secular trends in crash mortality and implementation of traffic safety laws (restraint laws, maximum posted speed limits, laws designed to limit drinking and driving), trauma systems had a significant impact on deaths due to

## 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





**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.

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 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).

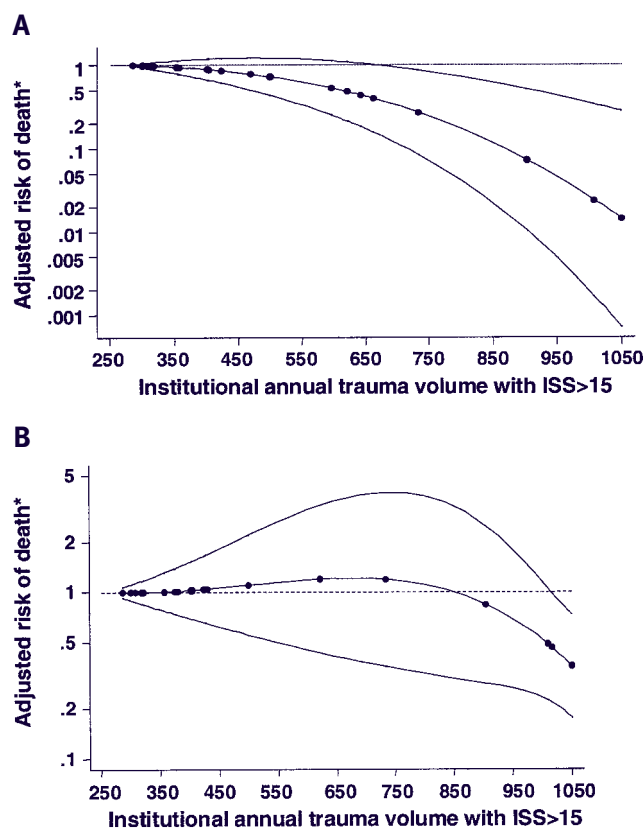
Similar advantages were also evident when hospital lengths of stay were assessed. The greatest benefits to these high-risk patients occurred when they were cared for in centers with greater than 650–700 major (ISS>15) trauma admissions per annum.

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



**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.

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 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 I 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 I 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).

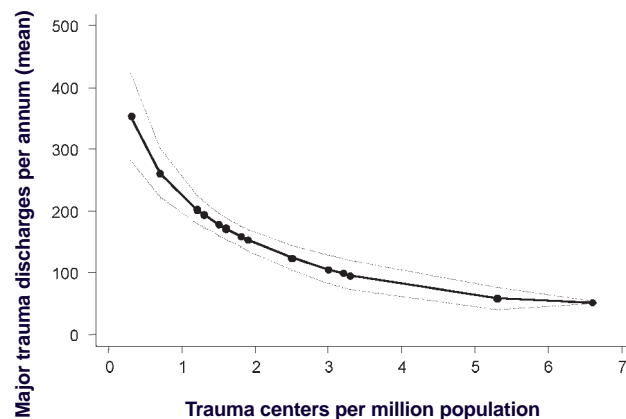
The variation in availability of designated centers affected the trauma experience of individual institutions. The mean trauma center volume (major trauma discharges per annum) dropped off significantly as the density of trauma centers per million population increased, from 348 discharges per annum in 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 I 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



**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).

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 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, 5310 patients were first assessed at the Level III or IV centers, and 281 (5%) were ultimately transferred to the Level I facility. The remaining 4439 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 (3000 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. Bronchoalveolar lavage fluid F<sub>2</sub>α<sub>8</sub>isoprostane 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- $\alpha$ , IL-1 $\beta$ , 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  $\alpha$ -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.

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  10. Nathens AB, Maier RV, Brundage SJ, Jurkovich GJ, Grossman DC. The effect of interfacility transfer on outcome in an urban trauma system. *J Trauma*. In press.
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# Grant O'Keefe, M.D., MPH

## • 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 ( $\leq 1$  hour) or early ( $\leq 24$  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

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

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*We have identified associations between gene polymorphisms and severe sepsis and septic shock after trauma.*

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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

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.

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. Individuals were genotyped for 3 polymorphisms in the promoter region of the IL-6 gene; each of which is



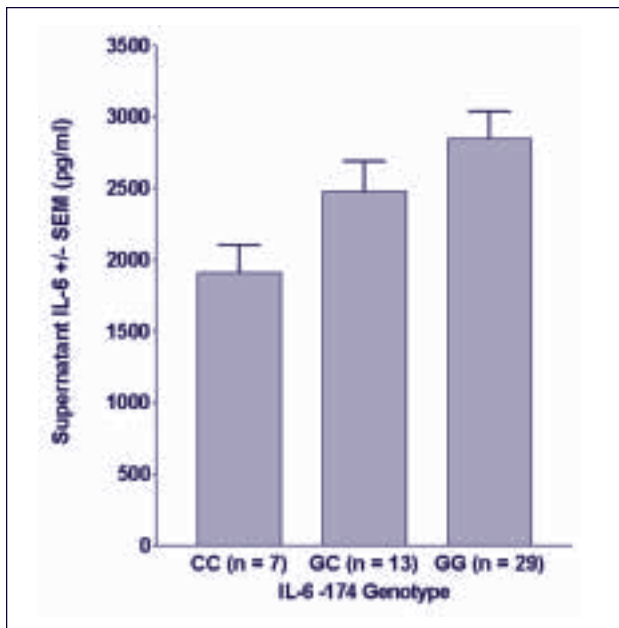


FIGURE 1

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- $\alpha$  (-308 G(A), IL-1 $\beta$  (-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- $\alpha$  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.

Logistic regression analysis of the association between single nucleotide polymorphisms and complicated appendicitis.

Interestingly, a "high-risk" genotype, defined by the presence of at least one A-allele at TNF- $\alpha$  (-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- $\alpha$  (-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.

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- $\alpha$ -308 A-allele	2.7	0.9–7.9	0.07

TABLE 1: Results of Multivariate Analysis

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 than 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.

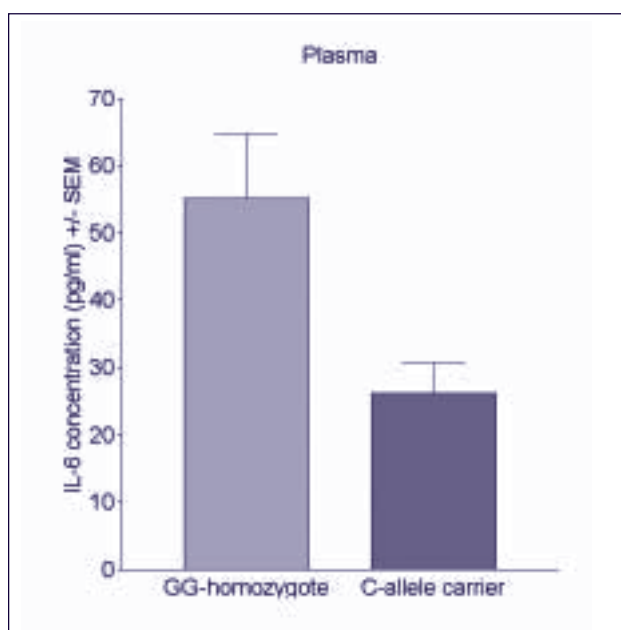


FIGURE 2: Plasma Concentrations

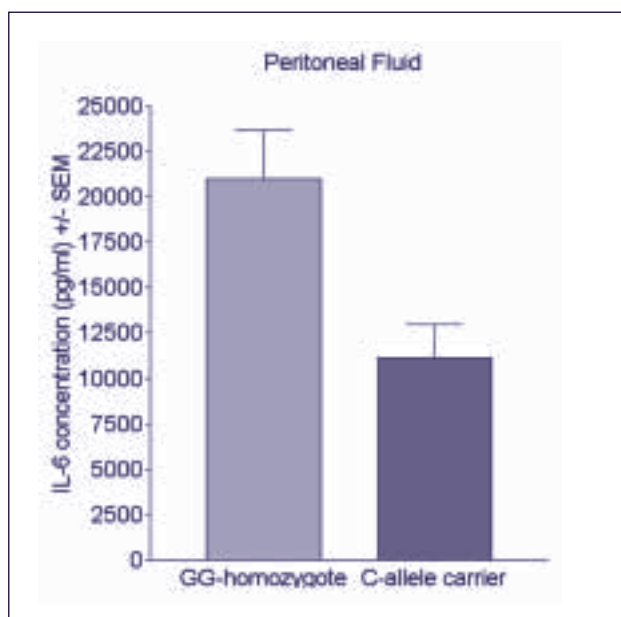


FIGURE 3: Peritoneal Concentrations

### The G→A Single Nucleotide Polymorphism at the -308 Position in the TNF- $\alpha$ 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- $\alpha$  promoter, in which a number of SNPs may affect transcriptional regulation of TNF- $\alpha$  production.

A SNP at the -308 position (G→A substitution) was shown to alter TNF- $\alpha$  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- $\alpha$  promoter SNPs may also be important in transcriptional regulation of TNF- $\alpha$  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 trans-fected 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- $\alpha$  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- $\alpha$  promoter is associated with an increased risk for complicated sepsis.

Of the three SNPs in the TNF- $\alpha$  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 developed severe sepsis, as did 15 of the 34 (44%)

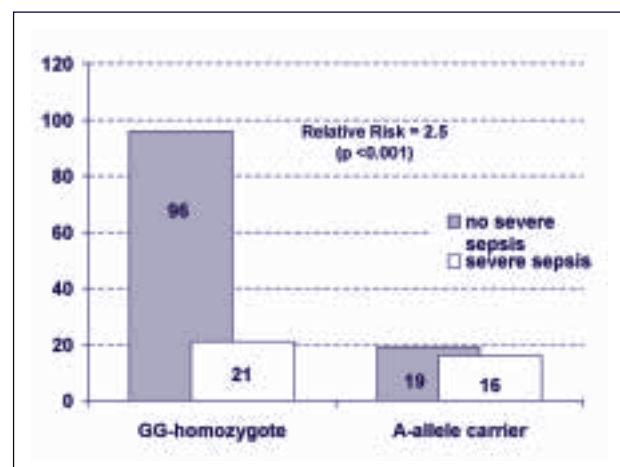


FIGURE 4: Sepsis Risk Factor



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- $\alpha$  -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.

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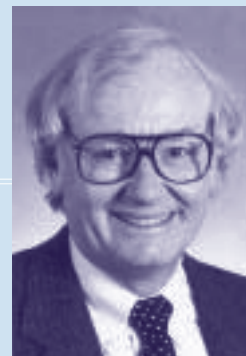
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# Robert K. Winn, Ph.D.

- 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 experi-

mental 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 considerably longer than 1 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 activation 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

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 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 neutrophil apoptosis and engulfment by macrophages

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*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.*

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provide information that will allow the development of therapeutics for the treatment of this devastating syndrome.

#### **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

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.

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# PEDIATRIC SURGERY

ROBERT S. SAWIN, M.D.

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# Robert S. Sawin, M.D.



## • NEUROBLASTOMA IN THE PEDIATRIC PATIENT

### AWARDS

Robert E. Condon Surgical Resident Competition, 1998  
Wisconsin Surgical Society  
• Resident Competition (Scientific Division), 1998

### FUNDING

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

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

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

Our laboratory effort has focused on a particular peptide growth factor, gastrin releasing peptide (GRP),

that is expressed in both adult and pediatric tumors that are derived from neural crest cells. Our work has shown that GRP and its receptor, GRP-R, are both expressed in abundance by neuroblastoma cells in culture and by tumor cells removed from children. Our cell culture studies have also shown that inhibitors of GRP retard neuroblastoma growth.

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

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*Despite treatments involving aggressive regimens of chemotherapy, and even bone marrow transplantation, the mortality for neuroblastoma remains 40 to 50%.*

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### OTHER CO-INVESTIGATORS

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# John Waldhausen, M.D.

• SURGICAL TREATMENT REVIEW IMPROVES CHILDREN'S HEALING PROCESS



Pediatric surgery is in general a very clinically oriented field. 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

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

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*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.*

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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

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

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 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.

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# PLASTIC AND RECONSTRUCTIVE SURGERY

LOREN H. ENGRAV, M.D.

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# Loren H. Engrav, M.D.

- 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 reported last year that 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. Gibran, Cole and Isik are significantly involved in these activities. The Visiting Scientists listed above have also played major roles within these projects.

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

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

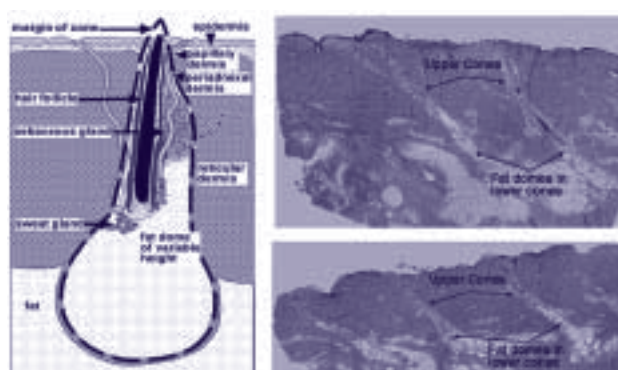


FIGURE 1: The Cones of Skin, Schematic and in vivo

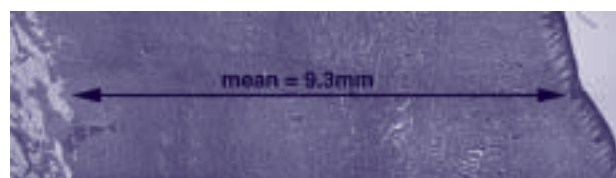


FIGURE 2: Thick Scar in Female, Red Duroc Pig

the only option to be reconstructive plastic surgery. It is clear that new, prospective approaches to this devastating problem, which allow us to intervene before permanent scarring occur, are necessary. In fact, the impact of scarring is so profound that until steps are taken to greatly reduce or eliminate scarring all together, efforts to enhance rehabilitation of burn survivors will remain palliative at best.

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:** Scarring in the female, red Duroc pig is a model of human, hypertrophic scarring and can be used to verify that the cones of skin are related to hypertrophic scarring and to determine the etiology of hypertrophic scarring.

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

One reason for our lack of understanding of hypertrophic scarring is that tissue for study has historically been obtained from humans undergoing scar revision. This has been necessary since there is no useful animal model. This means that tissues are obtained on a convenience basis without order or control, which prevents systematic study of the hypertrophic scarring process. Nearly 30 years ago, Silverstein, Goodwin, Raulston, and Pruitt reported that deep donor sites in 12/12 female, red Duroc pigs healed with hypertrophic scarring (Hypertrophic scar in the experimental animal. The ultrastructure of collagen. J.J. Longacre. Springfield, IL, Thomas). 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



FIGURE 3: Hypertrophic Scar

factor one in the scar of the female, red Duroc pig harvested on post-wounding days 0, 7, 14, 21, 28, 60, 90, and 150 to that reported in the literature for human, hypertrophic scar.

*Specific Aim 2:* To confirm that cutaneous scarring in the female, red Duroc pig and/or human, hypertrophic, cutaneous scarring involve the cones of skin.

A second reason for our lack of understanding of the etiology of hypertrophic scarring is that, in the past, most tissue has been minced and homogenized thereby destroying skin anatomy and homogenizing all cell populations. Thinking that skin architecture and cell location/orientation might be important to understanding the cause of hypertrophic scarring, we reviewed skin anatomy to include the cones. The cones were described in the early 1900s and re-discussed in the mid-1900s with little interest. In fact, most studies of human, hypertrophic scar either ignore the anatomy

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*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.*

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comparing the clinical appearance; histology; immuno-histochemical localization of decorin, versican, transforming growth factor beta one, insulin-like growth factor one, and constitutive and inducible nitric oxide synthase; in situ hybridization of decorin and insulin-like growth factor one; and quantification of transforming growth factor beta one and insulin-like growth

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. We will test the relationship by in situ hybridization of collagen I and III mRNA and immunohistochemical localization of transforming growth factor and insulin-like growth factor one in tissues from the female, red Duroc pig and humans.

Specific Aim 3: To determine which genes related to wound healing are up or down regulated in the cones in cutaneous scar in the female, red Duroc pig and in human, hypertrophic, cutaneous scar.

Third, 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. We will dissect the cones out of the surrounding mass of scar collagen by laser capture microdissection and study cone tissues in isolation utilizing gene arrays to assess gene expression within this cone tissue in tissues from the female, red Duroc pig and humans. 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.

## 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 five faculty including Drs. Engrav, Gibran,

Patterson, Esselman and Wiechman. The Research Nurse Supervisor is Gretchen Carrougner, RN, MN. Drs. Kowalske, Fauerbach, Herndon and Lezotte are the other NIDRR Burn Rehabilitation Model System PIs.

- *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 Years Report

#### Prevalence of Hypertrophic Scarring

**Abstract** – Hypertrophic scarring after burns remains a major problem and is considered to be “common”. Pressure garments are commonly used as treatment even though there is little sound data that they reduce the prevalence or magnitude of the scarring. In 1999 we began a study of the efficacy of pressure garments on forearm burns. After studying 30 patients, mainly white adults, we found no hypertrophic scar in either those treated with pressure or without.

This prompted us to review the literature on the prevalence of hypertrophic scarring after burns and found only four articles with a relatively small number of patients and only three geographical locations. It became clear that the prevalence of hypertrophic scarring is really unknown. We then did a retrospective study of 110 burn survivors and counted all hypertrophic scars of all sizes and locations in all races and found the prevalence of hypertrophic scarring to be 67% which conflicts with the published reports and our prospective study and suggests that further research is necessary. We concluded that a worldwide, prospective survey is necessary to establish the prevalence of hypertrophic scarring after burns. In this article we are calling for and offering to organize this survey.

#### Efficacy of Pressure Garment Therapy

Despite marked improvement in mortality statistics for major burn injuries over the past two decades, comparatively little progress has been made in improving

the scarring, which results from healed, burn wounds. Hypertrophic scars are raised, thickened and hard and are often associated with joint contractures and other anatomic disfigurement. They constitute a major source of morbidity for the otherwise “healed” burn patient, leading to significant functional impairment and cosmetic deformity. Of all the factors that hinder return to work after a burn, hypertrophic scarring is clearly among the most problematic.

The routine use of pressure garments has become the worldwide standard of care in treating healed burns, skin grafts, and donor sites. Yet, no controlled studies have been performed that verify the efficacy of such treatment. Pressure therapy is complex, problematic, and expensive. Therefore, its use cannot be justified solely on the basis of case reports, retrospective studies, and anecdotal data. Presently, the decision to prescribe pressure garments is made on the basis of individual/institutional experience and, in some centers, is ordered for most all patients. Other centers never use pressure garments. Clearly, there is a need to determine whether or not pressure therapy is truly efficacious in reducing hypertrophic scarring and the aesthetic and functional consequences of its use.

The project objective is to determine the efficacy of pressure garment therapy in the prevention of hypertrophic scarring in burn scars and skin grafts in burn-injured children and adults. We hypothesize that pressure garment therapy reduces the degree of hypertrophic scarring and will study the outcome with and without pressure on burns of the upper extremity allowed to heal spontaneously. The International Association of Fire Fighters has funded this project. Gretchen Carrougner and Marilyn Moore direct this project.

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#### RELATED PUBLICATIONS

1. Bombaro, K.M., Engrav, L.H., Carrougner, G.J., Wiechman, S.A., Faucher, L., Costa, B.A., Heimbach, D.M., Rivara, F.P., Honari, S. What is the Prevalence of Hypertrophic Scarring Following Burns? *Burns*, 2003; 29(4):299-302.

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# Richard A. Hopper, M.D., M.S.

- CLEFT LIP AND PALATE
- SYNDROMIC SEVERE MIDFACE HYPOPLASIA
- CRANIOSYNOSTOSIS

## FUNDING

CHRC 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.

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

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*Osteoblasts do not exist in isolation in the skull. Bone healing involves a complex coordination between osteoblasts and adjacent blood vessel, or endothelial, cells.*

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## 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

new guidelines for the nutritional care of infants with cleft lip and palate based on their individual needs.

### Syndromic Severe Midface Hypoplasia

Children born with syndromes such as those described by Apert, Crouzon, and Pfeiffer can have such poor growth of their upper facial skeleton, or midface, that it compromises the closure of their eyelids and therefore the protection of their vision, the airway of their nose and therefore their ability to sleep, and the relationship between their upper and lower jaws and their ability to chew. The recognized surgical treatment of these children is to separate the upper facial skeleton from the rest of the skull, known as a LeFort III osteotomy, then to move the upper face forward and secure it in place with bone graft harvested from the child's ribs.

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

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

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

Since midface distraction osteogenesis is a relatively new technique in evolution, we are actively researching ways to improve the process at the Craniofacial Center of Children's Hospital. A prospective Institutional Research Board (IRB) approved study is underway to examine the psychosocial impact of the three month long procedure on the patients and their families, and to suggest interventions to minimize the stress. Pre- and post-operative extensive sleep studies are being performed on all the children undergoing the procedure to examine the effect on quality of sleep.

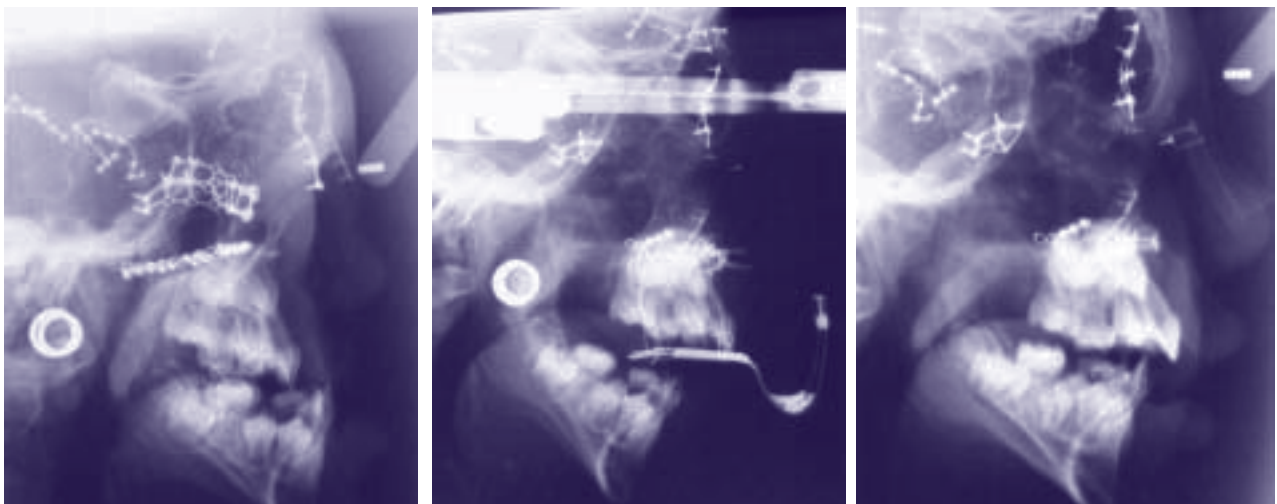
Sequential radiographic imaging is being used to learn how the facial bones adjust, remodel, and grow after they have been advanced such a large distance. Timing of how long it takes the new bone to form behind the advanced facial bones is also being studied to determine the optimum time to remove the distraction device.

## Basic Science Research

### Craniosynostosis

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

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.

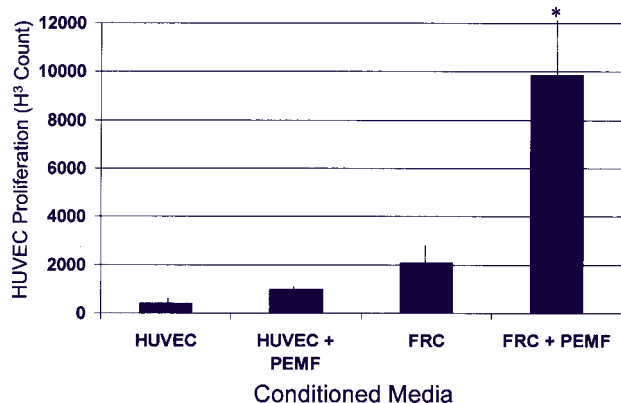
Osteoblasts cultured from fused sutures grow faster than osteoblasts cultured from open, or patent, sutures. The prevailing theory is that osteoblasts around fused sutures are abnormal, however our alternate theory is that there are cells within normal sutures that serve to inhibit the growth of surrounding 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.

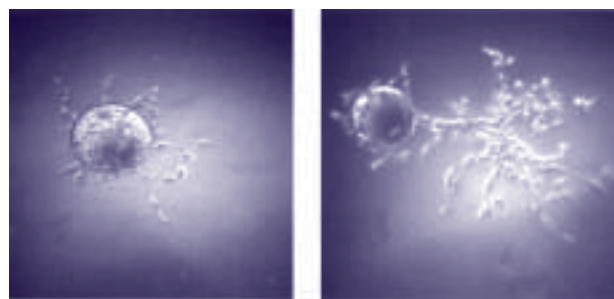
Osteoblasts do not exist in isolation in the skull. Bone healing involves a complex coordination between osteoblasts and adjacent blood vessel, or endothelial, cells. A collaborative project with Dr. Geoff Gurtner at New York University Medical Center has just been completed which examined the interaction 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).

#### OTHER CO-INVESTIGATORS

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# Anne Hocking, Ph.D. Frank Isik, M.D.



## • WOUND HEALING

### FUNDING

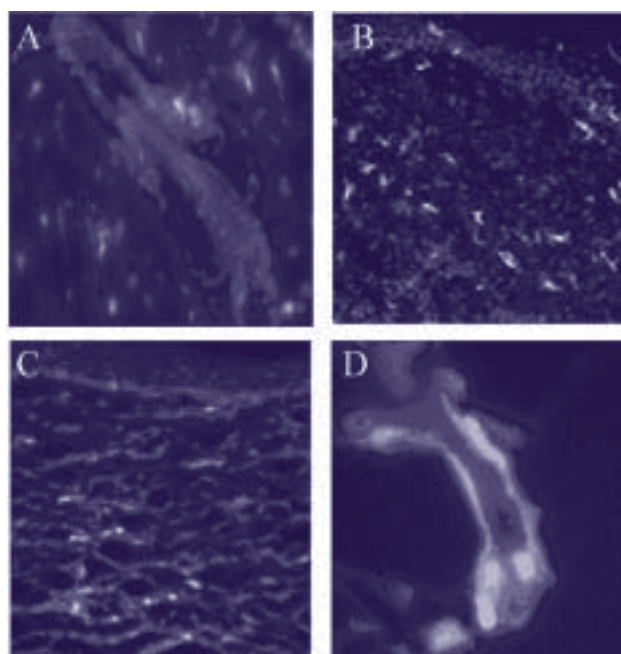
National Institutes of Health

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.

### Origin of Cells in a Healed Wound: Bone Marrow

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



**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).

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



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*We think stem cells in the epidermis and stem cells from bone marrow can provide the missing cell types following loss due to injury.*

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dendritic type cells and endothelial cells (Figure 1). These findings suggest that bone marrow derived cells in the wound, not only participate in the inflammatory response, but are an important source of cells for reconstituting the dermis. We are currently investigating this unique role of bone marrow-derived cells in wound repair and are also interested in identifying the signaling pathway responsible for the differentiation of the progenitor cells in the wound.

#### **Gene Expression Profiling of Normal Human Wound Healing**

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

We observed significant up-regulation of gene expression at thirty minutes after wounding: expression of 334/4000 genes was increased >3 fold. Expression of genes involved in the inhibition of cell signaling including SOCS and the suppressor of ras-1 were up-regulated. In addition, expression of genes encoding regulators of the cell cycle (e.g. Rb) and proteases (e.g. uPA) were down-regulated. At 1 hour post wounding, 471/4000 genes were increased > 3 fold. We observed down-regulation of transcriptional and signaling inhibitors, and up-regulation of multiple transcriptional activators. A searchable web site has been constructed to disseminate this data (<http://faculty.washington.edu/isik/research.html>).

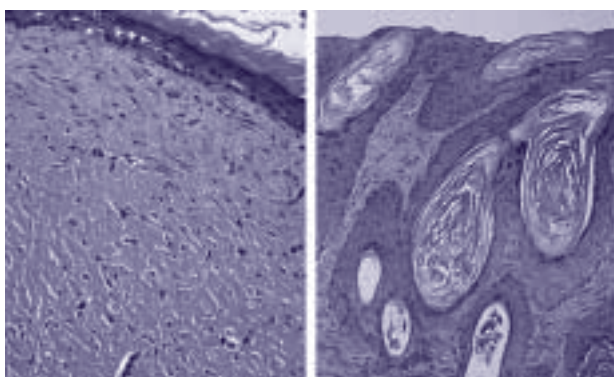
Our data demonstrate the complexity of the gene activation/suppression processes that occur early in the normal human wound healing process. Most of these genes have never been examined in wound healing research. Using this database, new targets have emerged that provide further insight into the study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

of the Wnt signaling pathway that are up-regulated in wound repair.

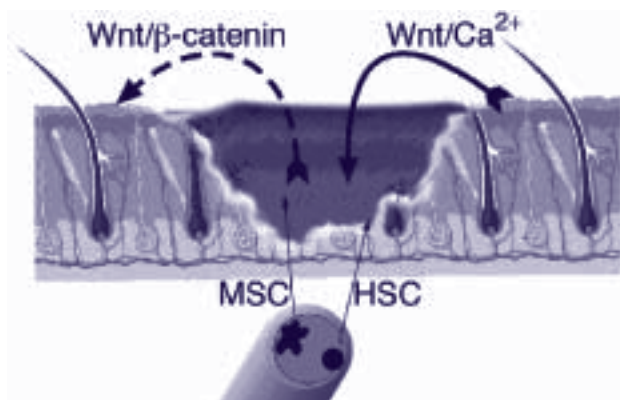
#### **Developmental Genes Reintroduced in Adult Wound Healing: Wnt Genes**

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

Several mechanisms for transduction of the Wnt signal have been elucidated. The Wnt/ $\beta$ -catenin pathway is the most well characterized. Activation of the Wnt/ $\beta$ -catenin signaling pathway promotes the stabilization of  $\beta$ -catenin in the cytoplasm. This pool of  $\beta$ -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/ $\beta$ -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  $\beta$ -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



**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.

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/ $\beta$ -catenin signaling is required for hair follicle morphogenesis and recent data also indicates that inhibition of Wnt/ $\beta$ -catenin signaling may be necessary for basal epidermal cell specification. Our microarray data revealed that components of the Wnt pathway including: TCF-4,  $\beta$ -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/ $\beta$ -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.

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# ROBOTICS

LILY C. CHANG, M.D.

RICHARD SATAVA, M.D.

MIKA SINANAN, M.D., PH.D.

# Lily C. Chang, M.D.



- INTRODUCTION OF SURGICAL ROBOTICS SYSTEMS
- INTEGRATION OF ROBOTICS INTO THE CLINICAL SETTING
- VIRTUAL REALITY SIMULATION IN LAPAROSCOPIC SURGERY
- OBJECTIVE MEASUREMENT OF SURGICAL SKILL USING LAPAROSCOPIC SIMULATORS

## AWARDS

### International Conference of Robotics and Automation

- The Blue DRAGON – A System for Measuring the Kinematics and the Dynamics of Minimally Invasive Surgical Tools In-Vivo, Best Paper Award Finalist

## Introduction of Surgical Robotics Systems

Many recent advances in surgery concentrate on minimizing the invasiveness of a procedure which improves outcome and patient satisfaction. Yet, in the conversion from traditional open surgery to minimally invasive surgery, the surgeon has lost several degrees of hand and wrist freedom as well as 3D visualization. As surgery enters the technology age, many innovations integrate computer systems to allow for enhancement of visualization, accuracy, dexterity and stability. Surgical robotic systems are an extension of this type of computer-aided surgery in combination with minimally invasive or endoscopic technique. These systems provide surgeons with the dexterity and depth perception that are lacking in standard endoscopic equipment. Moreover, maximal precision can be achieved through "motion scaling" in which large hand movements are translated and reduced into fine instrument movement. Further developments in technology will create virtual immobilization which gives the illusion of stillness to allow for beating-heart surgery. The potential clinical applications of surgical robotics are vast.

## Integration of Robotics into the Clinical Setting

Surgical robotics systems are being integrated into operating rooms all over the world. With the degree of sophistication in these systems, it is no longer sufficient to simply purchase the equipment and begin clinical use without proper training. While the surgeon may receive extensive instruction through courses and proctoring, the ancillary services are often overlooked. Our goal is to create a systematic approach for the clinical intro-

duction of a surgical robotics system which involves the entire surgical team.

Surgeons require hands-on training in both a dry lab setting as well as an animal or cadaver lab. For centers that do not have animal or cadaver labs, this type of training must be sought out in established centers. Instruction should not only be focused upon robotic skills practice, but also equipment setup, system capabilities and limitations, and troubleshooting. A checklist of tasks with established goals should be outlined. The amount of time needed to achieve mastery may be highly variable. Regardless, surgeons must be able to document their training and abilities in order to obtain privileges to operate these systems.

Operating room nurses also need detailed instruction in equipment setup, instrumentation, sterilization techniques, and patient safety issues. Practice sessions in the operating room with mock patients simulate the actual procedure and allow the staff to iron out any inefficiencies. In-servicing is also mandatory for the instrument room and clinical engineering to ensure the proper care, cleansing, servicing and storage of the system. In addition, proper education of clinic nurses and patient care coordinators who often address patients' concerns is paramount to a successful robotic operation. Informational handouts should be developed specifically for patients emphasizing systems operation, surgeon presence, and patient safety.

We have successfully incorporated robotic technology into our operating room at UWMC. We have shown that the clinical introduction of this technology can succeed through the use a systematic approach to training and education which includes surgeons, nurses, support staff, and patients.

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*Laparoscopic surgery has become a mainstay of the general surgery armamentarium. Its mastery, however, requires a unique set of operative skills.*

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### Virtual Reality Simulation in Laparoscopic Surgery

Laparoscopic surgery has become a mainstay of the general surgery armamentarium. Its mastery, however, requires a unique set of operative skills. The psychomotor challenges inherent in laparoscopic manipulations have frustrated skilled surgeons and trainees alike. Compared with traditional open surgery, laparoscopy requires the surgeon to perform tasks using a two-dimensional videoscopic image of the operative field while viewing only the tips of the instruments. Laparoscopic instruments are longer than traditional instruments and the surgeon's hands are therefore far removed from the working ends. These instruments also provide muted tactile feedback. Finally, a "fulcrum effect" exists where directional movements of the surgeon's hand result in contrary deflections of the working end of a laparoscopic instrument, creating a disparity between visual and proprioceptive feedback. These variables impact the laparoscopic novice until he or she learns to adjust through extensive psychomotor practice.

Another unique aspect of laparoscopic surgery is the limited ability of the mentor to control the operative field for the trainee. In order for the trainee to perform a procedure, he or she must be given full control of the operating instruments leaving the mentor to instruct by verbal coaching rather than physical guidance. This experience can provoke anxiety and frustration in trainee and mentor alike. In order to overcome these limitations, a number of inanimate and animate models have been developed to help the trainee hone their laparoscopic skills. The sophistication of these training devices has varied from periscope viewing and foam rubber organs to live animal labs. The development of powerful personal computers has made virtual reality training a more recent possibility for developing laparoscopic skills. These trainers can simulate many of the steps of laparoscopic surgery such as manipulating tissue, clipping, cutting, and even suturing. The potential for greatly enhancing laparoscopic surgical training is great. Now the trainee can work independently in a life-like environment on a platform that is available 24-hours a day. Basic tasks can be rehearsed until mastered without impacting patient outcome or undue utilization of operating room time.

Many studies have demonstrated that trainees who practice laparoscopic skills in a simulated environment will improve their mastery of those skills when tested in that same environment. Fewer studies have been able to demonstrate a direct correlation between such simulation training and improved performance in the operating room. Our group is participating in a multi-center trial which will investigate whether a training curriculum involving a virtual reality laparoscopic simulator (LapSim<sup>®</sup>, Surgical Science Ltd, Göteborg, Sweden) will help surgical residents gain mastery of the skills necessary to complete a laparoscopic cholecystectomy.



FIGURE 1: Virtual reality laparoscopic simulator

### Objective Measurement of Surgical Skill Using Laparoscopic Simulators

Providing a safe and efficient training environment for our surgical residents to become the future leaders in surgery is a primary goal of any academic institution. Attaining this goal can be a challenge given new reductions in resident work hours and societal distrust of resident training. The use of surgical simulators appears to improve surgical training, but few criteria have been established to document objective assessment of technical skill.



At the University of Washington, the departments of Surgery and Engineering have constructed and validated the BlueDRAGON™ system in a live swine model, which allows objective assessment of laparoscopic surgical performance. The system acquires force/torque (kinematics) and motion (dynamic) information from the tip of an instrument during performance of surgical tasks. Our previous analysis of data reveals that surgeons of different levels of skill from novice to expert show varying patterns of states while surgeons with similar levels of skill show characteristically similar patterns. Therefore, a particular surgeon's kinematic and dynamic

data can be analyzed and objectively correlated to a degree of technical expertise.

Our goal is to develop and validate *inanimate* surgical training models for use with the BlueDRAGON™ system to objectively measure surgical skill. Residents at all levels as well as attending laparoscopic surgeons will perform surgical tasks while their performance data is collected by our novel system and then analyzed. As residents proceed through training, their improvement in technical skill can be further evaluated. This will provide safe and efficient training with objective feedback to residents as they acquire surgical expertise.

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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 & Materiel Command

- Telemedicine & Advanced Technology Research Center



## Objective Assessment of Surgical Skills

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

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

cystectomy 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 room - one which decreases the number of personnel required, increases efficiency and quality control, and which incorporates the robotic system into the hospital information system. In addition the robotic systems will be used to train, objectively assess and certify competence of surgeons.

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*The conceptual change is to train residents not for a given time, but rather to a given criterion level.*

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**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**

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# Mika Sinanan, M.D., Ph.D.

- **SURGICAL TELEMEDICINE: PROJECTION OF SURGICAL EXPERTISE IN THE WWAMI REGION**

*A Collaborative Research Project with UW Telemedicine, UW Surgery and Biorobotics, UWMC, Harbor Surgical Associates, and Gray's Harbor Community Hospital in Aberdeen, WA.*



**T**elemedicine refers to the use of information-based technologies such as videoteleconference, computer, and communications systems, to project healthcare and specialty expertise across geographic distances. In the Pacific Northwest, concentration of subspecialty expertise—especially surgical expertise—in the only medical school servicing the WWAMI region offers a compelling opportunity for developing a surgical telemedicine program. There is increasing interest at a Congressional level in such methods of standardizing care, primarily as a means of improving patient safety.

Surgical disorders frequently require urgent intervention before a complete diagnostic evaluation can be done. Unexpected findings in the OR especially in critically ill or fragile patients often lead the surgeon to seek consultation. Technology limitations delay and limit the value of this consultation, frequently until after irrevocable decisions have been made. We propose a surgical telementoring program that addresses these issues, facilitating timely consultation on all aspects of the patient data and projecting specialty expertise to the hand of the remote surgeon. Such a system has potential for substantially improving patient outcomes and providing patient-specific education to our regional surgical community.

Despite the perceived benefits of such a system, many issues need to be resolved to create the basis for sustained, routine, and facile teleconsultation and telementoring. These include:

#### **Procedural issues and etiquette:**

- confidentiality;
- consent;
- security and safety;
- cost for services, set-up, maintenance, etc.;
- electronic protocol for medical documents;
- responsibility;
- liability; and
- patients' preference: teleservices vs. in-person services.

#### **Engineering issues:**

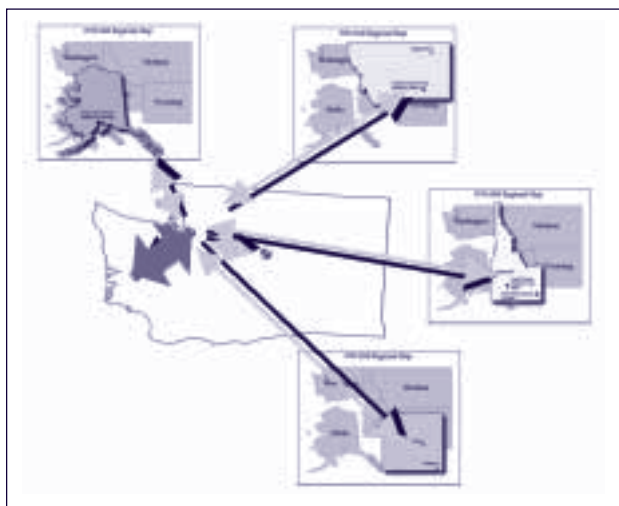
- communication/networking : latency due to time delay, bandwidth, traffic prioritization;
- efficient shared surgery environment for cooperation and telecollaboration;
- device optimization in both master and slave : high bandwidth, high fidelity, movement scale and reasonable force level;
- 3D visualization with high update rate;
- delay: effects of visual and haptic delay in bidirectional force (haptic) feedback;
- dynamic overlay feasibility of digital imaging data (MRI, CT, etc.) without performance degradation; and
- safety and durability of equipment.

Our research partner, Computer Motion, Inc. (<http://www.computermotion.com>), has the first device (Socrates(tm)) to be cleared by the FDA in the newly created category of Robotic Telemedicine Devices.

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*Unexpected findings in the operating room often lead the surgeon to seek consultation.  
Technology limitations delay and limit the value of this consultation, frequently until after  
irrevocable decisions have been made.*

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**FIGURE 1: Proposed Phased Surgical Telemedicine Consortium**

Socrates allows collaboration using audio and video conferencing, shared control of an endoscopic camera (when implemented with the Aesop Robotic Camera Control system), and video annotation of the surgical endoscopic image in the OR. It is an ideal platform for this project. In collaboration with Cara Towle and Dr. Tom Norris of the UW Office of Telemedicine, we have secured financial support for installation of two Socrates systems with appropriate telemedicine links for a one-year trial program between Gray's Harbor Community

Hospital in Aberdeen, and the University of Washington Medical Center.

Drs. Juris Macs, Akbar Ali, and Brent Rowe of Harbor Surgical Associates and members of the hospital OR leadership at Gray's Harbor Community Hospital will be our partners in this research project. We have developed a series of specific goals to demonstrate feasibility, utility, and economic viability of such a system and will incorporate them in the framework of a one-year clinical trial. Data on real-time, bedside surgical teleconsultation from this trial will include technical issues of connectivity, etiquette for surgical teleconsultation, patient factors including patient notification and consent, cost-benefit calculations to both patient and referring physician, and finally ancillary benefits including educational benefits, to the UW surgeons, residents, and to UW Medicine.

After analysis, if these data support measurable benefit from the projection of surgical teleconsultation to a regional partner institution, they will be used to gather support for a wider Surgical Telemedicine consortium, as illustrated in Figure 1. We hope to take this project to the UW Medicine, the University leadership, the WWAMI state legislatures, and to the federal government as a means of improving quality and reducing cost through projection of surgical specialty expertise in the WWAMI region.

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# TRANSPLANT SERVICE

CHRISTIAN S. KUHR, M.D.

WEI LI, M.D., PH.D.

JAMES D. PERKINS, M.D.



# Christian S. Kuhr, M.D.

- 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, 1997  
National Institutes of Health  
• Career Investigator Development Award, 1999

## 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

mitigate the effects of chronic non-specific immuno-suppression. 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

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*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.*

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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.

### 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

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 litter-mate donor, 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.

### 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

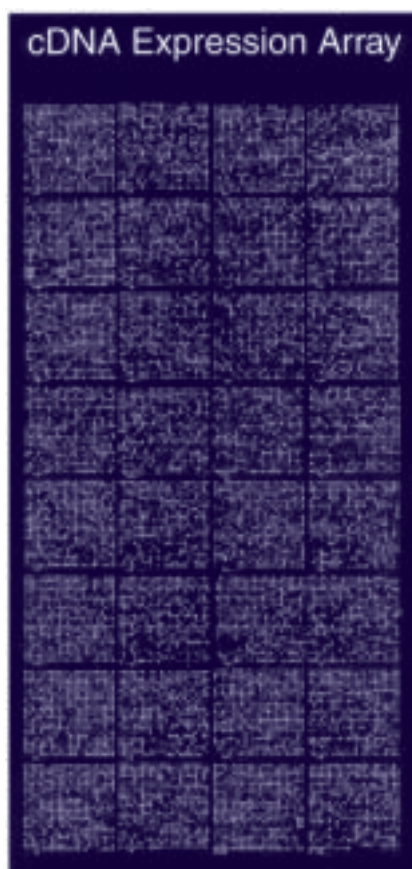


FIGURE 1

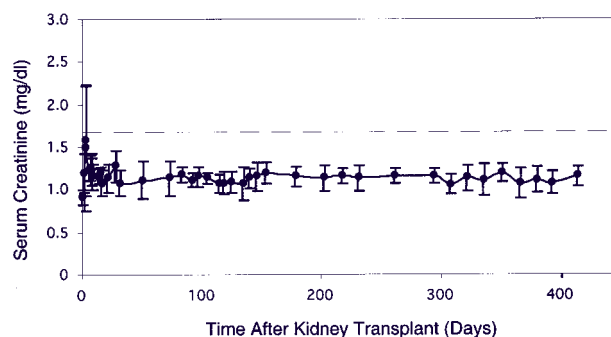


FIGURE 2

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

- MECHANISM OF MOUSE LIVER TRANSPLANT TOLERANCE
- THE ROLE OF COSTIMULATORY MOLECULES ON TOLERANCE INDUCTION
- THERAPEUTIC POTENTIAL OF DC IN ORGAN TRANSPLANTATION

## AWARDS

American Society of Transplant Surgeons

- Young Investigator Award, 1997



Although immunosuppressive drugs improve the short-term survival of organ transplants, 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 in which 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.

- To examine the ability of immature dendritic cell (DC) to induce T reg in vivo, and cytokines that modulate this activity, and to assess and maximize the therapeutic potential of DC and regulatory T cell in promoting tolerance induction in organ transplantation.

## Mechanisms of Mouse Liver Transplant Tolerance

It has been previously demonstrated that mouse liver grafts are accepted spontaneously across MHC barriers and induce donor-specific tolerance without immunosuppressive therapy (hepatic tolerance). Despite in vivo hyporesponsiveness to the liver graft and to subsequent grafts from the same donor, in vitro mixed lymphocyte culture (MLC) and cytotoxic lympholysis (CTL) assays

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*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.*

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I am currently focusing on studying the cellular and molecular basis of immune responses of organ transplantation using mouse orthotopic liver transplant (OLTx), heterotopic heart transplant (HTx), skin transplant (STx), or islet transplant (ITx) models:

- Taking the advantages from TCR transgenic or gene knockout mice and costimulatory molecule blocking antibodies to define and characterize the dominant factors which involved in the liver tolerance induction, such as T cell subsets, including T regulatory cell (T reg), the signals or pathways between antigen presenting cell (APC) and allo-reactive T cell, both in local (grafts) and in systemic (spleen and lympho nodes) ways.

- To further ascertain the mechanisms of liver transplant tolerance.

showed unimpaired antidonor reactivity (split tolerance). By contrast, livers from donors treated with Flt3 ligand (FL), which dramatically increase 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 and recipient T cells, and increased production of IL-12, IFN- $\gamma$ , 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 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.

We hypothesize that inducing activated T cell apoptosis and T regulatory cell differentiation both are critical in the liver tolerance, liver immature DC may be a key factor to induce regulatory T cell differentiation and mediate activated T cell apoptosis. To confirm our hypothesis, we have tested costimulation blockade on liver DC and T cell interaction by using CTLA4 Ig and anti-CTLA4 mAb recently. The results showed that blocking both B7-CD28/ B7-CTLA4 signals using CTLA4 Ig promoted liver allograft survival from FL pretreated donor. It was associated with increased alloreactive T cell apoptosis in the liver graft and recipient spleen, and increased IL-2, IL-10, decreased IFN- $\gamma$  levels in the recipient serum.

In contrast, blocking CTLA4 alone using anti-CTLA4 mAb, which was defined a negative signal to the T cell, broke the liver spontaneous tolerance and induce liver allograft acute rejection. This was associated with decreased alloreactive T cell apoptosis in the liver graft and recipient spleen, and increased IL-2, IFN- $\gamma$ , and decreased IL-4 in the recipient serum. These results suggest that co-stimulation between donor DC and recipient T-cells contribute to T cell immune devia-

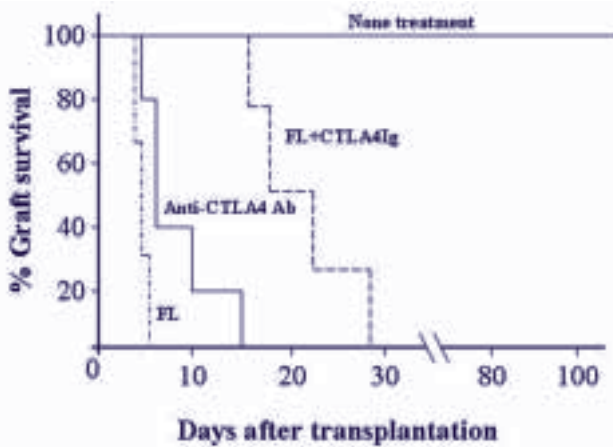


FIGURE 1

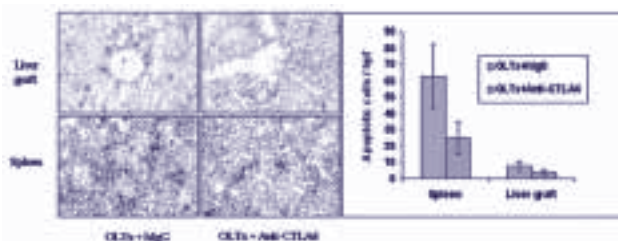


FIGURE 2

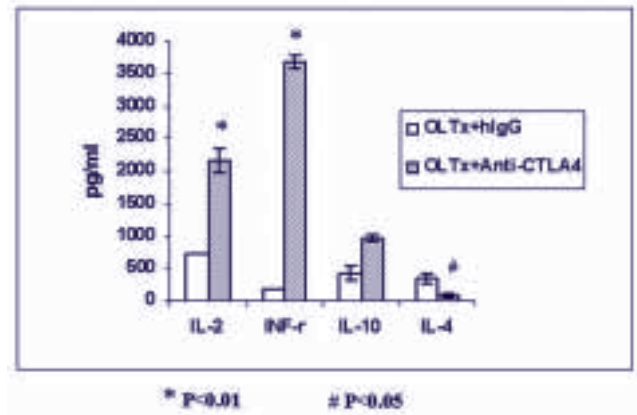


FIGURE 3

tion, alloreactive T cell apoptosis. If it lead to the production or generation of regulatory T cells remain to be further studied.

### The Role of Costimulatory Molecules on Tolerance Induction

Costimulatory molecules, in particularly B7/CD28 super family, has been extensively studied recently. A number of new members have been discovered and characterized, including B7/ CD28, B7/ CTLA4, and most recently PD-L/ PD-1, B7H/ ICOS, OX4o/ OX4oL, Tim3L/ Tim3. As we have know 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, induce tolerance. On the other hand, B7/CD28, B7H/ICOS, and OX4o/ OX4oL interactions provide a positive signal to the T cells, promote T cell proliferation and IL-2 production, induce immunity.

We are going to further target on these costimulatory molecules to precisely assess which signal is critical for liver tolerance induction and rejection. These studies on the role of these new accessory molecules and their effect on liver tolerance, activated T cell apoptosis, and possible promotion of T regulatory cells may provide a guide for a trial of DC, or antibody, or gene based therapy in patients receiving organ transplants.

### Therapeutic Potential of DC in Organ Transplantation

A recent report revealed DC is capable to induce T reg that inhibit immune responses. We also reported that the immunoregulatory cytokine, IL-10, induce T reg both in vivo and in vitro and promotes heart allograft survival in mice. Costimulation between donor DC and recipient T-cells may not only contribute to T cell immune deviation, alloreactive T cell apoptosis, but also

may lead to production of regulatory T cells. Thus treating the allograft recipient with immature donor DC in the presence of IL-10 or TGF $\beta$  may drive in vivo regulatory T cell generation and promote organ transplant tolerance.

We next will administer to mice donor immature DC (propagated from bone marrow or liver) plus treatment of these cells with IL-10 or TGF $\beta$  to examine the ability of immature DC to induce regulatory T cell in vivo, and possible cytokines that modulate this

activity. We will challenge DC treated recipients with allogeneic heart transplants or islet transplants (in NOD mice or STZ treated diabetes mice) to assess the therapeutic potential of DC-induced alloantigen specific tolerance.

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

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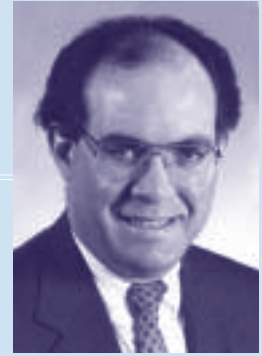
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# James D. Perkins, M.D.

• 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

## 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

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*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.*

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

the 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<sub>2</sub>O 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

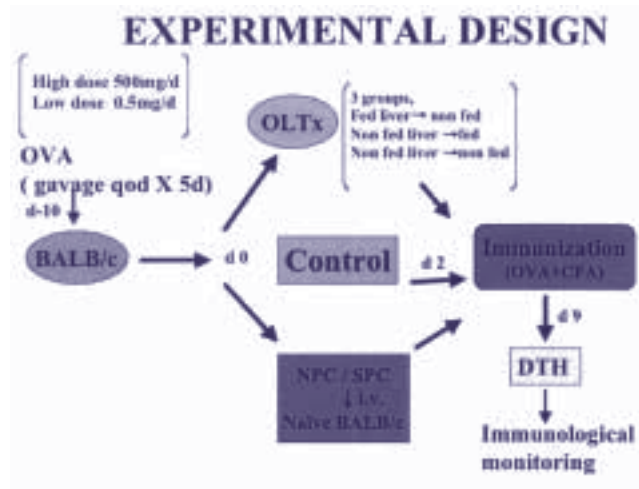


FIGURE 1: A model to study the liver's role in peripheral tolerance.

measure the cytokine levels of IL-2, IFN- $\gamma$ , and IL-10 from mixed lymphocyte reaction (MLR) culture detected by ELISA.

### How the Model Demonstrated Induction and Transfer of Tolerance

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

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

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- $\gamma$  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,  $\gamma\delta$  cells, and immature dendritic cells. The model could be manipulated to include transplanting livers between different genders, transplanting irradiated livers and other variations, and it could be used to study specific cell types previously unable to be evaluated.

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

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- THE BREAST HEALTH GLOBAL INITIATIVE FOR COUNTRIES OF LIMITED RESOURCES: CONSENSUS GUIDELINES FOR EARLY DETECTION, DIAGNOSIS AND TREATMENT

## FUNDING

AstraZeneca

Ethicon Endo-Surgery

Novartis Oncology

The Susan G. Komen Breast Cancer Foundation

Internationally, breast cancer is the most common cancer and the leading cause of cancer-related deaths among women. Much is known about methods for decreasing the morbidity and mortality of breast cancer. Unfortunately, most countries in the world lack the financial resources to implement appropriate breast health care interventions. This problem is compounded by social and cultural barriers, which obstruct women from seeking or receiving proper health care in a timely fashion.

*The Breast Health Global Initiative for Countries of Limited Resources: Consensus Guidelines for Early Detection, Diagnosis and Treatment* (originally titled the *Global Summit on International Breast Health*), was established to address breast healthcare measures in countries where access to healthcare is a challenge; where awareness is limited and where cultural barriers need to be overcome.

## Background

Women living in limited- and low-resource countries have a lower incidence of breast cancer but poorer survival, compared with women living in countries with higher levels of resources (Figure 1). The leading cause of higher breast-cancer mortality appears to be diagnosis at more advanced stages combined with limited access to treatment.

To begin to address this situation, the challenge of improving breast health care around the world is in recognizing that improvements need to be made in increments. It is unrealistic to expect that the health care changes made in the United States or Europe can be directly imported into a country of limited resources in Africa, Asia or the Middle East. Different countries have vastly differing social issues and financial needs

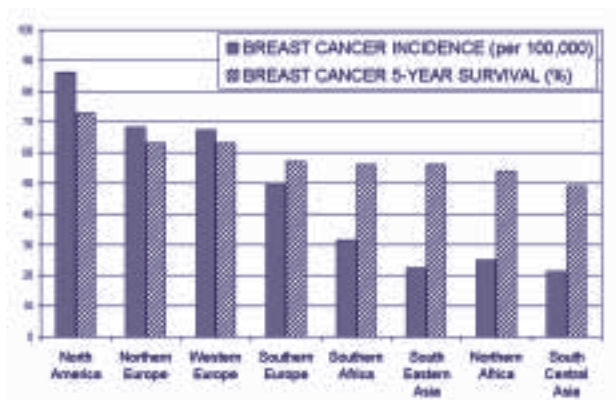


FIGURE 1: Breast cancer occurrence compared to five-year survival

that frame how breast health care changes should be implemented. A huge issue therefore is in sorting out where to begin.

Breast health care guidelines oriented to countries of limited resources have not existed in the past, yet they are a key step in improving health care particularly in developing countries of the world with limited financial resources. Evidence-based guidelines for breast health care provide a fundamental and flexible framework for change and improvement in medical health-care delivery. The development of these guidelines also cultivates international dialogue and understanding based on need for resources. Since health care systems cannot be transformed at once, guidelines help define where to begin in breast health care, providing an essential structural framework for dialogue and change, and a benchmark for growth and improvement.

## The Breast Health Global Initiative

In recognizing the growing global impact of breast cancer, and the shared challenges among countries

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*Breast health care guidelines oriented to countries of limited resources have not existed in the past, yet they are a key step in improving health care particularly in developing countries of the world with limited financial resources.*

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worldwide, *The Breast Health Global Initiative* was begun in 2002. The goals of the Initiative are:

### **Guideline development**

- Organize and host a biennial *Breast Health Global Initiative's* "Global Summit" as a venue to integrate physicians, scientists, healthcare providers, and advocates with expertise in breast health care, representing countries and regions throughout the developing and developed world, as well as representatives from world health and medical organizations, governmental health ministries and agencies.
- Through the biennial working meetings, create evidence-based breast health care guidelines that address core issues of finite resource allocation, in order to promote early detection, proper diagnosis and medically appropriate therapy for breast cancer in countries of limited resources.

### **Interdisciplinary linkage and alliance building**

- Build an organizational structure for linkage between three core groups:
  - clinicians and governmental health care agencies (health care systems, physicians, government agencies);
  - advocacy and non-governmental organizations (through communication, implementation, and patient education);
  - public health researchers (outcomes analysis, economic modeling, social impact studies).
- With the Guidelines as a framework, promote international outreach to underserved communities and political communities to advance change for improved breast health care, using education of the public and governmental decision makers as a tool for implementing change.
- Create new alliances and collective engagement through communication in multiple areas, including dialogue around social barriers to women's health and breast cancer advocacy.
- Continuous expansion of this program as a structure

for linkage and a "HUB" for ongoing communications, collaboration and collective engagement with focused international alliances, expanding global reach for continuing partnerships.

### **Communication**

- Write and publish the biennial multi-disciplinary international breast healthcare guidelines oriented to a) countries of low-level resources that have some existing breast health care delivery programs and infrastructure b) countries of medium-level resources, and c) the medically underserved population pockets and regions within countries with high level resources.
- Continuous worldwide dissemination of the Guidelines through a broad cross-section of channels of communication, targeting world-health and medical organizations and associations, developing nation embassies, thought leaders, media and breast cancer advocacy organizations.
- Facilitate the implementation of the Guidelines by providing relevant data and recommendations for pilot and fully funded research projects initiated by other organizations.

### **Public health sciences research**

- Generally provide a venue for public health sciences research directed at analysis of breast health care delivery in countries of limited resources.
- Specifically stimulate public health research projects in developing regions that specifically test the Guidelines, so as to 1) measure outcome, and 2) validate the Guideline recommendations.

### **Progress to Date: Global Summit 2002**

The initial step of the *Breast Health Global Initiative* was to hold the first Global Summit Consensus Conference in Seattle, Washington on October 2-4, 2002. The working meeting brought together breast cancer experts and patient advocates to develop consensus recommendations for the early detection, diagnosis, and treatment of breast cancer in countries with limited resources.



The panelists, representing 17 countries and 9 world regions, followed the process charged by the World Health Organization (WHO) to address breast cancer care in countries of low- or medium-level resources. Reviewing evidence-based data and consensus-defined breast care guidelines, the panelists debated approaches for breast health care, and specifically considered how this care may best be provided under the constraints of limited resources.

The results of the Global Summit Consensus Conference 2002 were published in *The Breast Journal* in the spring of 2003 (International Breast Health Care: Guidelines for Countries with Limited Health Care Resources,” *Breast J*, May/June 2003, Vol. 9, Supplement 2). Major findings and recommendations can be summarized as follows:

#### OVERVIEW

All women have the right to access to health care, but considerable challenges exist in implementing breast health care programs when resources are limited.

All women have the right to education about breast cancer, but it must be culturally appropriate, and targeted and tailored to the specific population.

In countries with limited resources, most women have advanced or metastatic breast cancer at the time of diagnosis.

Because advanced breast cancer has the poorest survival and is the most resource-intensive to treat, efforts aimed at early detection can reduce the stage at diagnosis, potentially improving the odds of survival and cure, and enabling simpler and more cost-effective treatment. These efforts are likely to have the greatest overall benefit in terms of both survival and costs.

There is a need to build programs that are specific to each country's unique situation.

The development of cancer centers can be a cost-effective way to deliver breast cancer care to some women when it is not yet possible to deliver such care to women nationwide.

Collecting data on breast cancer is imperative for deciding how best to apply resources and for measuring progress.

#### EARLY DETECTION

Early detection entails both early diagnosis in symptomatic women and screening in asymptomatic women.

Key prerequisites for early detection are ensuring that women are supported in seeking care, and that they have access to appropriate, affordable diagnostic tests and treatment.

The panelists propose the following action plan: 1) promote general activities to the empowerment of women to obtain health care, (2) develop infrastructure for diagnosis and treatment of breast cancer, (3) begin early detection efforts through breast

cancer education and awareness, (4) when resources permit, expand early detection efforts to include mammographic screening.

Public education and awareness can promote earlier diagnosis, and these goals can be achieved in simple and cost-effective ways, such as dissemination of messages through mass media.

When resources become available for screening, they should be invested in screening mammography, as it is the only modality that has thus far been shown to reduce breast cancer mortality.

Clinical breast examination (CBE) and breast self-examination (BSE) are important components of routine breast care in countries with access to mammography; and are important for general breast health education in all countries. However, the evidence does not support the use of CBE and BSE as sole screening methods at this time, recognizing that data from the most extreme limited-resource settings are lacking.

There is tremendous diversity among and within countries, and a program to promote early detection must therefore be tailored to each country's unique situation.

#### DIAGNOSIS

Accurate diagnosis avoids erroneous treatment for breast cancer, which can have devastating consequences for the woman and unnecessarily consumes resources.

The panel distinguishes between a *clinical diagnosis* of breast cancer (one based on signs and symptoms and imaging findings) and a *pathology diagnosis* of breast cancer (one based on microscopic examination of cellular or tissue samples).

All women should have a pathology diagnosis of breast cancer before they are given definitive treatment for the disease, no matter how strongly their clinical findings suggest cancer.

Mastectomy should not be used to diagnose breast cancer, noting that accurate diagnosis can be made by less invasive means.

Mammography and ultrasound also help determine the extent of disease within the breast, which is essential when breast-conserving therapy can be offered to women.

Expertise in pathology was identified as a key requirement for ensuring reliable diagnostic findings.

The tools for pathology diagnosis include fine needle aspiration biopsy, core needle biopsy, and standard surgical biopsy.

The panel noted that each of these tools has potential benefits and limitations in the limited-resource setting, and concluded that the choice among them must be based on the available tools and expertise.

#### TREATMENT

Local treatment of *early-stage breast cancer* involves either mastectomy or breast-conserving surgery followed by whole-breast irradiation.

Substantial support systems are required to optimally and safely

use breast-conserving approaches to local therapy or cytotoxic chemotherapy as systemic therapy.

Cytotoxic chemotherapy also improves recurrence rates and survival with the magnitude of benefit decreasing with increasing age.

*Locally advanced breast cancer* (LABC) accounts for at least half of all breast cancers in countries with limited resources and has a poor prognosis.

The treatment of LABC requires multiple disciplines and is resource intensive.

Most women with LABC will require a radical or modified radical mastectomy after neoadjuvant (preoperative) chemotherapy and/or hormonal therapy.

In those women who cannot receive preoperative chemotherapy because of resource constraints, mastectomy with node dissection, when feasible, may still be considered in an attempt to achieve local-regional control.

After local-regional therapy, most women should receive additional systemic chemotherapy.

Ovarian ablation or suppression with or without tamoxifen is an effective endocrine therapy in the adjuvant treatment of breast cancer in premenopausal women with estrogen-receptor positive or unknown breast cancer.

In postmenopausal women with estrogen and/or progesterone receptor-positive or unknown breast cancer, the use of tamoxifen or anastrozole are effective adjuvant endocrine therapies.

Efforts to reduce the number of breast cancers diagnosed at an advanced stage thus have the potential to improve rates of survival while decreasing the use of limited resources.

These practical written guidelines are a crucial end-product of this collective process and they function as a vital medical tool and, importantly, as a flexible framework for implementing and expanding programs that can be tailored to each country and region's unique circumstances. It is important to recognize that populations differ, social environments differ, the types of resources that can be made available differ among different parts of the world, and so the solutions in two different parts of the world as to what is the next step may well be different.

#### Future Directions: Global Summit 2004 and Beyond

Now sponsored by the Fred Hutchinson Cancer Research Center, and co-sponsored by The Susan G. Komen Breast Cancer Foundation, *The Breast Health Global Initiative* is growing steadily. Collaborating organizations now include the World Health Organization (WHO), World Society for Breast Health (WSBH), International Society of Breast Pathology (ISBP),

American Society for Breast Disease (ASBD), and the U.S. National Committee/International Union Against Cancer (USNC/UICC).

*The Breast Health Global Initiative* is providing a flexible framework for a long-term partnership of organizations that actively support continuing growth, development and dissemination of these international breast care guidelines which serve as practical instruments for improving breast health policy, planning and implementation. These linkages and process are essential to cultivate lines of communication for a deeper understanding of the complexities of the global environment, and initiate fundamental research and pilot projects in developing regions.

In the Global Summit 2004, there will be added components of research, economic analysis and pilot projects. *The Breast Health Global Initiative* has evolved to operate as a means to produce progress and multiple outcomes and at the same time to create linkages for strategic, multifaceted public and private partnerships (Figure 2).



FIGURE 2: Framework for Breast Care Guidelines

The international breast health guidelines published in 2003 are progressively being disseminated through national and international health care and medical organizations and government health ministries. The next *Breast Health Global Initiative* is slated for fall 2004 in Washington, DC. The universal objective of this global initiative is as a continuing international alliance and inquiry into the challenge of improving breast health care around the world.

#### Additional Information

- **Website:** [www.fhcr.org/phs/global\\_summit/](http://www.fhcr.org/phs/global_summit/)
- **Contact** Leslie Sullivan @ 206-221-2350 or [sullivan@u.washington.edu](mailto:sullivan@u.washington.edu)
- **To view an international briefing** held in Washington, DC on *The Breast Health Global Initiative for Countries of Limited Resources* click on the Komen website to view their webcast: [www.komen.org](http://www.komen.org).

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- 

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# David R. Flum, M.D., MPH

- 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.

epidemiology and biostatistics. To address this goal of system-level quality improvement for all areas of clinical interest, we use these tools to answer four necessary questions.

## 1. Can We Determine the Way Surgical Procedures Impact the Average Patient?

Risk of adverse outcome is a component of all surgical procedures. While the informed consent process tries to address this by providing the patient with a summary of the expected risk, in fact what we really offer in the consent process are the results found in the published case series of the best practitioners in the field. For the vast majority of general surgical procedures we simply don’t know the community level risk of adverse outcome. As such, we are unable to determine what should be considered the standard, who are the outliers (both good and bad) and what techniques work out of the

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*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.*

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

To do this, outcomes researchers use a set of tools borrowed from health economics, decision analysis,

research environment. In the absence of a tracking system for outcomes we often rely on estimates derived from randomized trials (which for most general surgical procedures have not been completed) or administrative data. Only by understanding the real level of risk can we determine the opportunities for improvement in the system.

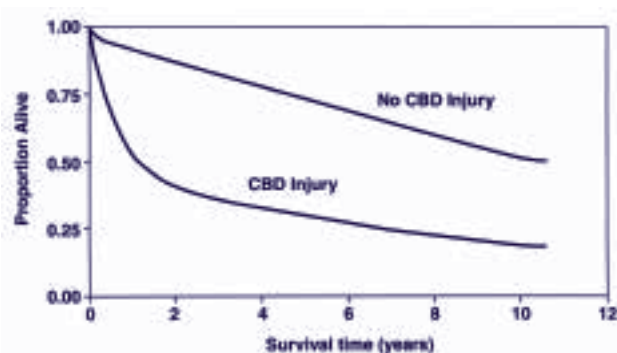
Research I’ve been involved with during the last year has addressed this issue of community-level risk in commonly performed general surgical procedures by using administrative data. Determining population-level risk requires the analysis of large databases. For example, in evaluating rates of misdiagnosis in 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.

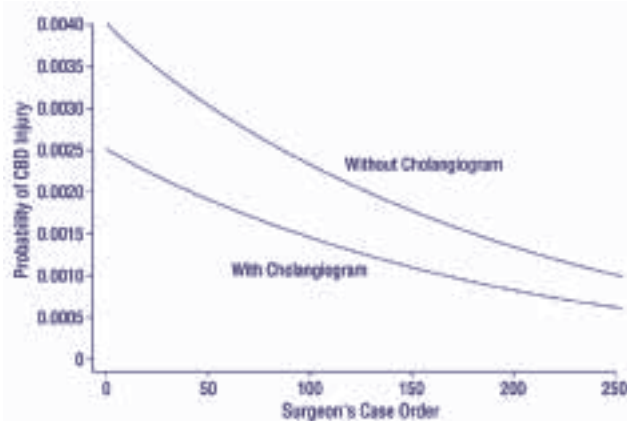
We recently evaluated the risk of death after bile duct injury among all Medicare beneficiaries nationwide and found they were 2.5 times more likely to die within



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

the first few years after an injury compared to uninjured patients (Figure 1).

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



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

## 2. What are the Avoidable Factors Associated with These Adverse Outcomes?

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

For example, using administrative data we have quantified the degree to which both surgical inexperience and the failure to use a cholangiogram are associated with CBD injury. Surgical inexperience (the surgeons'



1st through 19th cholecystectomy) and failure to use a cholangiogram result in a 60–70% increase in the likelihood of CBD injury. When combined, these factors have even greater impact. Surgeons are 2.2 times more likely to have a CBD injury during their first 20 operations if they do not use a cholangiogram compared to procedures performed at later points in the experience curve. Defining the risk relationship associated with CBD injury is also important in informing patients and surgeons of the predicted probability of this adverse outcome (Figure 2). This may be a more effective way of “informing” the informed consent process.

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

### 3. What Are the Implications (Using Cost/Decision Analysis and Randomized Trials) of Avoiding Those Factors?

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

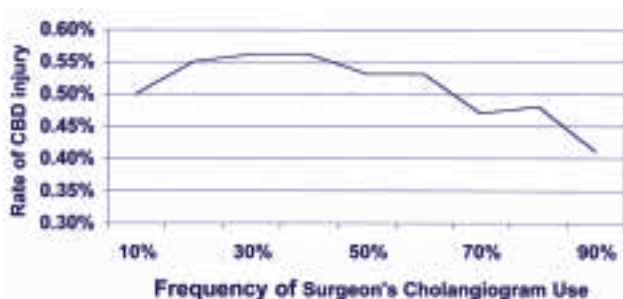


FIGURE 3: The effect of increasing the surgeon's frequency of cholangiogram use on the rate of common bile duct (CBD) injury

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

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

### 4. How Can We Make System Level Changes and Monitor the Impact of Those Changes?

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

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

Involving the financial stakeholders may be the most effective way in improving system level care, but it may not be the best way. Over the last century, the surgical community has shown real leadership in



addressing adverse outcomes and taking responsibility for them. The morbidity and mortality conference, for so long a part of the surgical culture, was ahead of its time in trying to improve the results of future interventions by avoiding past mistakes. Unfortunately, it has

become apparent that conferences alone cannot deal with system-level factors involved in adverse outcome. Outcomes researchers are doing just that, and the surgical community has an opportunity to use this research in leading the way towards quality improvement.

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# 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, 1999



## 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

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

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

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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

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

rather than on measurements of function and quality-of-life; and incorrect statistical methods.

My primary interest is in surgical outcomes research utilizing clinical trials and quality of life assessments of surgical treatments. A current NIH-funded project involves a multi-institutional, phase II study of video-endoscopic assisted retroperitoneal debridement (VARD) for infected peripancreatic fluid collections following necrotizing pancreatitis. Open surgical necrosectomy for infected pancreatic fluid collections is highly effective, but is associated with significant morbidity primarily related to the large abdominal incision. Percutaneous catheter techniques are much less morbid, but require long treatment times and intensive drain manipulations to produce moderate success. Effectiveness is limited because the large amount of necrotic tissue debris cannot be easily drained via small diameter 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 (HbA1C, 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.

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# Brant K. Oelschlager, M.D. 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, 2001

### FUNDING

Gore, Inc.

Cook Surgical

Tyco, United States Surgical Corporation

### Multichannel Intraluminal (esophageal) Impedance

**M**ultichannel 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 "normal" manometry tracings and vice versa. This phenomenon was unrecognized before this new technology.

### 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

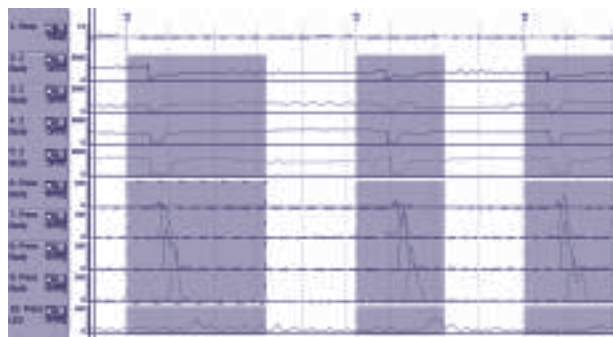
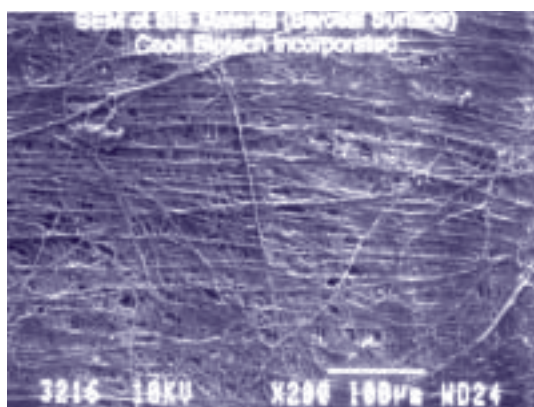


FIGURE 1: Impedance and manometry tracing showing complete bolus transit (MII) with a normal manometric tracing.

*Intestinal metaplasia or Barrett's esophagus is condition associated with significant gastroesophageal reflux disease. Its presence confers an increased risk of developing adenocarcinoma of the esophagus.*

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.



**FIGURE 2:** Small intestinal submucosa (SIS).

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.

### 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 4. There may be episodes of reflux, so called "volume reflux", in which the pH does not drop to or below 4, thus are undetected by pH sensors. Before MII, these episodes were unrecognized. 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 study showed that pharyngeal reflux was present in 40% of patients with airway symptoms and abnormal reflux. This result leads to the thought that patients with abnormal pharyngeal reflux may go undetected by pharyngeal pH testing. Artifact and the fact that respiratory symptoms may be due to volumetric reflux rather than acid pharyngeal reflux, support this theory.

Pharyngeal reflux detection with MII is being carried out in our department. Our preliminary results in normal subjects are showing that the previously thought "normal value" (1 pharyngeal episode) might not be the case if non acid reflux is taken into account.

### Ongoing Studies with Impedance

- 1) Measurement of esophageal motility measuring bolus clearance
- 2) Evaluating patients with GERD and Respiratory Symptoms
  - a. Normal amount of nonacid reflux in the pharynx. (Figure 3)
  - b. Nonacid reflux in the pharynx as a predictor of success or failure of medical therapy
- 3) Patients with GERD who do not respond to medical therapy

### Barrett's Esophagus

Intestinal metaplasia or Barrett's esophagus is a condition associated with significant gastroesophageal reflux disease (GERD). Its presence confers an increased risk



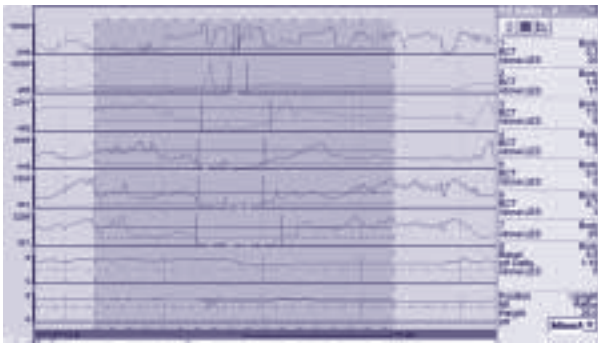


FIGURE 3: Pharyngeal reflux episode detected by impedance.

of developing adenocarcinoma of the esophagus. This risk is estimated to be approximately 0.5% per year. The treatment of Barrett's is focused at treating symptoms of GERD and surveillance for progression to dysplasia or cancer. Both medical and surgical therapy are fairly effective in controlling symptoms, but neither is thought to decrease the risk of progression to cancer. Both acid and bile have been implicated in the pathogenesis of Barrett's. Proton pump inhibitors effectively reduce the acidity of gastroesophageal reflux, but gastric secretions and bile reflux still occur. Antireflux surgery creates a barrier to all reflux, therefore limiting both acid and bile reaching the esophagus, therefore theoretically is the best method of halting the progression of Barrett's esophagus. Most reports of the outcomes of antireflux surgery have not been designed to address whether regression of metaplasia or decrease in the progression to cancer occurs. Recently two separate studies have suggested that Barrett's may regress in a significant number of patients after an effective anti-reflux procedure.

We recently analyzed the course of 106 consecutive patients with Barrett's Esophagus who underwent Laparoscopic Antireflux Surgery (LARS) between 1994 and 2000, representing 14% of all LARS (n=754) performed in our institution during that period. All 106 patients agreed to clinical follow-up in 2002 at 40 mo. (median; range: 12-95 mo). Fifty-three patients

(50%) agreed to functional evaluation (manometry and 24-hour pH monitoring); and 90 (85%) patients to thorough endoscopy, with appropriate biopsies and histologic evaluation to determine the status of BE.

Our results revealed that heartburn improved in 94/98 (96%) and resolved in 69 patients (70%) after LARS. Regurgitation improved in 58/69 (84%) and dysphagia improved in 27/33 (82%). Distal esophageal acid exposure improved in 48/53 (91%) patients tested and returned to normal in 39 patients (74%). One patient underwent re-operation two days after fundoplication (gastric perforation). Pre-operatively, biopsy revealed Barrett's without dysplasia in 91 patients, Barrett's indefinite for dysplasia in 12 patients, and low-grade dysplasia in 3 patients. Fifty-four of the 90 patients with endoscopic follow-up had short segment Barrett's (< 3cm) and thirty-six had long segment Barrett's (> 3cm) preoperatively. Post-operatively, endoscopy and pathology revealed complete regression of intestinal metaplasia (absence of any sign suggestive of BE) in 30/54 patients (55%) with short segment Barrett's but in 0/36 of those with long segment BE. Among patients with complete regression, 89% of those tested with pH monitoring had normal esophageal acid exposure. This was observed in 69% of those who failed to have complete regression. One patient developed adenocarcinoma within 10 months of LARS.

In conclusion, patients with Barrett's, LARS provides excellent control of symptoms and esophageal acid exposure. Moreover, intestinal metaplasia regressed in the majority of patients who had short segment Barrett's and normal pH monitoring following LARS, a fact that was, heretofore, not appreciated. LARS should be recommended to patients with Barrett's to quell symptoms and to prevent the development of cancer. We are currently embarking on a prospective trial of medical versus surgical therapy in patients with Barrett's to further elucidate the differences and mechanisms of progression and regression of this disease.

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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

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 tuberlin, has a sequence of 1807 amino acids showing significant homology to the Rap1GAP protein over a 200 amino acid region near the C-terminus.

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

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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

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 tuberlin in the PI3K/mTOR signaling pathway that is pivotal to the cellular response to growth factors (e.g., insulin) and nutrients. Genetic screens in mosaic flies for cell size control identified loss-of-function mutants of the *Drosophila* homologs of TSC1 and TSC2 that exhibit increased cell size in a cell-

autonomous fashion. Conversely, over-expression of dTSC1 and dTSC2, but neither alone, effectively rescued this phenotype (i.e., reduced cell size). Genetic epistatic experiments in flies showed that the effects of dTSC1 and dTSC2 were dominant over dInR and dAkt but not dTor and dS6K. Biochemical studies confirmed a negative regulatory role of the hamartin-tuberin complex in mTOR-dependent protein synthesis.

The current model suggests that tuberlin inhibits mTOR activity by serving as a GTPase activating protein for Rheb, a Ras-related protein, and consequently reduces p70S6K and 4E-BP1-dependent protein translation (Figure 1). Upon growth factor stimulation of PI3K, downstream activation of Akt results in phosphorylation of tuberlin and releases its inhibition on mTOR. In TSC tumors, cells have lost TSC1 or TSC2 activity, thus resulting in uninhibited cell growth associated with elevated levels of mTOR and p70S6K activities. Indeed, pharmacologic blockade of mTOR with rapamycin, an immunosuppressant drug, causes profound anti-tumor response in vivo. However, it is not currently known how up-regulation of mTOR results in tumor formation, nor do we understand the mechanisms of tumor response to rapamycin.

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

### The $\beta$ -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/ $\beta$ -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  $\beta$ -catenin canonical pathway is best understood for its role in various human cancers (e.g., colon, skin, liver).  $\beta$ -Catenin is a highly conserved 95-kD protein involved in cell-cell adhesion and intracellular signaling. In its latter role,  $\beta$ -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  $\beta$ -catenin and cyclin D1. In 293T renal epithelial cells,

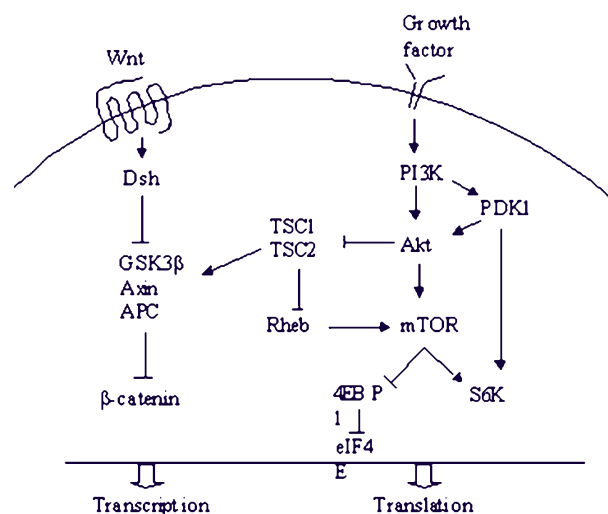


FIGURE 1: Model of TSC1/TSC2 pathway

expression of TSC1 and TSC2 reduced  $\beta$ -catenin levels by promoting its degradation. Correspondingly, TSC1/TSC2 inhibited  $\beta$ -catenin dependent activity of the LEF/Tcf transcription factors. Evidence suggested that TSC1 and TSC2 act at the level of the  $\beta$ -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 tuberlin act on distinct molecular targets in various pathways, how may their function be regulated? One possible mechanism for separating multiple activities within the cell could be on the basis of unique subcellular localization of the proteins. Since signaling complexes function as modules, the context in which they interact with other proteins depend on their localization. For example, insulin stimulation of PI3K leads to localized increased concentration of PIP3 at the plasma membrane. This, in turn, recruits Akt from the cytosol to the membrane where it becomes activated.

In studying the subcellular localization of hamartin and tuberlin, we found that they indeed reside in multiple compartments (i.e., cytosol, microsome, cytoskeleton). Of particular interest is the vesicular component in which tuberlin was previously shown to interact with rabaptin-5 to modulate endocytosis. Biochemical analyses showed

that the microsomal fraction of TSC2 belongs to the lipid raft domains and interacts with caveolin-1, a cholesterol-binding, structural protein of caveolae. Cells devoid of tuberin have mis-localized caveolin-1 and reduced formation of caveolae at the plasma membrane.

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

### Genetic Modifiers and Phenotypic Heterogeneity

One of the unexplained observations of the TSC syndrome is the variability in disease severity. This so called

phenotypic heterogeneity can be seen in related individuals carrying the same genetic mutations, thus implicating the presence of other modifying factors.

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

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

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# VAPSHCS/GENERAL SURGERY

LORRIE LANGDALE, M.D.

MICHAEL SOBEL, M.D.



# Lorrie Langdale, M.D.

## • MECHANISMS OF INJURY CONTROL AFTER LIVER ISCHEMIA-REPERFUSION

### FUNDING

VA Merit Review Grant – submitted for funding



Trauma is the leading cause of death in the United States for those between the ages of 1 and 45. The majority of trauma patients' deaths (75%) occur as a result of their initial injuries within the first 24 hours. The remaining 25–40,000 patients die within two days to several weeks after injury as a result of multiple organ failure. Death from cardiac, pulmonary or renal failure may be averted in many patients by aggressive support of these organs. However, the development of hepatic failure is associated with a virtually uniform mortality without liver transplantation. As with trauma, hepatic failure after elective liver resection or liver transplantation carries a high morbidity and mortality.

for global immunosuppression, as evidenced by their failure to express major histocompatibility complexes. These subjects fall victim to multiple organ failure when they fail to recognize a "second hit" challenge due to inhibition of their inflammatory response to injury. Thus, uncontrolled pro-inflammatory or anti-inflammatory states result in poor clinical outcomes.

Therapeutic strategies to improve outcomes after IR have been aimed at disrupting single components of a widely redundant inflammatory cascade. To date, however, laboratory successes have not translated to clinically relevant therapies. Given that many patients present for treatment after the pro-inflammatory phase of injury is well underway, a more recent approach

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*We hypothesize that negative regulators of cytokine and chemokine signaling play a pivotal role in the evolution of hepatic IR injury, and that it is the lack of appropriate negative regulation which leads to the uncontrolled inflammation associated with multiple organ failure.*

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Organ failure is often preceded by inadequate tissue and cellular perfusion which produces an ischemic phase of injury and culminates in a gradual loss of cellular homeostasis. Reperfusion has been postulated to initiate a complex chain of "second-phase" events that leads to a neutrophil-mediated injury. The risk of multiple organ failure (MOF) with its attendant high mortality has been postulated to significantly increase when pro-inflammatory cytokine and chemokine expression is sustained and unchecked, as might be expected with serial inflammatory stimuli. At the opposite extreme of the systemic inflammatory response spectrum is the less well defined anti-inflammatory response syndrome (CARS). Animals and patients subjected to prolonged periods of hemorrhagic shock or simulated post-operative infection show evidence

focuses on understanding the mechanisms of inflammation regulation and control. Understanding the mechanisms of cellular signaling that precede, trigger and control the inflammatory response to an injury could be key to effective clinical modulation of ischemia-reperfusion injury, extravascular inflammation, and subsequent organ failure. Further, identification and potential exploitation of natural inflammatory control mechanisms offers a new avenue for clinical management of a spectrum of ischemia-reperfusion injuries.

### Hepatic Ischemia-Reperfusion: Evolution of an Injury

The liver is particularly vulnerable to the effects of ischemia-reperfusion (IR) because of its "open architecture". The early phase of IR injury reflects the direct effects of local toxic reactive oxygen products that injure

tissue and mediate neutrophil activation and chemoattraction. The hypoxia/re-oxygenation of ischemia-reperfusion also initiates the recruitment of leukocytes to the specific site of injury through a spectrum of cytokines and chemotactic proteins expressed by Kupffer cells and hepatocytes. Activated PMNs alter microcirculatory perfusion through adhesion to endothelium, and adherent PMNs diapedese from the sinusoid, through the fenestrated endothelium, into the parenchyma where release of additional reactive oxygen products compounds the local injury. This PMN-mediated phase of hepatic IR occurs relatively late in reperfusion, with neutrophil infiltration dominating between 8 and 24 hr of reperfusion. This neutrophil-mediated phase of liver injury either continues to progress, culminating in necrosis and liver failure, or resolves, with resumption of normal liver function and a return to homeostasis and health.

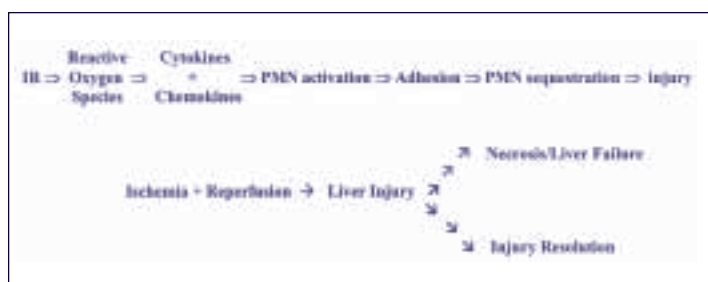


FIGURE 1

The events which determine whether injury progresses to irretrievable necrosis or resolves are poorly understood. Based on the hypothesis that uncontrolled inflammation is largely responsible for the morbidity and mortality associated with ischemia-reperfusion injury (IR), the vast majority of investigations addressing mechanisms of IR have focused on understanding and modulating the pro-inflammatory phase of injury. Laboratory studies have advocated inhibition of reactive oxygen products, neutralization of inflammatory mediators with cytokine specific antibodies or soluble receptors, or blockade of neutrophil adhesion mechanisms to control the acute inflammation associated with IR.

Translation into clinically relevant therapies, however, has been disappointing, primarily because therapeutic strategies have been aimed at disrupting single components of a widely redundant inflammatory cascade. Given the critical role that cytokines and chemokines play in both early and late events after ischemia-reperfusion, an alternative approach to control of inflammation is to examine and potentially

exploit the natural mechanisms that regulate and inhibit cytokine and chemokine production.

### JAK-STAT Cell Signaling and Suppressors of Cytokine Signaling (SOCS proteins) in Inflammation

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

STAT dimer/tetramer translocates to the nucleus and binds with a specific DNA sequence and/or other transcription factors to effect target gene transcription.

A regulated response to injury requires both active inflammation, with the expression of pro-inflammatory cytokine and chemokine mediators and neutrophil activation and trafficking, and active inflammation control. In addition to effecting cytokine signaling, STAT-mediated cell signaling induces the

expression of Suppressors of Cytokine Signaling (SOCS) proteins that serve as classic negative feedback mechanisms for cytokine expression. Numerous cytokines important to acute inflammation activate cells through JAK-STAT, including TNF $\alpha$ , IFN $\gamma$ , IL-1, IL-6, IL-10 and Growth Hormone (GH). These mediators are, in turn, controlled, at least in part, by SOCS proteins.

The pattern and time-course of SOCS mRNA observed following cytokine stimulation appears to be both stimulus and tissue dependent. For example, although constitutively expressed in thymus and spleen, the level of SOCS-1 mRNA are very low in un-stimulated liver cells. Injection of IL-6 or IFN $\gamma$  results in dose dependent increased levels of SOCS-1, SOCS-2, SOCS-3 and CIS RNA. Both SOCS-1 and SOCS-3 mRNA are detectable in liver within 20 min after IL-6 injection in mice. SOCS-1 mRNA levels return to baseline within 4 hr while SOCS-3 mRNA is sustained for up to 8 hr. SOCS-2 and CIS mRNA remains elevated for 24 h.

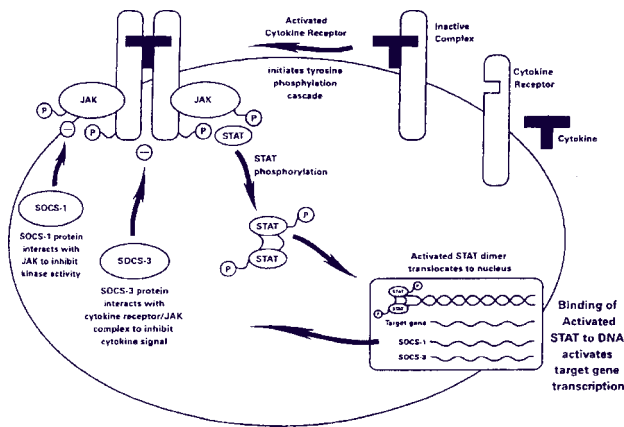


FIGURE 2

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

### Current Laboratory Focus

Most laboratory models of acute hepatic IR utilize a fixed period of ischemic injury, applying the variables of reperfusion time and treatment regimens to delineate and understand the cascade of inflammatory events that follow ischemia. Clinically, the response to hepatic IR injury varies with the injury itself, and the balance between pro-inflammatory mediators and negative control mechanisms likely determines whether tissue heals or proceeds to cellular apoptosis and/or necrosis. We hypothesize that negative regulators of cytokine and chemokine signaling play a pivotal role in the evolution of hepatic IR injury, and that it is the lack of appropriate negative regulation which leads to the uncontrolled inflammation associated with multiple organ failure. To

this end, our current work is focusing on determining the role of JAK/STAT signaling and SOCS-mediated negative regulation of inflammation on the evolution of liver injury severity. We have developed a mouse model of lobar IR with a spectrum of ischemic injury and are testing our hypothesis by applying measurements of SOCS gene and protein expression and immunohistochemical techniques to longitudinal time course studies of injury severity in normal mice, mice treated with interferon gamma (IFN $\gamma$ ), and mice conditionally deficient for SOCS-1 or SOCS-3.

Early or sustained induction of SOCS proteins would potentially significantly alter the evolution of increasingly severe injury. If true, exploitation of these natural inflammatory control mechanisms offers a potential new avenue for clinical management of IR injuries. We have previously shown that IFN $\gamma$  is protective in a model of liver IR when given in a dose known to restore clinical immunocompetence. High dose IFN $\gamma$  pre-treatment of normal, immunocompetent rabbits blunts progression of liver IR injury, as evidenced by decreased glutamate pyruvate transaminase concentrations, while lower dose IFN $\gamma$  pre-treatment or saline control is associated with a significantly increased cellular injury 24 hr after liver IR. Histologic injury, characterized by midzonal and centrilobular 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 IFN $\gamma$ . Late neutrophil infiltration is virtually eliminated. Our data have since been corroborated by other investigators utilizing high dose IFN $\gamma$  in a rat model of liver IR. They further showed amelioration of the associated secondary lung injury. Proinflammatory cytokine and chemokine expression in both liver and lung is markedly attenuated by high dose IFN $\gamma$  pre-treatment. Although the mechanism(s) by which IFN $\gamma$  influences the progression of acute inflammation after IR has not been elucidated, given that IFN $\gamma$  is a potent inducer of SOCS-1, early or sustained expression of SOCS-1 may account for these observations.

### Summary of Significance

We hypothesize that the relative balance between acute inflammatory mediators and negative regulating cell signaling events determines whether an ischemia-reperfusion injury progresses or resolves. Expanding our understanding of normal mechanisms of inflammation control will potentially open new therapeutic strategies to the management of acute injury.

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# Michael Sobel, M.D.

## • 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

laboratories have found that heparin directly influences platelet function by at least two separate mechanisms.

### Heparin Interactions with von Willebrand Factor

Using biophysical methods, binding assays, and molecular modeling, they demonstrated that heparin binds to a specific domain of von Willebrand factor (vWf) (1;2). This plasma protein is essential for normal platelet hemostatic function, and mediates the adhesion of platelets at sites of vascular injury (especially under high shear, arterial conditions). When heparin binds vWf it interferes with the platelet hemostatic properties of the protein. Specific sub-species of heparin were purified that bound vWf with especially high affinity. Through scientific collaborations with Dr. Yasuo Suda, a carbohydrate polymer chemist in Japan, a structurally defined disaccharide motif was identified that was responsible for heparin's binding to vWf. A refined heparin with high affinity for vWf (and low affinity for antithrombin-

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*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.*

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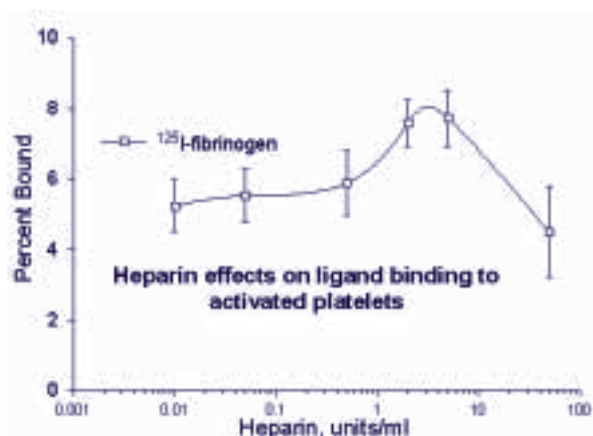
and cells have not been as well characterized. In part, the structural complexity of carbohydrates and heparin in particular has hindered efforts to better understand its structure-function relations. Also, the biological effects of heparins have often been contradictory or confusing, due to the complexity 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

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, GpIIb/IIIa (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).

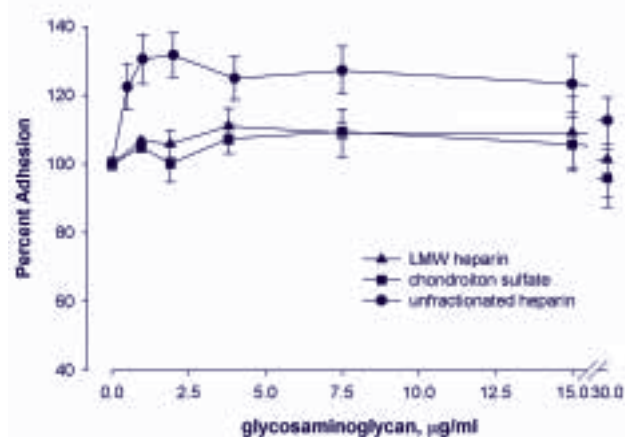
### Heparin Modulates $\alpha_{IIb}\beta_3$ Integrins

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  $\beta_3$  containing integrins ( $\alpha_{IIb}\beta_3$ ,  $\alpha_V\beta_3$ ) but the type of  $\alpha$  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.

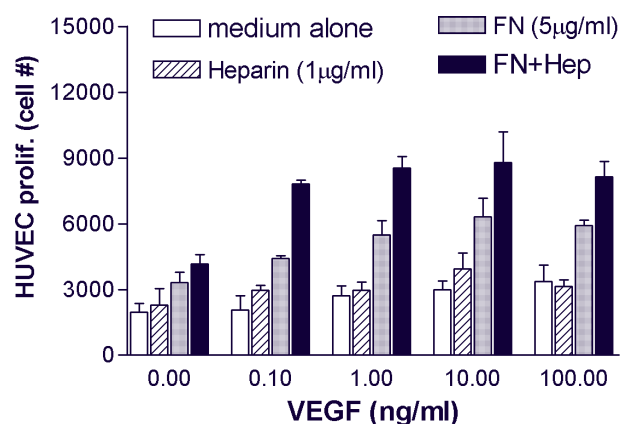
### Heparin Modulation of Endothelial Cell Migration and Proliferation

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.



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# VASCULAR SURGERY

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- TISSUE PULSATILITY IMAGING FOR THE DETECTION OF SOLID TUMORS
- FUNCTIONAL TISSUE PULSATILITY IMAGING OF BRAIN
- HIGH INTENSITY FOCUSED ULTRASOUND (HIFU) LESION MONITORING
- ULTRASOUND READING CENTER FOR CAROTID STENTS

## FUNDING

DARPA/Office of Naval Research

National Cancer Institute

National Institute on Biomedical Imaging and BioEngineering

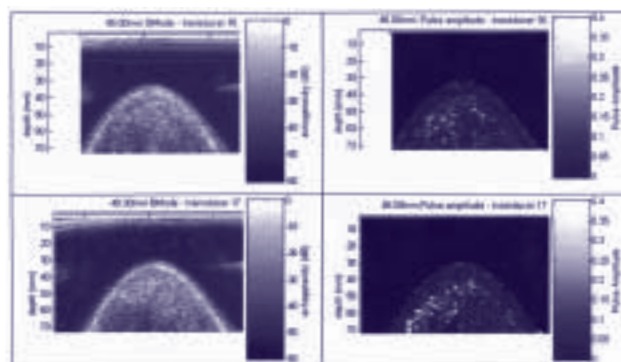
National Institutes of Health



Based on the pioneering work of D. Eugene Strandness, Jr., M.D. in the Department of Surgery between 1961 and 2002, 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 disorders.

## Tissue Pulsatility Imaging for the Detection of Solid Tumors

Forty years ago, Judah Folkman postulated that solid tumors could not grow larger than 1 mm in diameter without generating a new blood supply. The creation of new blood vessels to supply the tumor is a process called angiogenesis. Now it is accepted that angiogenesis is a marker for growing tumors. The new blood vessels are abnormal: large, poorly innervated, and arranged in irregular patterns. We hypothesize that the cardiac cycle pulsations of tumors with new vessels formed by angiogenesis have a greater amplitude than surrounding normal tissues. Our preliminary data suggests that the pulse amplitude of breast tumors averages three times as great as normal surrounding breast tissues. Normal pulsations in tissue are about 0.1% by volume. Using ultrasound methods, we can measure volume pulsations of 0.005%. The National Cancer Institute Unconventional Innovations Program is supporting the construction and testing of an ultrasound instrument to form 3-dimensional pulsation images which are expected to display breast tumors as regions of increased pulse amplitude. Patients who have had tumors detected with



**FIGURE 1: Ultrasound B-mode and Pulsatility Images of Breast Cancer**  
Conventional ultrasound “brightness mode” images are shown on the left, pulsatility images are shown on the right from the same section

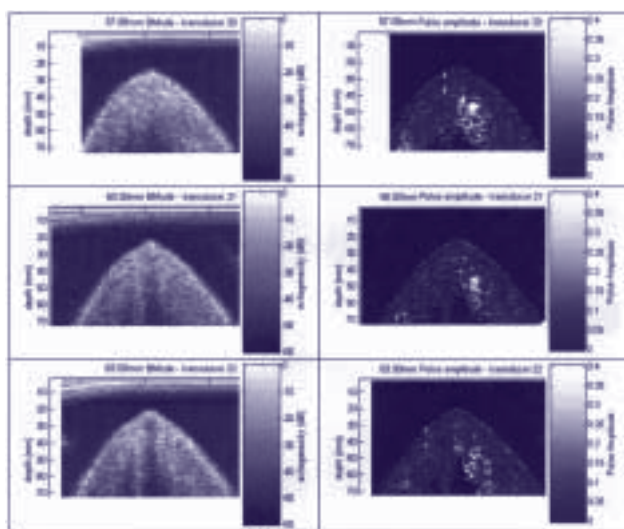
breast MRI are asked to volunteer to have 3-dimensional ultrasound “Tissue Pulse Images” under a Human Subjects approved protocol.

Three-dimensional ultrasound pulsatility images of breast tissue are made with a special 3-dimensional ultrasound imaging system constructed for this purpose. A series of sectional images of a breast with lobular carcinoma is shown in Figures 1 and 2.

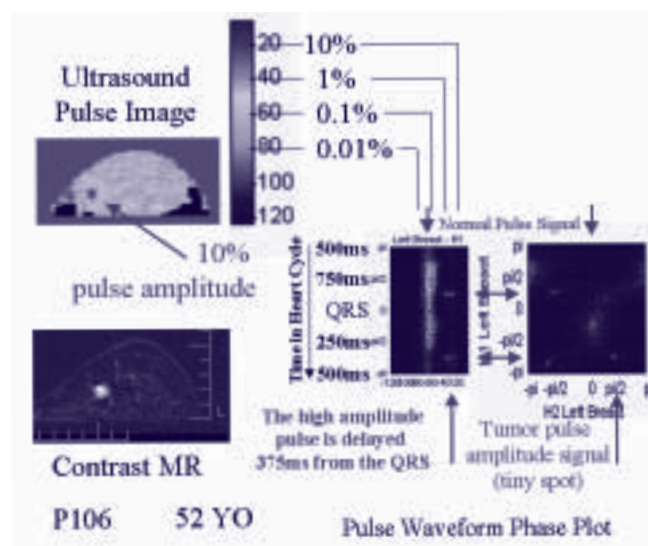
In Figure 1, the pulsatility image on the right shows a marked 12 millimeter layer of adipose tissue under the skin with a pulse amplitude less than 0.05%. Under that layer, in the glandular tissue, pulse amplitudes of 0.4% can be seen.

In other cross sectional pulsatility images of the breast (Figure 2), large regions of pulse amplitude of 0.4% can be identified. When compared to MRI contrast images of the breast, these high pulse regions seem to be located at the margin of bright contrast regions in the MR images.

In addition to pulse amplitude, the timing or “phase” of the pulse with respect to the ECG is used to separate normal signals from abnormal signals. From previous experience, we expect that tissue with an obstructed or limited supply will have a delayed pulse. The pulse waveshape can be classified by a mathematical technique called Fourier Analysis, which provides



**FIGURE 2: Ultrasound B-mode and Pulsatility Images of Breast Cancer**  
Conventional ultrasound “brightness mode” images are shown on the left, pulsatility images are shown on the right from the same section.



**FIGURE 3: High Pulse Amplitude of Tumor**

**Upper Left:** Ultrasound Pulse Amplitude image of the same tumor showing the pulse amplitude is 10%.

**Lower Left:** Contrast MR image of a 9 mm diameter breast tumor

**Center:** Plot of pulse timing vs amplitude showing pulse of tumor compared to pulse of normal tissues.

**Right:** Plot indicating the shape of the pulse waves.

information on the phase and amplitude of higher frequencies in the pulse signal.

In figure 3, the Center plot and the Right plot require explanation. Pulse images of the breast are formed by measuring the pulsation in each of 10,000 rectangular volumes in the breast. The Upper Center plot shows the pulse amplitude (horizontal axis) and pulse time (vertical axis). Each dot on the plot, shown as brightness, displays the data from one of the 10,000 volumes in the breast. A mark at ZERO in the center of the vertical axis indicates pulse with an expansion maximum at the ECG QRS wave. The pulse waves shown at “ $-\pi/2$ ” have peak expansion at 250 milliseconds after the QRS wave. The normal amplitude ( $-60 \text{ dB} = 0.1\%$ ) pulse waves are also indicated by points on the Right plot. Those points appear as a “cloud” at “ $-\pi/2$ ” on the vertical axis and just to the right of ZERO on the horizontal axis. The waveforms at this location are normal in shape, expanding during systole and relaxing in diastole.

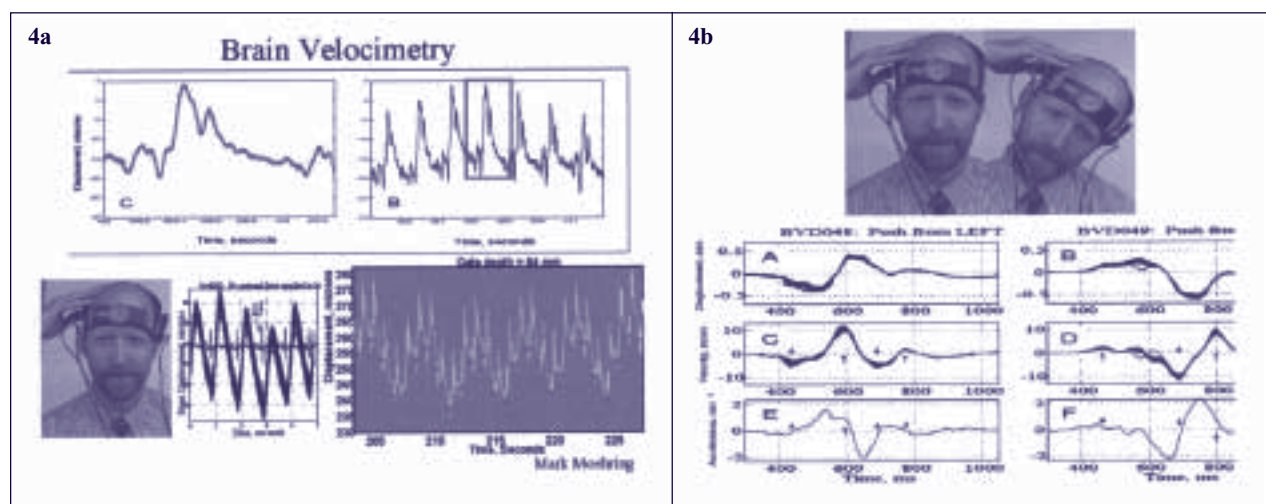
The group of points in the Right plot forming a “cloud” at  $+\pi/2$  on the vertical axis and  $-\pi$  on the horizontal axis have a pulse wave shape and timing exactly like the normal wave shape but the pulse wave is inverted. This is a surprising result. We believe that the normal wave shape occurs in normal tissues dominated by arterioles and that the inverted wave shape occurs in normal tissues dominated by venules. The pulsations in the arteriole dominated tissue are compressing the venule dominated tissue producing the inverse waves.

The two “dashes” at  $-20$  on the Upper Center horizontal axis (pulse amplitude of 10%) at times  $-3\pi/4$  and  $+\pi/4$  (cardiac cycle time on the vertical axis) represent tumor waveforms. In some volumes the waveform is upright and in others the waveform is inverted indicated by the two tiny spots on the Upper Right plot. Another unusually strong waveform appears slightly later. This also appears upright and inverted. The shape is different from the normal and the tumor shape.

Currently this breast pulsatility imaging system is being tested clinically. A similar instrument for prostate tumors is about to begin clinical testing.

### Functional Tissue Pulsatility Imaging of Brain

The same measurement methods use in the breast and prostate can be used in the brain, kidney and placenta to investigate perfusion in those tissues. Based on the preliminary data in Figure 4. Pulse imaging of the brain will be tested under a new NIH/NIBIB grant awarded this year for the identification of ischemic brain regions



**FIGURE 4: Brain Motion and Pulsatility**

*Fig. 4a: Brain Pulsations from a Trans Cranial Doppler – The brain moves 50 microns with each heartbeat and an additional 20 microns with each respiratory cycle.*

*Fig. 4b: Brain Motion from a Head Push/Tilt – The brain floats in the Cerebral Spinal Fluid with respect to the skull when the skull accelerates.*

in stroke patients and for detecting functional perfusion changes in the brain during different activities.

#### Monitoring Tissue Response to High Intensity Focused Ultrasound (HIFU) Therapy

Our colleagues at the University of Washington Center for Industrial and Medical Ultrasound (CIMU) are developing High Intensity Focused Ultrasound (HIFU) systems to cauterize tissue. HIFU treatment can deliver intensities of 2,000 Watts/cm<sup>2</sup> to a treatment volume measuring 1 mm by 1 mm by 10 mm at a depth of 10 cm below the skin. This intensity, 20,000 times the intensity used in diagnostic ultrasound and 4,000 times the intensity of sunlight, can heat tissue in the treatment volume to 90 degrees centigrade in 2 seconds. Potential applications include sealing arterial punctures in two seconds and ablating tumors. The HIFU methods have the advantage that deep tissue can be treated without penetrating the overlying tissues with instruments.

The lesions formed by HIFU (Figure 5) are about the size of a grain of rice; the size is proportional to the intensity and the treatment time.

Our research group is charged with monitoring the treatment so that the therapy can be stopped at the most efficacious time. When HIFU is applied to tissue, in addition to heating the tissue, associated radiation force pushes the tissue away from the transducer. The radiation pressure can be higher than blood pressure so that blood can be pushed backwards along an artery. When HIFU is applied to solid tissue, the tissue is pushed away,

when the HIFU is turned off, the tissue “rebounds” or bounces back to the original position, as long as the tissue remains elastic.

When HIFU is applied, the lesion may be pushed more than half a millimeter further away from the transducer. When the HIFU is stopped, the tissue rebounds to a final position in about half a millisecond. By interrupting the treatment every second for a few milliseconds, the rebound can be monitored without extending the required treatment time. The amount of rebound increases for the first few seconds of the treatment as the temperature of the tissue in the treatment volume increases and then the rebound decreases as the tissues “cook” at the elevated temperature.

The loss of rebound verifies that treatment is complete so that the system can, if necessary be advanced to a new location to treat a new volume.

#### Ultrasound Reading Center for Carotid Stents

One of the major treatable causes of stroke is stenosis or 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 formed by an atherosclerotic plaque. If left untreated, the plaque can fall apart sending debris to occlude smaller arteries in the brain, causing a 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 artery to open the stenosis, has been under development since



*We hypothesize that the cardiac cycle pulsations of tumors with new vessels formed by angiogenesis have a greater amplitude than surrounding normal tissues.*

1995. The artificial lining device is called a stent. A stent is usually made of metal. It is placed with a catheter entering the body through the femoral artery in the leg.

Stents are now used clinically in many arteries. Several clinical trials are evaluating their effectiveness for treating carotid stenosis. One of the trials is CREST (Carotid Revascularization Endarterectomy versus Stenting Trial), a major, National Institutes of Health funded multicenter trial. Duplex Doppler ultrasound examination, developed here in the Vascular Division, is used for long term follow-up of the patients in the CREST trial. CREST is one of ten clinical trials served

by the Ultrasound Reading Center. The Ultrasound Reading Center in the Vascular Division is responsible for assuring that the follow-up examinations performed at over 130 field centers around the United States are performed and interpreted according to protocol.

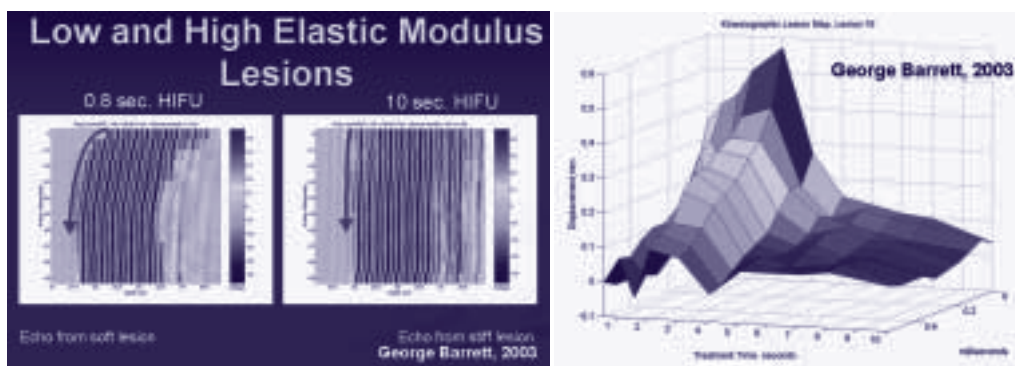
The ultrasound reading center publishes protocol manuals for the ultrasound field centers, provides training for field center personnel, receives examination images and interpretations from the centers, evaluates those data for adherence to protocol, assigns classifications to the results, tabulates the data, and reports the results to the trial sponsors.



**FIGURE 5:** High Intensity Focused Ultrasound Lesions formed in Liver

*Left:* Cross sectional appearance of the lesions for different exposures.

*Right:* 3-dimensional computer reconstruction of lesion shapes for different exposures.



**FIGURE 6:** Lesion Rebound when HIFU is Interrupted

*Left:* Rebound from immature and mature lesion.

*Right:* Rebound magnitude and rate as a function of treatment time.



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# Alexander W. Clowes, M.D.

## • REGULATION OF VASCULAR SMOOTH MUSCLE CELL GROWTH

### AWARDS

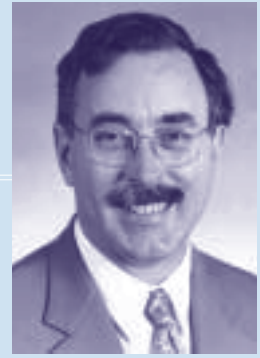
National Heart, Blood and Lung Institute MERIT Award

National Institutes of Health

- Vascular Surgery/Cardiology Training Grant

### FUNDING

National Institute of General Medical Sciences



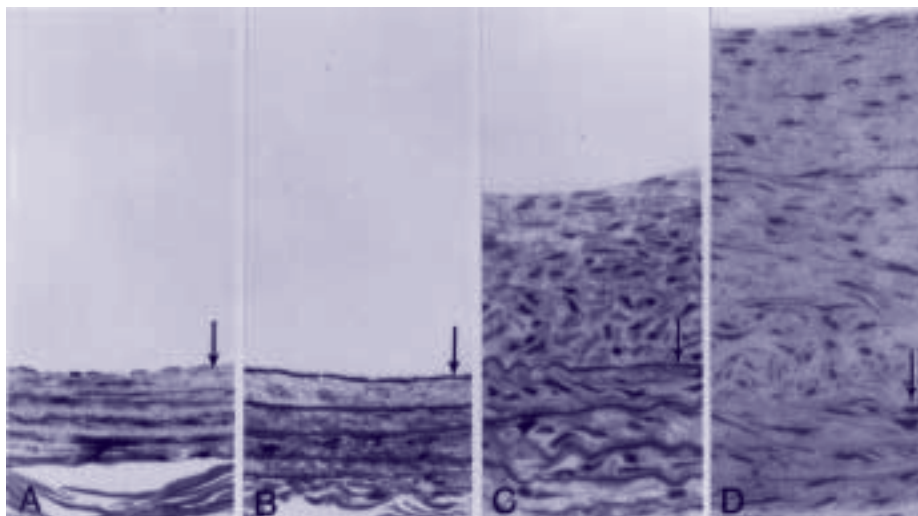
Vascular surgical procedures are designed to rebuild diseased blood vessels and improve blood flow. While these procedures restore the circulation, they also cause injury. This injury induces a wound healing response that in some instances is associated with accumulation of scar tissue (intimal hyperplasia) 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.

Recent studies in the laboratory have defined a novel pathway of smooth muscle cell activation which



**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).

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

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- $\beta$  family, by array analysis. BMP-4 is expressed by endothelium, is upregulated by increased shear stress, and inhibits growth and at times kills smooth muscle cells.

Recent experiments using a mouse monoclonal antibody that recognizes and blocks the beta form of the

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*We have been studying heparin as a paradigm for drugs that inhibit smooth muscle cell proliferation and migration.*

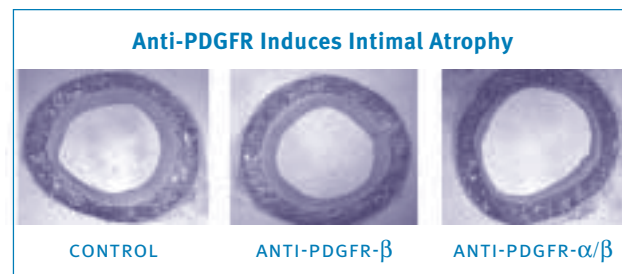
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human vascular smooth muscle cells. We are currently pursuing experiments designed to understand "crosstalk" between growth factor and cytokine pathways.

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

**Regulation of smooth muscle growth in grafts by blood flow:** 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,

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



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

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- 

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# Günter Daum, Ph.D.



- MODULATION OF GROWTH FACTOR SIGNALING IN VASCULAR SMOOTH MUSCLE CELLS BY INFLAMMATORY CYTOKINES

FUNDING  
National Institutes of Health

**R**estenosis 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.

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,

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*Inhibiting proliferation and survival of smooth muscle cells could promote plaque rupture and increase the risk of thrombotic events.*

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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

inhibits intimal hyperplasia in the balloon-injured baboon carotid artery.

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

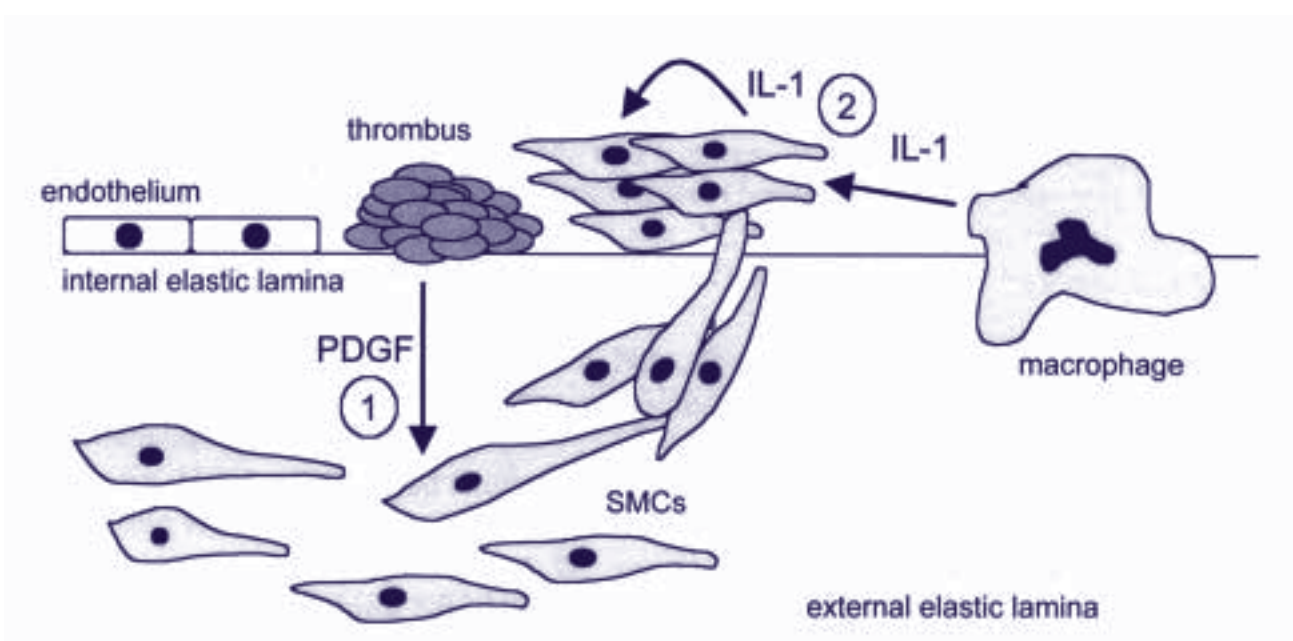
IL-1R2 is considered a decoy receptor, sequestering IL-1R1 ligands.

Several lines of evidence suggest a role for IL-1 in restenosis. In humans, a polymorphism in the IL-1RA locus has been discovered, which may protect from restenosis after coronary angioplasty and after coronary stenting. Although the consequences for this polymorphism for IL-1RA expression in the vessel wall is not known, observation in IL-1RA-deficient mice point to an anti-inflammatory function of IL-1RA 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- $\kappa$ B 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- $\kappa$ B also decreased intimal growth. Together, these data suggest that IL-1 may contribute to intimal hyper-

plasia 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-1 $\beta$  by itself is not mitogenic with or without cyclooxygenase inhibitors. When administered together with PDGF-BB, however, IL-1 $\beta$  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-1 $\beta$  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-1 $\beta$ . Analysis of cell cycle regulatory proteins showed that IL-1 $\beta$  suppressed expression of cell cycle-dependent kinase (CDK) inhibitors, p21(WAF1/CIP1) and p27 (KIP1). Consistent with this observation is our finding that IL-1 $\beta$  enhanced CDK2 activation by PDGF-BB.



**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.



These data suggest that IL-1 $\beta$  promotes proliferation of SMCs in the presence of PDGF-BB by inhibiting expression of CDK inhibitors. Experiments to address the question whether IL-1 $\beta$  affects p21/p27 transcription, translation, or stability of message or protein, are currently under way in our laboratory.

### Conclusion

IL-1 receptors, agonists, and antagonists appear to be expressed upon arterial injury in various animal models, strongly indicating a role for IL-1 in restenosis.

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

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# Thomas Hatsukami, M.D.



## • MAGNETIC RESONANCE IMAGING OF THE HIGH-RISK ATHEROSCLEROTIC PLAQUE

### FUNDING

National Institutes of Health  
Astra-Zeneca Pharmaceutical  
Esperion Pharmaceutical  
Pharmacia Pharmaceutical  
Pfizer Pharmaceutical

Our research work involves a collaborative effort between members of the Departments of Medicine, Pathology, Radiology, and Surgery, and investigators at the Armed Forces Institute of Pathology, PLA Hospital in Beijing China, Massachusetts General Hospital, Mayo Clinic, and University of Utah. Our long-term goal is to develop high-resolution cardiovascular MRI into a tool that can be used in large population-based studies and clinical trials for atherosclerosis prevention and treatment.

**Background:** Recent developments in therapy for atherosclerosis have improved the prognosis of cardiovascular disease and decreased cardiovascular morbidity and mortality. Further progress in eliminating atherosclerotic disease depends significantly on the development of accurate, reproducible and preferably non-invasive imaging techniques to identify high-risk lesions of atherosclerosis, and to evaluate the results of interventions aimed at slowing aggressive disease. Serial in vivo assessment of the atherosclerotic lesion is essential for studies examining mechanisms of disease progression. A better understanding of the underlying mechanisms of progression may lead to more precise identification of high-risk populations for more targeted intervention, as well as the development of novel therapies.

	NC/IPH PRESENT ON HISTOLOGY	NC/IPH ABSENT ON HISTOLOGY
NC/IPH Present on MRI	56	2
NC/IPH Absent on MRI	10	22

**TABLE 1:** Test performance of multispectral MRI for identifying regions of lipid-rich necrotic core (NC) and acute intraplaque hemorrhage (IPH). Accuracy (95% C.I.) = 87% (80-94%), sensitivity = 85% (78-92%), specificity = 92% (86-98%), and Kappa = 0.69 (0.53-0.85).

### HISTOLOGIC STATE OF THE FIBROUS CAP

MR APPEARANCE OF THE FIBROUS CAP	THIN OR RUPTURED	THICK	COLUMN TOTALS
Thin or Ruptured	25	6	31
Thick	6	54	60
Row Totals	31	60	91

**TABLE 2:** 2 x 2 performance table for identifying an unstable fibrous cap: comparing the MR findings with the histologic standard. Of the 91 exam locations, the MR findings suggested the presence of 33 unstable fibrous caps, twenty seven of which were confirmed histologically. The data result in a sensitivity of  $0.81 \pm 0.06$ , specificity of  $0.90 \pm 0.02$ , and a calculated Kappa statistic of 0.71 (95% CI = 0.56 to 0.86).

Over the past 10 years, we have developed and validated MRI techniques for characterizing advanced carotid atherosclerosis (Figure 1). We have shown that MRI is capable of quantifying lesion size with high precision and identifying plaque features such as the necrotic core, intraplaque hemorrhage, and fibrous cap status, and can categorize carotid plaque types according to the AHA atherosclerosis lesion classification with good sensitivity, specificity, and reproducibility (Table 1, 2, 3 and Figure 2). We have shown that fibrous cap thinning and rupture, identified by MRI, is highly associated with recent transient ischemic attack and stroke (Table 4), and that intensive lipid lowering therapy is associated with markedly decreased lipid content in carotid atherosclerosis by MRI. Furthermore, we have shown that plaque regions that enhance following gadolinium infusion are associated with areas that have increased plaque neovascularity (Figures 3 and 4). Neovascularity is believed to play an important role in the pathogenesis of the unstable plaque, serving as a conduit for recruitment of inflammatory cells into the lesion. In summary, MRI is capable of non-invasively

*Further progress in eliminating atherosclerotic disease depends significantly on the development of accurate, reproducible and preferably non-invasive imaging techniques to identify high-risk lesions of atherosclerosis, and to evaluate the results of interventions aimed at slowing aggressive disease.*

identifying morphologic and compositional features of advanced human atherosclerosis *in vivo*, and provides more biologically meaningful measurements than other currently accepted imaging modalities such as intima-media thickness.

**Conclusions:** MRI shows great promise as a non-invasive tool to examine the development of high-risk plaque features *in vivo*. Large, multi-center population-based studies will be needed to assess factors leading to more rapid atherosclerosis progression and unstable disease, not only in individuals with clinically

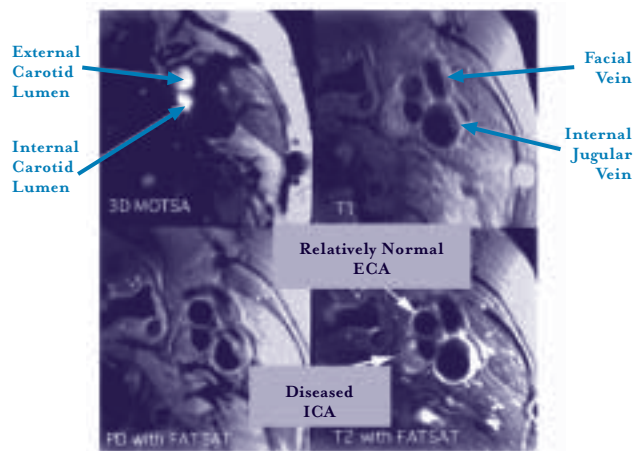
evident disease, but also amongst those with clinically silent, earlier stage atherosclerosis. Such studies, using direct, quantifiable, objective assessment of atherosclerotic lesions will be critical for the development and testing of novel therapies, and to more precisely define high-risk populations for targeted, early intervention and prevention.

RATER 1	RATER 2			ROW TOTAL
	THICK	THIN	RUPTURED	
Thick	109	1	0	110
Thin	20	94	0	114
Ruptured	4	2	5	11
Column total	133	97	5	235

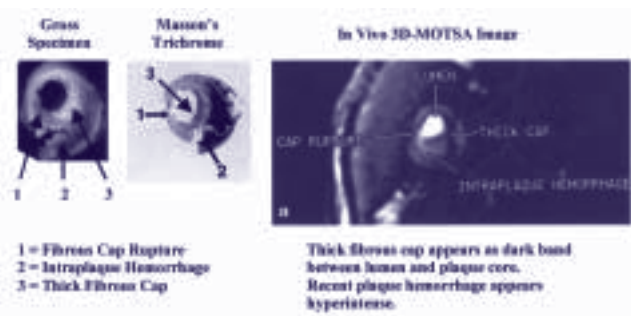
**TABLE 3:** Inter-rater reproducibility for categorizing the fibrous cap as intact thick, thin, or ruptured on MRI. There was a high level of agreement between raters, with Kappa = 0.78 (95% CI = 0.71-0.87).

CAP STATUS BY MRI	SYMPTOMATIC	ASYMPTOMATIC	PERCENT WITH SYMPTOMS	OR FOR SYMPTOMS	95% CI
Thick	1	10	9%	1	---
Thin	6	6	50%	10	1.0, 104
Ruptured	21	9	70%	23	3, 210

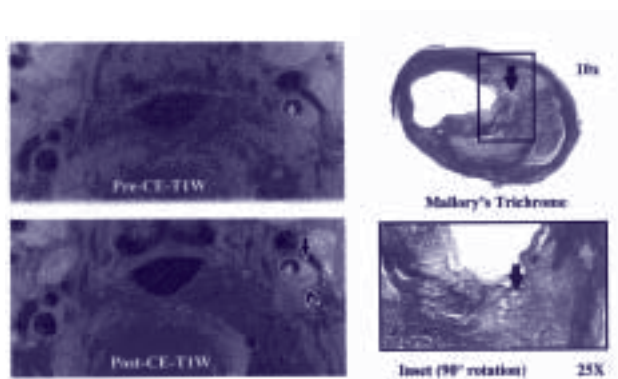
**TABLE 4:** Association between fibrous cap status on MRI and history pre-operative TIA or stroke. Pre-operative carotid artery MRI was performed on 53 consecutive patients and the fibrous cap was categorized as thick, thin or ruptured using previously published MRI criteria. Twenty-eight subjects had 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 and higher odds ratio (OR) for 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. There was an estimated 23-fold increase in the likelihood of having recent symptoms if MRI identified a ruptured fibrous cap, compared to those with thick caps.



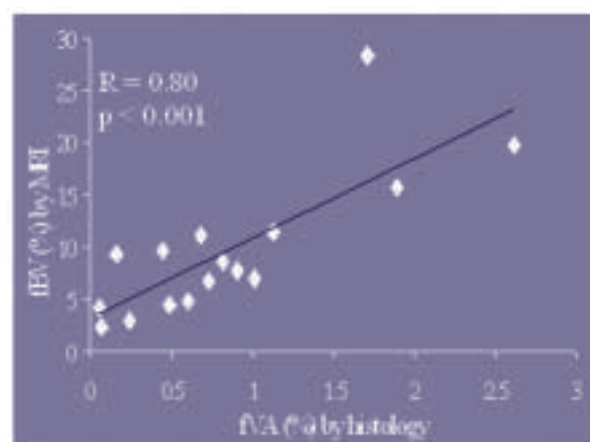
**FIGURE 1:** Appearance of eccentric plaque in the internal carotid artery on time-of-flight MRA (3D MOTSA), T1-, proton density (PD-), and T2- weighted MRI. The four cross-sectional views are taken at the same level of the carotid artery. MOTSA is an ME angio technique where the lumen is bright. T1, PD and T2 weighted images highlight the plaque features. ICA = internal carotid artery, ECA = external carotid artery.



**FIGURE 2:** Appearance of a thick fibrous cap and adjacent region of fibrous cap rupture (at the 8:00 position) on MRI, with corresponding gross and histological cross sections.



**FIGURE 3:** Enhancement in the common carotid artery (asterisk in lumen) at the 2:00 position seen after administration of gadolinium contrast. The enhanced region corresponds to an area rich in neovascularity seen on the histological section.



**FIGURE 4:** Dynamic contrast-enhanced MRI with gadolinium correlates well with the fractional area of neovascularity in carotid plaques ( $R=0.8$ ,  $p<0.001$ ).

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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

**E**ndovascular 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

### 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

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*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.*

---

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.

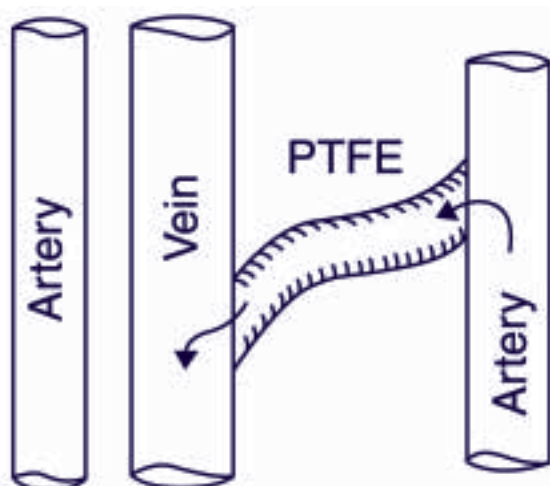
Dr. Kohler and Dr. David Glickerman, from interventional radiology, began the endovascular therapy program at the Seattle VA hospital. We are one of several centers in the country participating in an FDA-sponsored trial of the AneuRx endovascular graft. Dr. Kohler was on the planning committee for the VA Cooperative Trial of Open versus Endovascular Repair of abdominal aortic aneurysms. This trial will begin in October, 2002 at the Seattle VA.

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.

### 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,

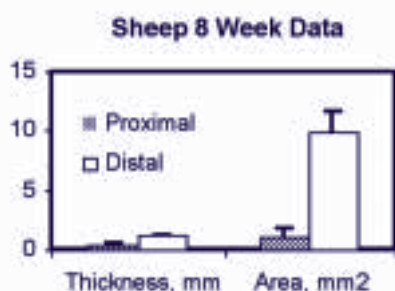




**FIGURE 1:** Fistula created between artery and vein to provide high-flow conduit

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



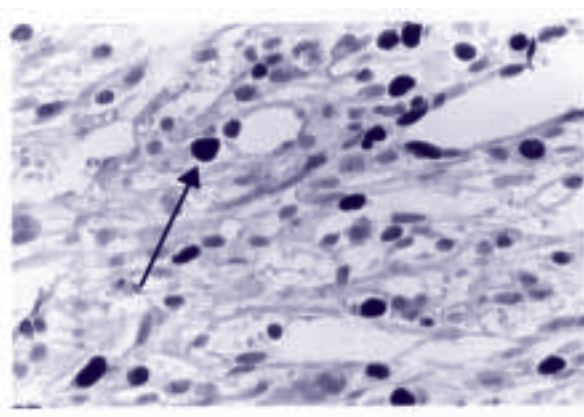
**GRAPH 1:** Sheep Eight Week Data

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**

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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  
National Cancer Institute  
Seattle Foundation



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

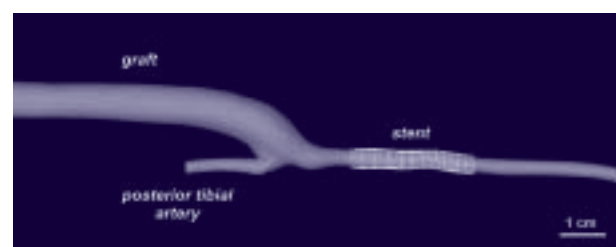
Vein grafts are placed to bypass diseased arteries in the lower limb when symptoms such as pain during walking, rest pain, and tissue necrosis occur. While vein grafts provide effective relief of lower extremity ischemia for the majority of patients, approximately 30-40% of these grafts fail due to focal 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, luminal 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



**FIGURE 1:** 3D surface reconstruction of the distal anastomosis of a bypass graft and a stent placed distal to the graft to treat a stenosis. The scan was performed one week after placement of the stent. The vessel lumen is displayed as a surface; the stent is displayed as a mesh surrounding the lumen.

difficult to perform and interpret. Quantitative monitoring of vein graft morphology may provide a means to distinguish normal remodeling from pathologic changes that threaten vein graft patency.

We are also able to track changes following endovascular interventions. Figure I 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

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 both for diagnosis and in the follow-up studies needed

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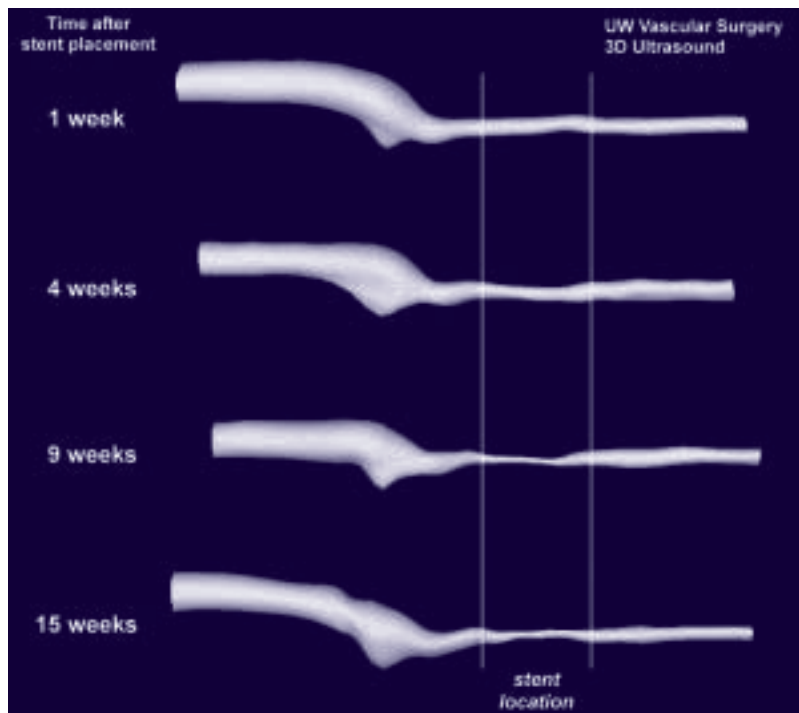
*Ultrasound is an attractive imaging modality for screening and monitoring abdominal aortic aneurysm patients since it does not involve radiation or contrast agents.*

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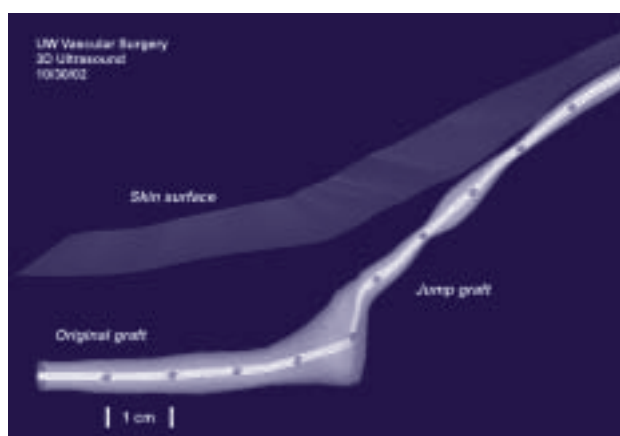
of the vessel within the stent (Figure 2). Enhanced displays of the 3D computer reconstructions are also being developed for more intuitive presentation of vessel anatomy. Figure 3 shows the proximal anastomosis of a jump graft with a calibrated '3D tape measure' placed along the center axis of the graft. 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

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.

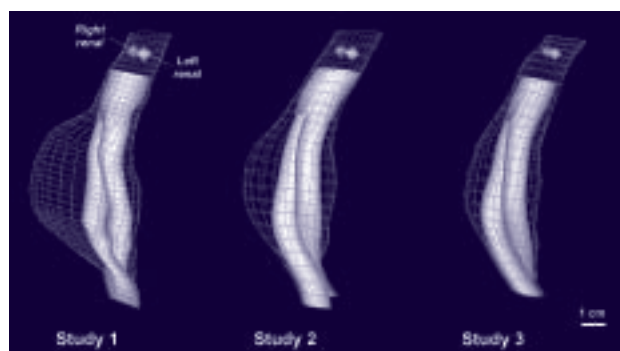
In contrast to the traditional open surgery, an endovascular graft is deployed using a catheter system



**FIGURE 2:** Serial 3D surface reconstructions of the site of the stent repair shown in Figure 1. Narrowing of the lumen within the stent is evident.



**FIGURE 3:** 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.



**FIGURE 4:** 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.

passed into the aorta through the femoral arteries. This procedure is associated with significantly reduced morbidity and recovery time. However, extended post-treatment monitoring is generally required to ensure that the endograft is stable and that there are no leaks. While decrease in aneurysm 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.

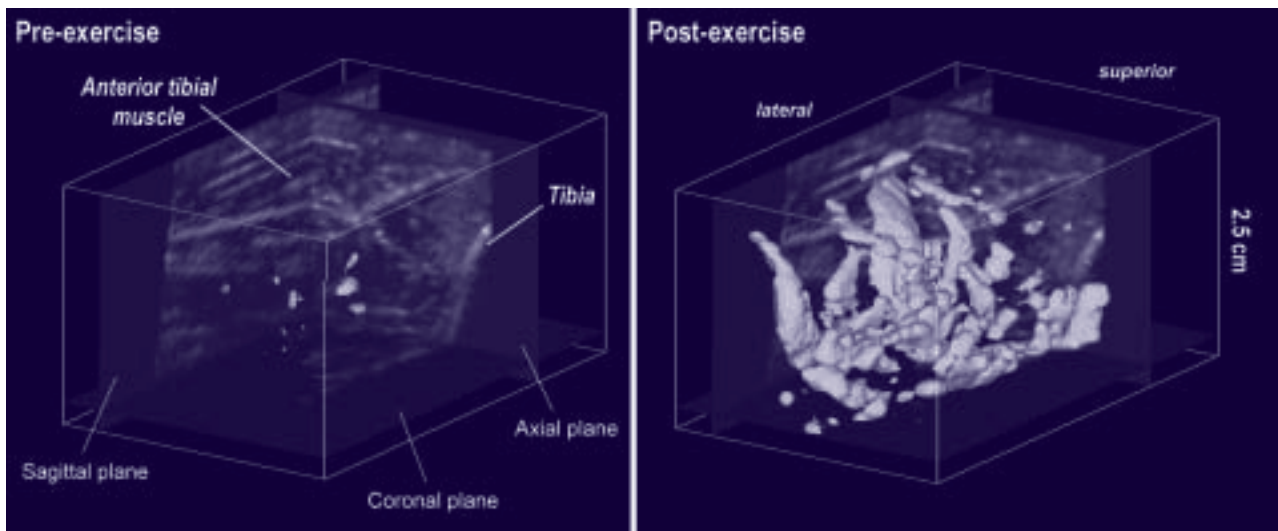
### Muscle Blood Flow Assessment with 3D Ultrasound Imaging

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 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  $2.3 \pm 1.1$  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.

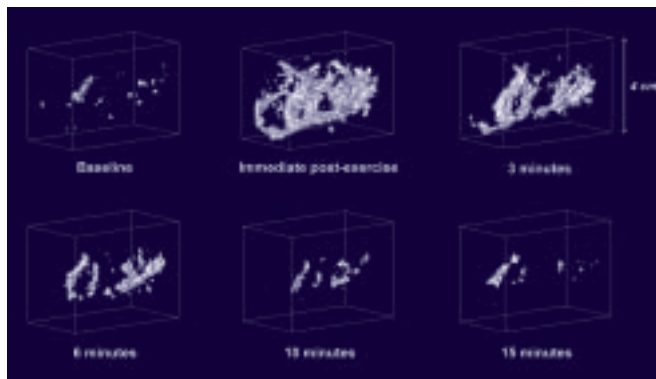
### Automated Measurement of Flow-Mediated Vessel Dilation

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

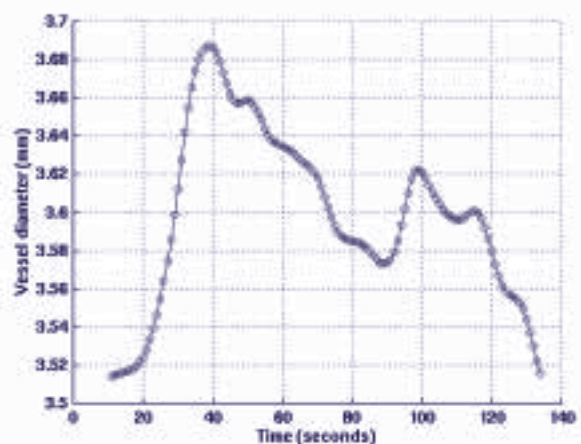
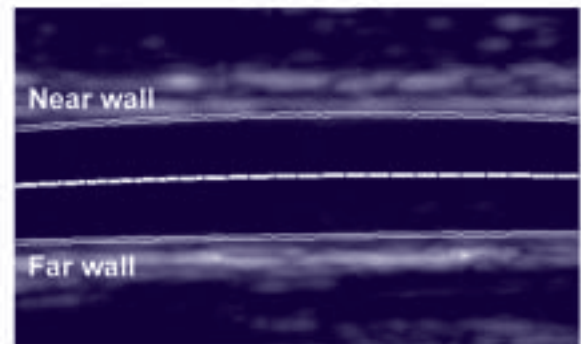


**FIGURE 5:** 3D volume reconstructions of the anterior tibial muscle before exercise (left) and immediately after exercise (right). Gray-scale and color data, representing tissue and blood flow respectively, were reconstructed separately and re-combined in a single display as orthogonal slices (anatomy) and surface rendering (blood flow). Voxel size = 0.5 mm.

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.



**FIGURE 6:** 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 7:** 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).

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  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.
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