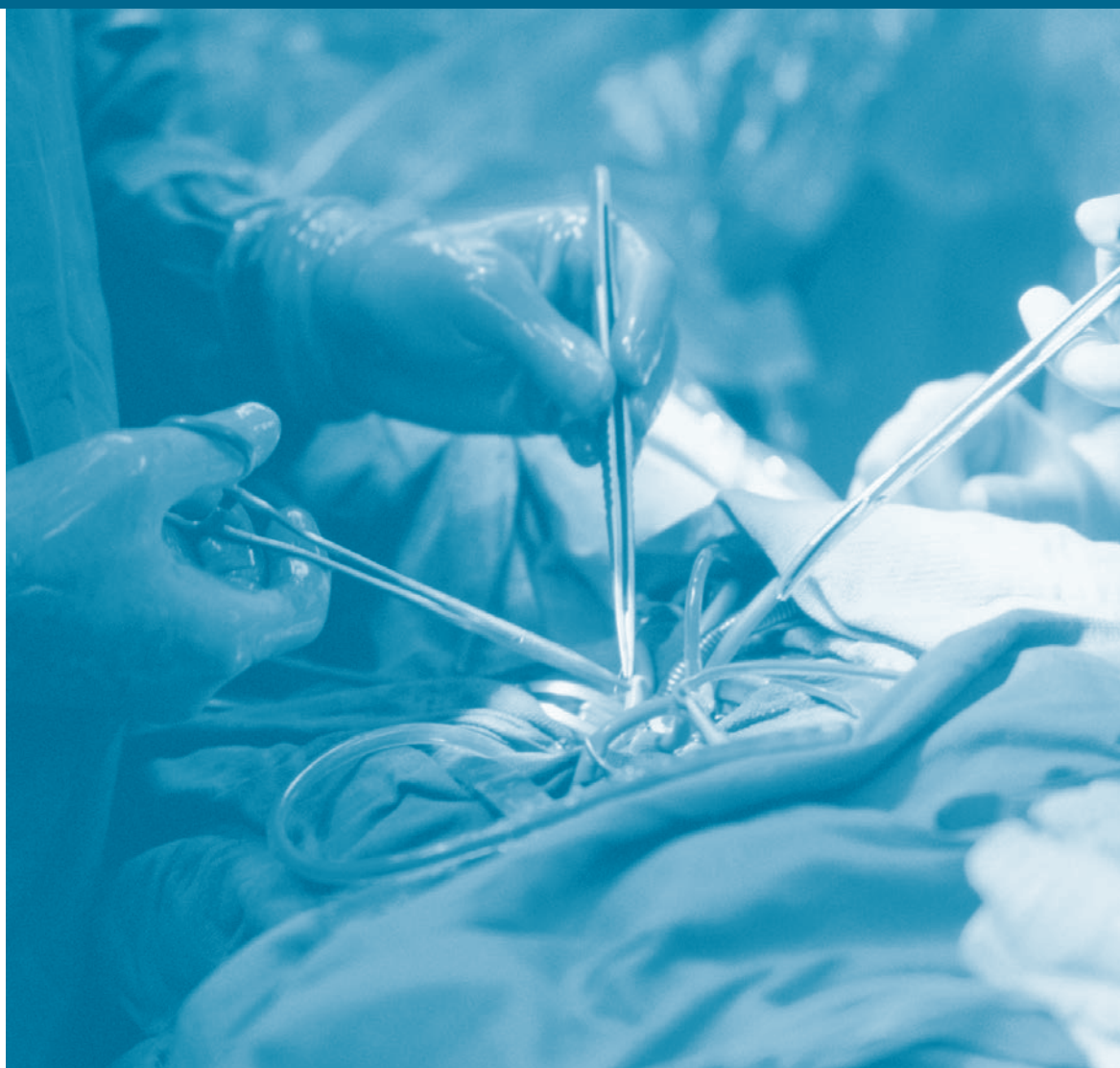
SURGERY RESEARCH

DEPARTMENT OF SURGERY 2003 REPORT



UNIVERSITY
OF WASHINGTON
SCHOOL OF
MEDICINE

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

2003 REPORT

UNIVERSITY OF WASHINGTON
DEPARTMENT OF SURGERY
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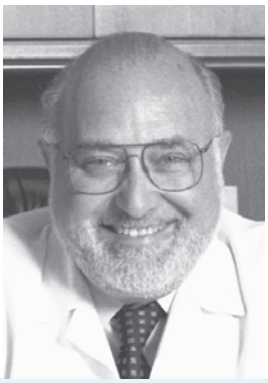
Research is Gateway to Healthy Nation



Clinical and scientific investigations are important because they help repair the ravages of illness and trauma. When our friends and loved ones become seriously sick or injured, we rely upon proven treatment protocols to offer them hope for renewed health. These protocols are created after years of dedicated and comprehensive medical research. The report you hold in your hands gives you more than an overview of the research currently being conducted in our department—it gives you a sneak-preview into tomorrow's miracle cures.

Despite today's economic climate where an increasing number of highly-qualified investigators are competing for fewer funding sources, our consistent track record of attracting research dollars continues. Almost all of our investigators receive funding from the National Institutes of Health (NIH), National Science Foundation, Centers for Disease Control, U.S. Army & Navy, and other federal & private peer-reviewed sources. Because of its uniformly-accepted reputation, many institutions refer to their level of NIH funding as a benchmark of academic vigor. In terms of dollars funded, the NIH ranked our department number 10 out of 90 (top 11%) for departments of surgery in fiscal year 2001. Although this is down from last year's ranking of 7 out of 86 (top 8%), the decrease is slight and was caused by faculty attrition and a number of grants completing multi-year awards.

Selected additional areas in which our researchers have been honored are in the sidebar accompanying this letter. I invite you to take a few moments to read about some of the research awards received by our faculty and staff during this past year.

One area where we achieved an aggressive goal was to formally expand our existing structured research divisions to include an emerging specialty field: surgical robotics. Technological advancements continue to offer us opportunities to further improve minimally invasive surgery (MIS) procedures. MIS is better for patients because it is less invasive than traditional surgery and it greatly reduces the patient's pain & recovery period. The surgeon, however, must compensate for a loss of dexterity and depth perception. Robotic systems use advanced computer innovations to enhance the operating fields of vision, accuracy and stability. In addition, Surgical Robots are information systems, and as such, can be made to interact with all other sources of information (i.e., databases from the radiology department, laboratory, and soon our electronic medical record) and to also integrate themselves with other components of the operating room (inventories, dispensers, etc). The clinical applications promise to be as significant to the practice of surgery as was the discovery of anesthesia. In the *Robotics* section of this report, three of our faculty investigators introduce you to their own particular area of expertise in this exciting new field.

Each of our other seven research divisions have submitted a summary of the high-caliber work being conducted in their laboratories. We are fortunate to have some of the brightest individuals in this country conducting research in the areas of Cardiothoracic, General, Pediatrics, Plastic & Reconstructive, Trauma, Transplant, and Vascular Surgery. While reading this report of collected ongoing departmental investigations, I could not help but ponder the incredible possibilities and improvements their work offer in the not too distant future. I hope you will share in my enthusiasm and pride as you read for yourself about some of their truly amazing studies.

We are dedicated to providing you the very best in surgical care. Your sustained support of all our academic activities provides crucial assistance for our research, clinical, and training programs. If you would like more information about how you can help further any of the research projects in this report, please contact Megin Flaherty Edwards, Associate Director of Development, at (206) 221-2847, email: megin@u.washington.edu.

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

Research Honors & Awards

Eileen Bulger, M.D., assistant professor: received the John Davis Award and $35K funding from the American Association for the Surgery of Trauma for her project entitled, "Modulation of alveolar macrophage activation in ARDS".

David Gourlay, M.D., research fellow: won the Young Investigator of the Year award from the Shock Society for his work investigating the effect of male hormones on the innate immune response and intracellular signaling of the immunoinflammatory response of monocytes and macrophages. **(Dr. Ronald V. Maier, mentor)**

Thomas Hatsukami, M.D.; associate professor:

- awarded $6 million over 5 years to support Project 2 of an NIH Program Project Grant "Genomic and Genetic Approaches to Plaque Rupture", (Hatsukami, PI for Project 2, **Steve Schwartz, M.D., Ph.D.,** overall program PI). Project 2 will identify new risk factors for atherosclerosis disease progression to better understand the mechanisms leading to the development of high-risk plaques;
- received a $2 million, two-year extension from Astra-Zeneca Pharmaceutical to continue work on project entitled, "A randomized double-blind single-center trial to assess the effect of high and low doses of ZD4522 on progression of carotid artery atheroma in moderately hypercholesterolemic subjects with asymptomatic carotid stenosis after 24 months of dosing";
- secured a $55,000 one-year pilot study funding from Pharmacia Pharmaceutical to determine the reproducibility of MRI for assessing carotid atherosclerosis between two centers, UW and the Massachusetts General Hospital, in preparation for a subsequent multicenter clinical trial.

Karen Horvath, M.D., assistant professor: awarded a two-year, $200K NIH grant for a multicenter study investigating, "Videoscopic-assisted retroperitoneal debridement of infected pancreatic fluid collections". **Co-PI's —Drs. Eileen Bulger, Mika Sinanan, & Matthias Stelzner.**

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".

Christian Kuhr, M.D., assistant professor: awarded a one-year, $40K grant from the Northwest Kidney Foundation for Centers for his research project entitled, "Immunological tolerance to kidney transplantation induced through mixed hematopoietic chimerism."

Michael Mulligan, M.D.; assistant professor: received a jointly-funded five-year, $875K NIH & Thoracic Surgery Foundation for Research & Education grant for project entitled, "The role of calcineurin in lung reperfusion injury".

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." The Institute is a division of the NIH.

Raminder Nirula, M.D., research fellow: won the best resident paper in the clinical category at the national American College of Surgeons Resident Paper Competition for his work entitled, "Crash test dummy analysis does not reliably predict the likelihood of traumatic brain injury in frontal motor vehicle crashes". **(Drs. Avery Nathens & Charles Mock, mentors)**

CARDIOTHORACIC SURGERY

GABRIEL S. ALDEA, M.D.

RIYAD KARMY-JONES, M.D.

MICHAEL S. MULLIGAN, M.D.

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Gabriel S. Aldea, M.D.

• MINIMIZING MORBIDITY OF CARDIOPULMONARY BYPASS

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

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 address the concerns and problems associated with conventional CABG.

Many of the complications of CABG are related to the biologic response of the body to artificial perfusion and gas exchange through the non-endothelialized cardiopulmonary bypass (CPB) circuit. Within seconds of CPB, formed and unformed blood elements come into contact with the large surface area of the CPB circuit. Despite anticoagulation with heparin, this interaction results in extensive activation of platelets, neutrophils, complement, cytokines and the fibrinolytic system, producing a complex and intense "inflammatory" response. Although these responses are usually short lived and leave no residual deficits, they can lead to long-lasting cardiac, pulmonary, renal and neurologic dysfunction in a subset of patients.

Using recent advances in perfusion technology and research in biomaterial sciences we have developed specific surgical techniques which 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.

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

Using recent advances in perfusion technology, we have developed specific surgical techniques which have resulted in the routine application of more biocompatible circuits.

Figure 1 shows a representative scanning EM at 200-fold magnification of the arterial filter (the last barrier to debris before the blood from the CPB circuit reaches the systemic circulation). This comparison demonstrates dramatic reduction (quantified in 60 patients to be >80% reduction) in debris and inflammation resulting from the use of biocompatible hep-

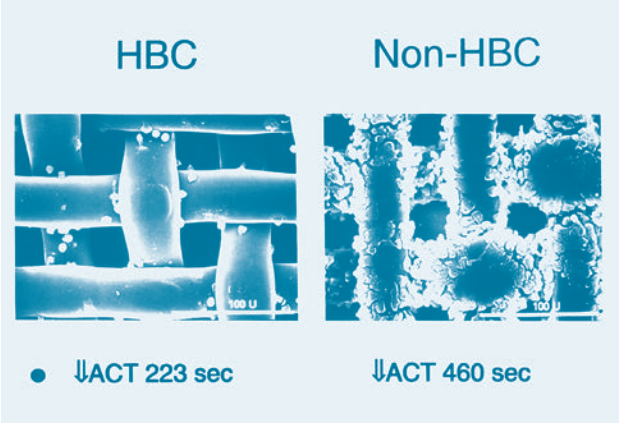

FIGURE 1: Scanning electron micrographs at 200 fold magnification of arterial filter. Lowest activating times (ACT) in seconds are noted. HBC= heparin-bonded circuits. Non-HBC- control non-heparin-bonded circuits.

arin-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. We continue to study ways to disassociate the contribution of biocompatible circuits from the specific surgical techniques (the effects of cardiotomy suction vs. use of cell saver technology) on markers of hemostasis, inflammation, neurological and 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.

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 (b-thromboglobulin) and neurological injury (neuron specific enolase, S-100b) are all nearly completely blunted when HBC are used and cardiotomy suction is eliminated during CPB. Our results suggest that cardiotomy suction should be eliminated whenever possible. Our results challenge long held precepts that adverse outcomes possibly associated with thrombin generation, inflammation and platelet activation are inevitable whenever CPB is used (Figures 2-4).

We continue to investigate novel targeted pharmacological interventions as well as further biomaterial

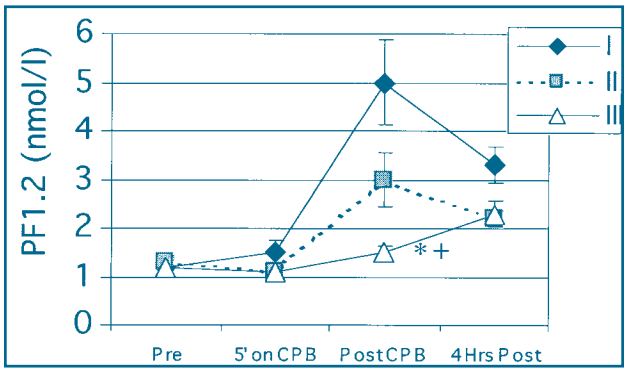

FIGURE 2: PF1.2 for thrombin generation

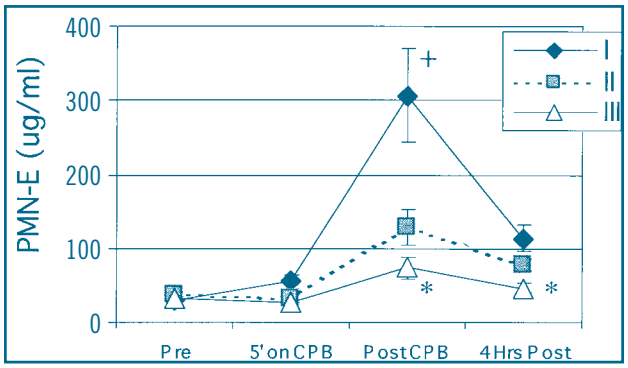

FIGURE 3: PMN-E for elastase

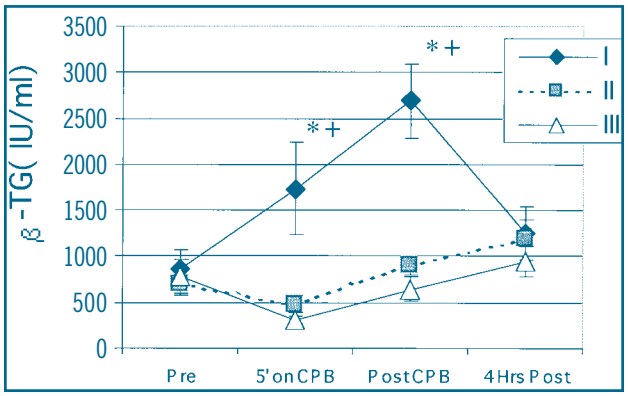

FIGURE 4: ß Thromboglobulin for platelet activation

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 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). By sampling and exposing serum and pulmonary lavage specimens to specific, graded stimuli prior to surgery, we hope to develop reliable biological essay to predict an individual patient's response to artificial perfusion.

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OTHER CO-INVESTIGATORS:

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

Riyad Karmy-Jones, M.D.

- INDICATIONS AND OUTCOME FOLLOWING 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 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).

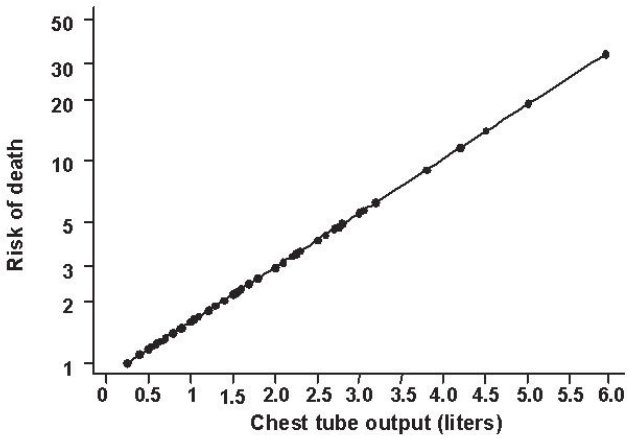

FIGURE 1: Risk of death and volume chest tube output

Harborview is unique in that it has one of the largest volumes of aortic trauma in the nation. The average trauma center treats on average less than two patients/year with aortic trauma while HMC, on average, treats 15.

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

We have also reviewed specific techniques of lung resection following traumatic injury. Considering all mechanisms of injury, there is an incremental risk of death with progressively complex resections (Figure 2). A related review focusing on patients with penetrating injury alone found that anatomic resections were associated with a lower incidence of septic complications compared to "stapled" approaches. The implications of this work are that a) lesser resections are favored, including damage control techniques but b) surgeons

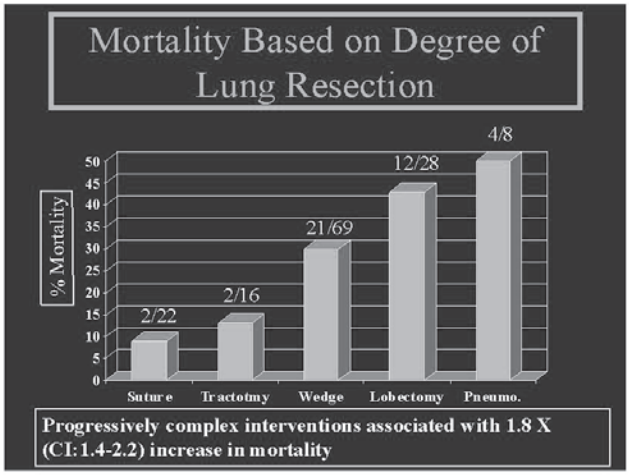

FIGURE 2: Mortality based on degree of lung resection

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 psueodoaneurysms that persist greater than two weeks will not resolve and will ultimately follow the pattern of chronic thoracic aneurysms. We are currently leading a multi-center prospective study of non-operative management of aortic trauma.

An exciting option in the management of thoracic vascular diseases is the role of endovascular stent grafts (EVSG). A review of 50 angiograms of patients with aortic rupture identified some key anatomic details with regards to modeling grafts. The mean distance along the lesser curve to the superior aspect of the tear was 5.8

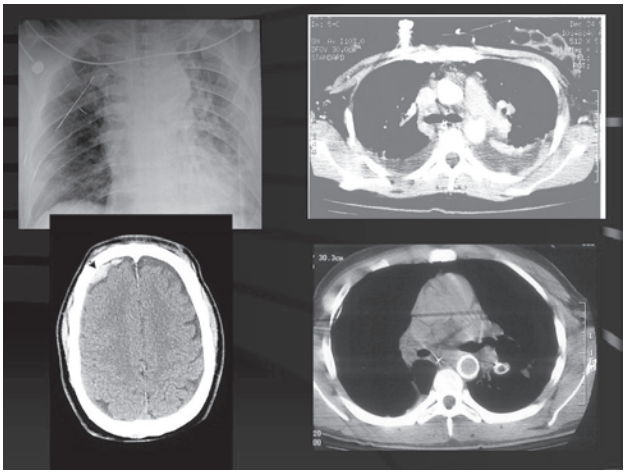

FIGURE 3: 18 year old female involved with MVC. Patient sustained closed head injury with elevated ICP, severe pulmonary contusion and required splenectomy. Her pelvis was unstable and angiographic embolisation needed for arterial bleeding. EVSG was utilized to control her TRA.

mm, aortic diameter 19 mm and mean degree of curvature 27°. The majority of aortic tears will require the origin of the subclavian to be covered. We have treated 10 patients with this approach (Figure 3) and have made the following observations: Commercially available grafts are superior to "home made ones"; most aortic injuries can be treated by commercially available "cuff-extenders"; tears near the aortic curvature of > 1.5 cm are associated with "telescoping" resulting in increased risk of endoleak. The ideal thoracic grafts are not yet available but our group, 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 occlusion to demonstrate that ischemia-reperfusion to one lung results in an indirect injury to the contralateral lung with release of NF*k*B (Figure 4).

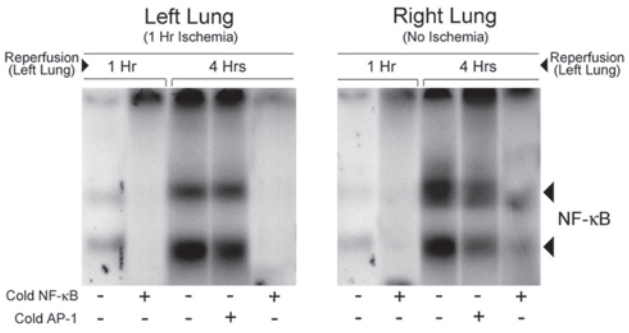

Figure 4: Left lung with ischemia compared to right lung with no ischemia

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

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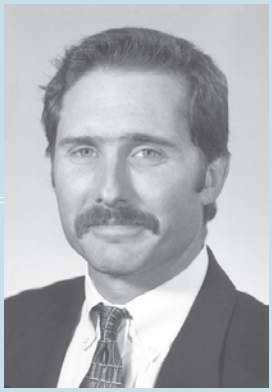

• CYTOKINES AND CHEMOKINES IN DIRECT ISCHEMIA REPERFUSION INJURY OF LUNG AND CARDIOTHORACIC TRANSPLANT REJECTION

AWARDS:

Schilling Lecture, University of Washington, Prize, 2000
Seattle Surgical Society, Best Presentation, 1999
Resident Teaching Award 2000

FUNDING:

Bayer Corporation
Novartis
PrimeSource Surgical
Thoracic Society Directors Association

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

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

The chemokines are a family of chemotactic cytokines with a high degree of specificity for subpopulations of leukocytes. Four groups of chemokines have been characterized based on the structure of the peptides, CC, CXC, CX_3C, and C. The CC chemokines or the β chemokines have two adjacent cysteine residues, and function primarily as monocyte and lymphocyte chemotactic agents. Members of this family include MCP-1, RANTES and MIP-1α, MIP-1β, to name just a few. The second group, the CXC chemokines, are also referred to as the α chemokines. This group is characterized by the presence of an amino acid between the two cysteine residues, and includes powerful neutrophil chemoattractants, such as IL-8, MIP-2, and CINC. Two recently discovered groups of chemokines include the C and CX_3C families. These chemokines are chemotactic for T lymphocytes and monocytes and include lymphotactin (C) and fractalkine, also known as neurotactin (CX_3C).

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

Likewise three C-C chemokines, MCP-1, MIP-1α, and RANTES, have been shown to play roles in the development of several of these models, but only IL-8 has been investigated for any potential role in lung ischemia reperfusion injury. MIP-1α is upregulated *in vitro* following hypoxic stress and increased MIP1α messenger RNA is found in liver allografts shortly after reperfusion. Secondary lung injury develops following reperfusion of ischemic limbs, and liver that is at least partially regulated by C-C and potentially C-X-C chemokines. These findings would suggest that chemokines are likely to play some role in regulating direct ischemia reperfusion injury of the lung.

A model of hilar isolation for the study of ischemia reperfusion injury of rat lung has been reproducibly established and standardized in our laboratory. A pattern of mRNA expression for MIP-1α in reperfusion

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

Lung injury as assessed by vascular leakage of ^{125}I labeled BSA has been determined as a measure of injury severity. The permeability index among negative (unmanipulated) controls is consistently 0.09±0.05. Permeability doubled in animals undergoing only thoracotomy and mechanical ventilation. Ninety minutes of ischemia did not significantly increase mean permeability values; however, four hours of reperfusion resulted in an eight-fold rise in lung permeability to a mean index of 0.75±0.01 ($p<.001$ compared to controls). In contrast, animals treated with blocking antibody to MIP 1α, experienced a mean 35% reduction in permeability compared to injured controls ($p<.001$).The lungs were also analyzed for myeloperoxidase (MPO) content as a measure of tissue neutrophil accumulation.

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

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

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

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

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

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

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

A heterotopic rat heart transplant model is also being used to determine the role of CC chemokines in heart allograft function and rejection. This model, which is technically challenging, involves a precise dissection of the donor heart using a 10x operating microscope followed by a hand sewn anastamosis using 8-0 suture. The hearts are explanted at various time points and the laboratory is currently gathering data on the role of chemokine blockade on cytokine expression and abrogation of rejection.

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

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• 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:

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Bayer Corporation
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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.

Ischemia-reperfusion injury: Ischemia/reperfusion (I/R) injury contributes significantly to morbidity and mortality in surgical patients. I/R injury is the principal pathogenetic event in stroke, complications of peripheral vascular disease, hemorrhagic shock, and early transplant graft dysfunction. Paradoxically, reperfusion of oxygen-deprived tissue, the mainstay of therapy for ischemia, causes further tissue injury by inciting a deleterious inflammatory reaction in and around reperfused tissue. Because restoration of oxygen delivery to ischemic tissue is critical to survival, a substantial amount of research in the last decade has focused on treating or preventing this important consequence of reperfusion.

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

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-

During severe systemic illness, inflammatory reactions may become generalized, initiating a distinct pathologic state called the "Systemic Inflammatory Response Syndrome" (SIRS).

selectin); and leukocyte activation and chemotaxis caused by the release from I/R injured endothelium of chemokines (for example, Interleukin-8) and growth factors. One component of our research is based on the transcription factor, NF-κB, that regulates transcription and expression of the genes that encode these proteins.

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

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

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

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

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

In addition, cell culture gives us the capability to move DNA sequences into human cells in a controlled fashion to deduce cellular mechanisms of activation based on the effect of the protein encoded by the transfected DNA on cellular function. Finally, by employing differential array and DNA microchip technology, we can identify and characterize novel protein kinases or transcription factors that, in concert with NF-kB, regulate the cellular response to hypoxia and reoxygenation. Interpretations of findings *in vitro* are provisional, however, until they can be confirmed *in vivo*.

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

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

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

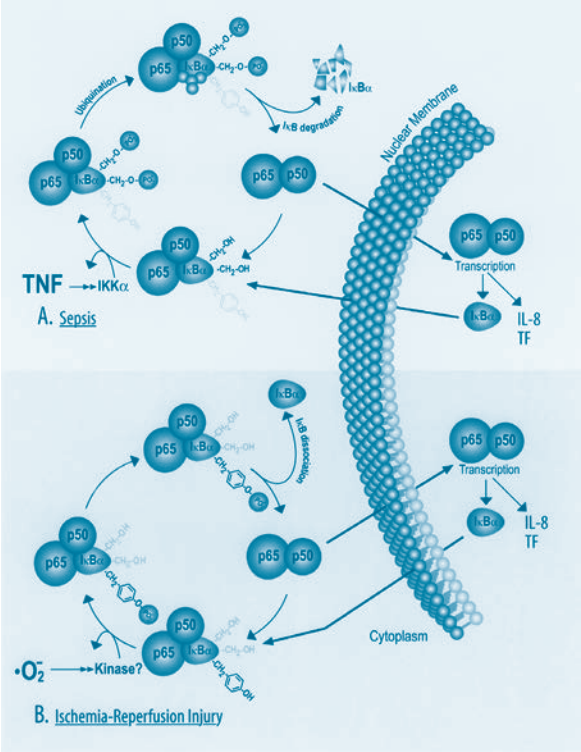

FIGURE 1: Parallel pathways of NF-κB activation. In (A), septic stimuli, such as TNF-α (or, IL-1 or LPS), activate transmembrane signaling pathways in responsive cells leading to the phosphorylation of a serine/threonine kinase, IKKα. This kinase in turn phosphorylates IκBα on serine residues 32 and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

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

In (B), reactive oxygen intermediates activate signaling pathways yet to be determined that lead to tyrosine phosphorylation of IκBα. The tyrosine kinase (or kinases) responsible for this reaction has not yet been identified. Tyr-phosphorylated IκBα, in contrast to Ser-phosphorylated I IκBα, dissociates from NF-κB without degradation. NF-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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VASCULAR SURGERY

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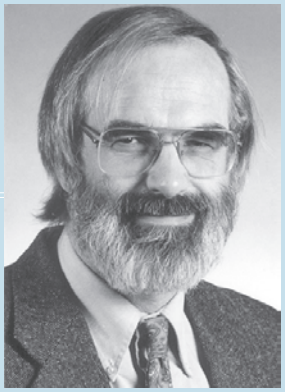

- TISSUE PULSATILITY IMAGING FOR THE DETECTION OF SOLID TUMORS
- ULTRASOUND LATERAL COHERENCE IMAGING
- THE EFFECT OF DOPPLER ANGLE ON DOPPLER VELOCITY WAVEFORMS AT THE FEMORAL BIFURCATION

FUNDING:
DARPA/Office of Naval Research
National Cancer Institute

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

Tissue Pulsatility Imaging for the Detection of Solid Tumors in Breast

Forty years ago, Judah Folkman postulated that solid tumors could not grow larger than 1 mm in diameter without generating a new blood supply. That process is 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 believe that their pulsations with the cardiac cycle 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 having other breast imaging or biopsy are asked to volunteer to have 3-dimensional ultrasound to make "Tissue Pulse Images" prior to the biopsy under a Human Subjects approved protocol.

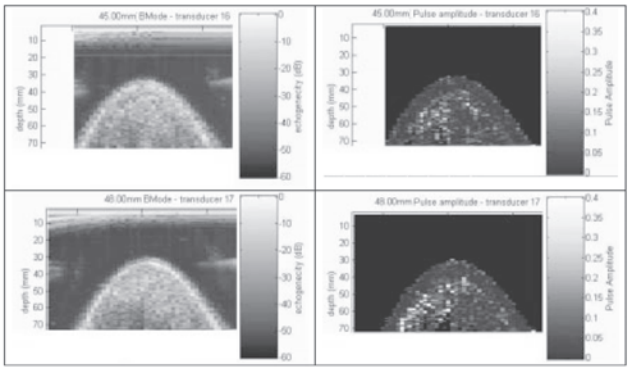

FIGURE 1: Ultrasound B-mode and Pulsatility Images of Breast Cancer

Conventional ultrasound "brightness mode" images are shown on the left, pulsatility images are shown on the right from the same section of the breast.

Three-dimensional ultrasound pulsatility images of breast tissue are made with a special 3-dimensional imaging system. 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.

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

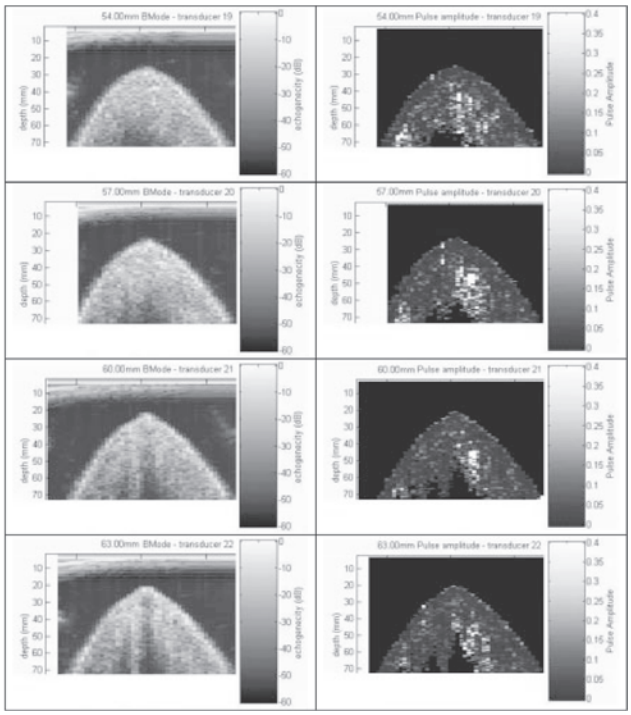

FIGURE 2: Ultrasound B-mode and Pulsatility Images of Breast Cancer

Conventional ultrasound "brightness mode" images are shown on the left, pulsatility images are shown on the right from the same section.

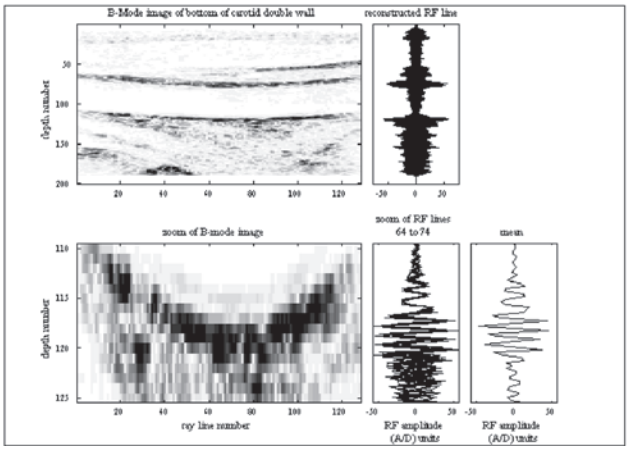

FIGURE 3: Coherence of Ultrasound Echoes from the Deep Arterial Wall

Upper left: 2 dimensional B-mode ultrasound image of an artery. The artery is the light colored region running horizontally across the center of the image. The dark streaks at the upper and lower boundaries of the artery are the artery walls. Muscle is superficial and deep to the artery.

Upper right: "Radio Frequency (RF) echo from one line in the center of the 2 dimensional (2D) B-mode image. Strong signals (wide parts of the pattern) are shown as dark on the 2D ultrasound image, weak signals (narrow parts) are shown as light on the 2D image.

Lower left: Magnification in the vertical direction of the deep wall of the artery from the 2D image.

Lower center: Details of the 11 RF lines (lines 64 to 74) around the deep wall of the artery. Notice that echoes from the wall are all aligned, echoes from the blood (portion above) and muscle (portion below) are not aligned.

Lower right: Horizontal average of the 11 RF lines.

Ultrasound Lateral Coherence Imaging

While exploring the Radio Frequency (RF) echoes from arterial walls, we found that the ultrasound echoes that return from arterial wall have aligned phase at adjacent locations in the image (Figure 3). This phase alignment is likely due to the laminar structure of the arterial wall. The random phase of echoes from deeper muscle and superficial blood suggests that these other structures do not have a laminar structure. The phase alignment of adjacent ultrasound echo lines is called lateral coherence.

By measuring the lateral coherence of tissues in ultrasound images, we expect to be able to detect and measure total arterial wall thickness and to identify "normal" (laminar structure) arterial wall (Figure 4).

The lateral coherence image (Figure 4 right) shows features of the tissue around the arterial wall which are not obvious in the B-mode image. The image on the left of Figure 4 is a conventional ultrasound B-mode (brightness-mode) image. The brightness of the image shows the strength of the ultrasound echo. Echoes from inside the artery across the middle of the image are weak showing the blood as "black". Solid tissues in other parts of the image reflect more ultrasound so they are shown in gray. Some of the brightest echoes come from portions of the artery wall, shown as white. Both images are made of 128 lines of data running from the transducer on the top of the image to the bottom. In the image on the right, each "RF" line in the image is compared with their neighbors to the right on to see if they are coherent. If a section of coherence is detected, then a horizontal streak is placed on the image to indicate that coherence. If the coherence persists over more lines, then the streak is made longer and darker to indicate that greater coherence. Notice the streak patterns in the right image correspond to the arterial walls in the left image. This technique may be useful in the early detection of atherosclerotic plaques and other wall abnormalities.

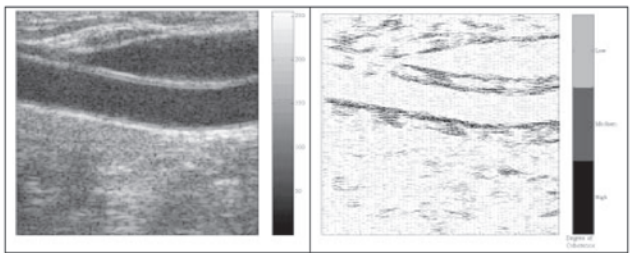

FIGURE 4: Ultrasound B-mode (left) and Lateral Coherence (right) Images

Blood Velocity Measurements of the Femoral Bifurcation with Vector Doppler

Blood velocities at arterial bifurcations are complex as the blood in part of the artery heads in one direction and blood in the other part heads in another direction. The pattern is much more complex because of the pulsatility of arterial blood flow. The flow reversals in peripheral arteries make bifurcations like the common femoral artery into the superficial femoral artery, which supplies the calf and the foot below the knee and the deep femoral artery, which supplies the thigh muscles, even more complex. The images here were taken in a resting leg. During systole, blood flows down the arteries from the heart. During diastole, there is no net arterial blood flow in the legs. As systole ends, before the flow stops, there is a period of flow reversal, as the pulse wave reflects from the distal ends of the arterial system. The reversal occurs first in the deep femoral artery.

The femoral bifurcation was studied with a vector Doppler developed in the Department of Surgery (Figure 5). It consists of a central ultrasound transmitting transducer flanked by a pair of ultrasound receiving transducers. The two receiving transducers view the blood velocity from different perspectives. The ultrasound frequency returning to the receiving transducers is different from the frequency transmitted; the difference is due to the Doppler shift. By subtracting the frequencies, the velocity perpendicular to the transmitting beam is measured, but adding the frequencies, the velocity parallel to the transmitting beam is measured.

In Figures 6, the headings of the blood velocity vectors can be seen just proximal to the femoral bifurca-

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.

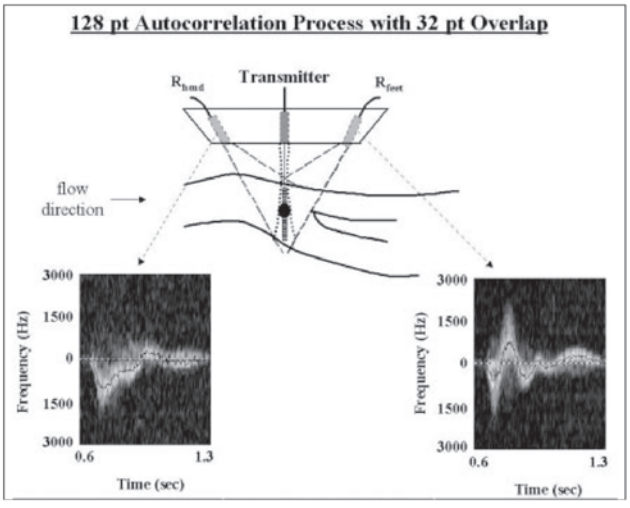

FIGURE 5: Vector Doppler Geometry

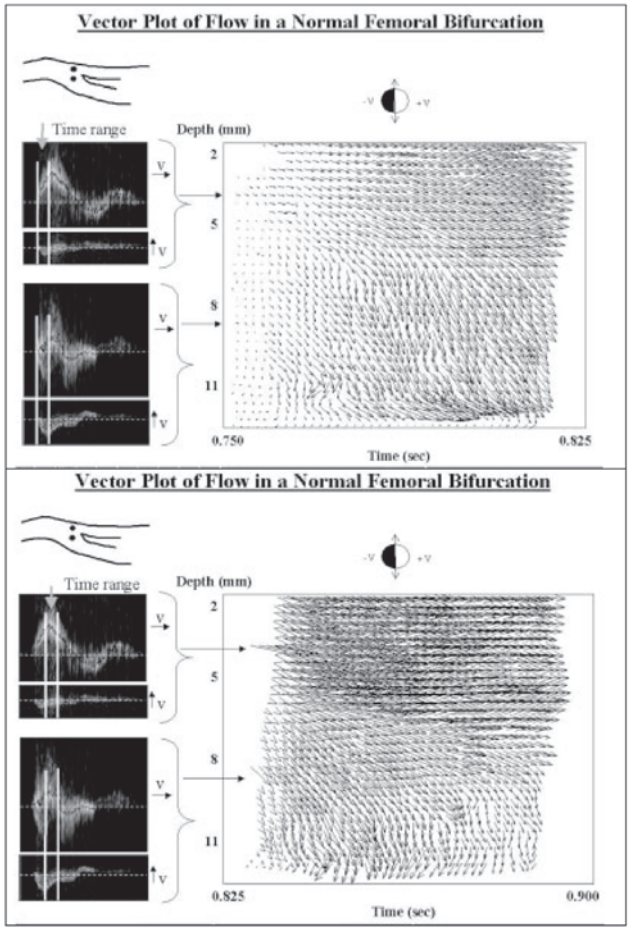

FIGURE 6 A, B: Velocity Vectors in the Femoral Bifurcation, Early Systole

LEFT: spectral waveforms, RIGHT: depth (vertical) vs. (time) horizontal

tion. As the blood is accelerating during early systole (Figures 6 A, B) the blood velocity vectors are heading into both the superficial femoral and deep femoral arteries. During late systole as the blood is decelerating (Figures 6 C, D), flow reversal can be seen from the shorter deep femoral artery while forward flow continues in the longer superficial femoral artery. When flow reversal is a maximum in the superficial femoral artery, there is little flow reversal in the deep femoral artery (Figures 6 E, F). This is one of the many rotational flows in the normal arterial circulation.

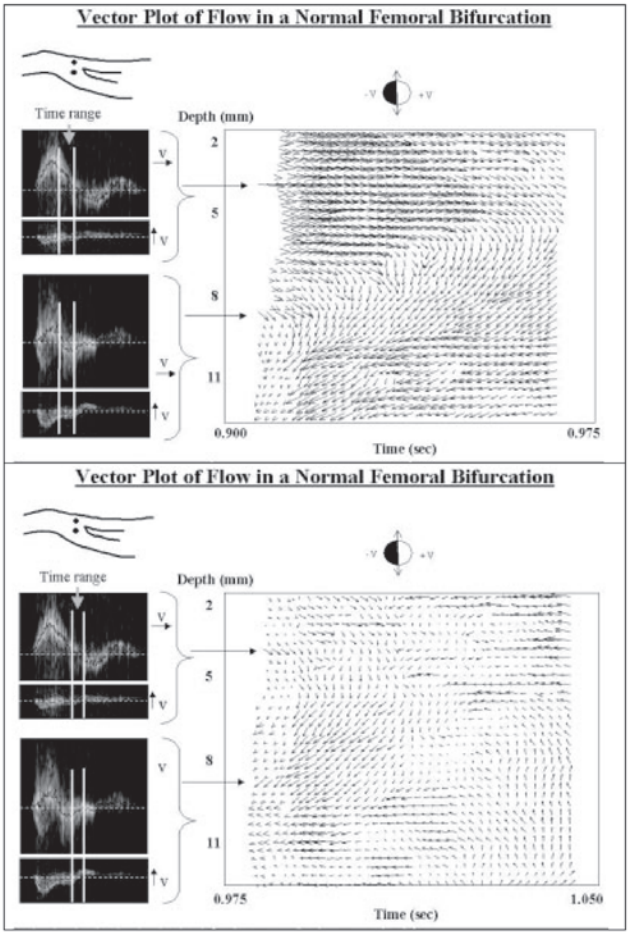

FIGURE 6 C, D: Velocity Vectors in the Femoral Bifurcation, Late Systole

LEFT: spectral waveforms, RIGHT: depth (vertical) vs. (time) horizontal

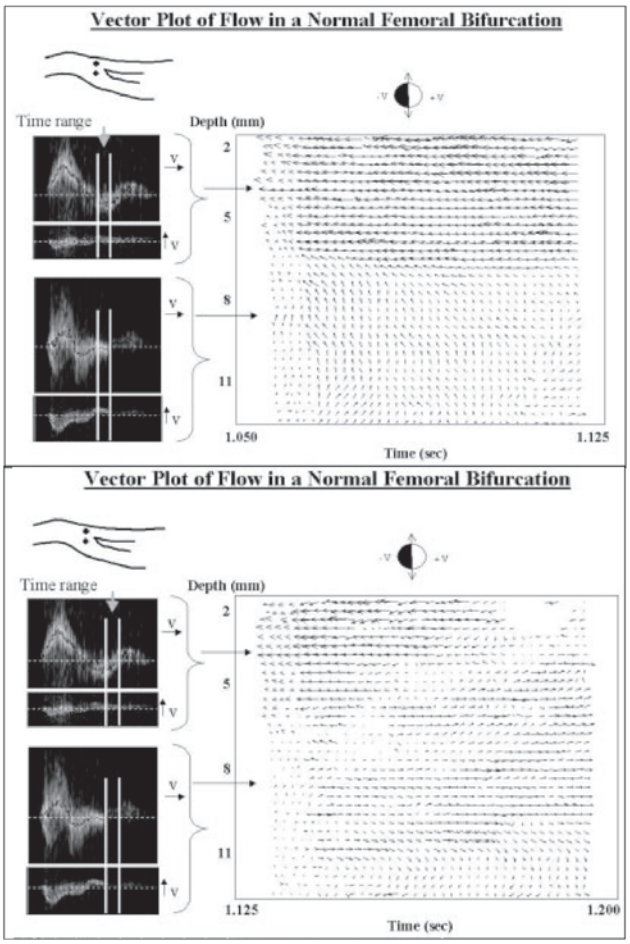

FIGURE 6 E, F: Velocity Vectors in the Femoral Bifurcation, Early Diastole

LEFT: spectral waveforms, RIGHT: depth (vertical) vs. (time) horizontal

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Alexander W. Clowes, M.D.

• REGULATION OF VASCULAR SMOOTH MUSCLE CELL GROWTH

AWARDS:
National Heart, Blood and Lung Institute MERIT Award
National Institutes of Health
• Vascular Surgery/Cardiology Training Grant

FUNDING:
National Institutes of Health

Vascular surgical procedures are designed to rebuild diseased blood vessels and improve blood flow. While these procedures restore the circulation, they also cause injury. This injury induces a wound healing response that in some instances is associated with accumulation of scar tissue (intimal hyperplasia) and significant luminal narrowing (e.g. 20-40% of coronary arteries treated by angioplasty). Smooth muscle cells living in the arterial wall proliferate in response to injury and are largely responsible for the intimal hyperplasia (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.

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

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

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

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.

(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, under some circumstances, the FGF receptor also mediates the cellular stimulus induced by PDGF. 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 arteril vasorelaxant. It is

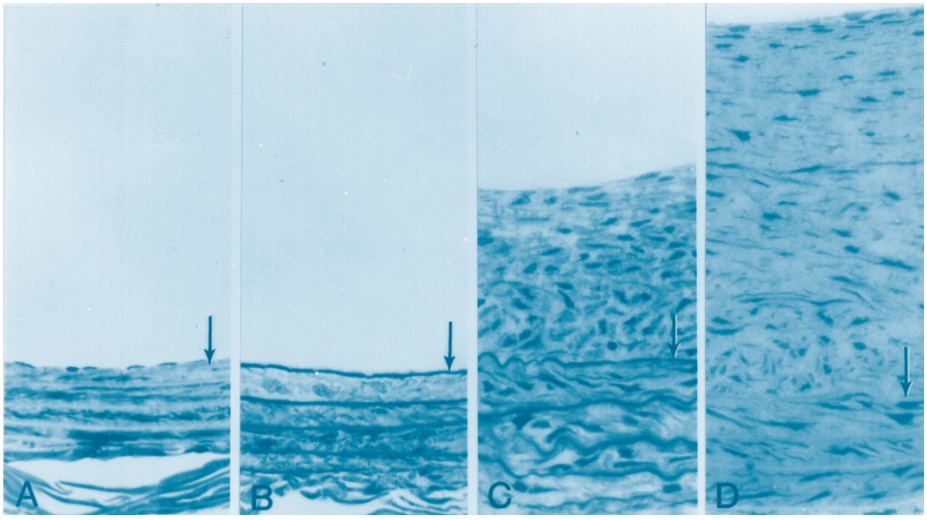

FIGURE 1: This series of photographs shows how a normal rat carotid artery (panel A-histologic cross-section) responds to injury. Angioplasty of the artery removes the surface endothelium (panel B). By two weeks (panel C), smooth muscle cells have migrated from the media into the intima (region above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

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 an intracellular use in cyclic GMP and the activation of a cGMP-dependent protein kinase (PKG). We are currently studying a downstream target of NO and PKG, vasodilator stimulated phosphrotein (VASP). Overexpression of VASP mutated to prevent phosphorylation by PKG makes cells unresponsive to NO, while overexpression of VASP mutated to prevent phosphorylation by PKC makes the cells sensitive to NO but unresponsive to serum. Thus, VASP may prove to be pivotal in the response of smooth muscle cells to growth stimulants and inhibitors, and pharmacological manipulation of this pathway might be a fruitful approach to controlling the arterial response to injury.

Regulation of smooth muscle growth in grafts by blood flow: We have found that smooth muscle cell proliferation and neointimal hyperplasia in primate PTFE grafts are exquisitely regulated by changes in blood flow. In the grafts, smooth muscle cells proliferate when endothelial cells are present, whereas in injured arteries they proliferate only when the endothelium is missing. Thus, depending on the physiological state, endothelial cells can have a positive or a negative effect on smooth muscle cell growth.

Using molecular arrays, we are attempting to define the molecules altered by changes in blood flow that might regulate smooth muscle cell proliferation (e.g., PDGF, NO). Recent experiments using a mouse monoclonal antibody that recognizes and blocks the beta form of the PDGF receptor (PDGFR-b) have demonstrated conclusively that intimal hyperplasia in grafts as well as in injured arteries depends on PDGF. In collaboration with Celltech, Ltd., and ZymoGenetics, Inc., this antibody has been genetically engineered to resemble a human immunoglobin; this "humanized" antibody has been tested in a human trial for the prevention of restenosis after coronary stent angioplasty and *failed*. We are astonished by this result and, in consequence, have gone back to the laboratory to investigate it further. Blockade of both PDGF receptors may be necessary. When we block both PDGFR-β and PDGFR-α, 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.

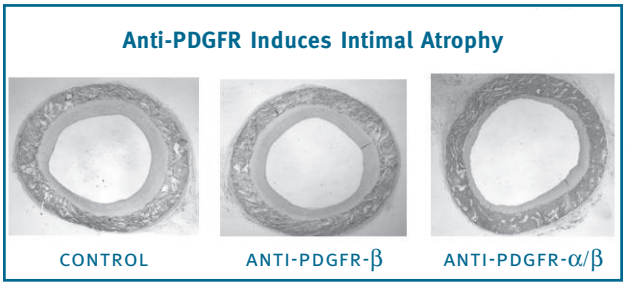

Figure 2: Histological cross-sections of normal flow PTFE grafts at 2 weeks following initiation of treatment with vehicle control, blocking antibodies to PDGFR-β, or blocking antibodies to both PDGFR-α and PDGFR-β. (H&E staining, 16X).

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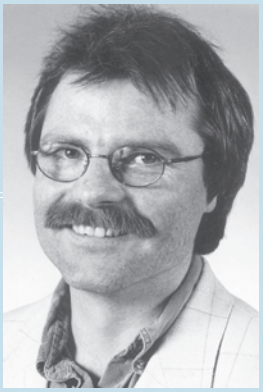

• MODULATION OF GROWTH FACTOR SIGNALING IN VASCULAR SMCs BY OXIDATIVE STRESS

FUNDING:
National Institutes of Health

In the normal vessel wall, arterial smooth muscle cells (SMCs) are quiescent because of the lack of activating factors and the presence of growth inhibitory stimuli produced by the endothelium (e.g. nitric oxide). In atherosclerosis, SMCs are exposed to a different environment that activates cells and induces migration, proliferation, or programmed cell death (apoptosis). All of those contribute to pathological aspects of the disease.

In the early stages of atherogenesis, for example, SMC proliferation contributes to plaque formation, whereas at later stages, SMC death may increase the risk of plaque rupture and the formation of occlusive thrombi. A major determinant of SMC function in atherogenesis appears to be oxidative stress. Low levels of oxidative stress are pro-proliferative and may also be part of growth factor signaling. In contrast, oxidative stress over time results in lipid peroxidation products which kill SMCs. Our laboratory is interested in identifying molecular mechanisms induced by oxidative stress that regulate SMC function.

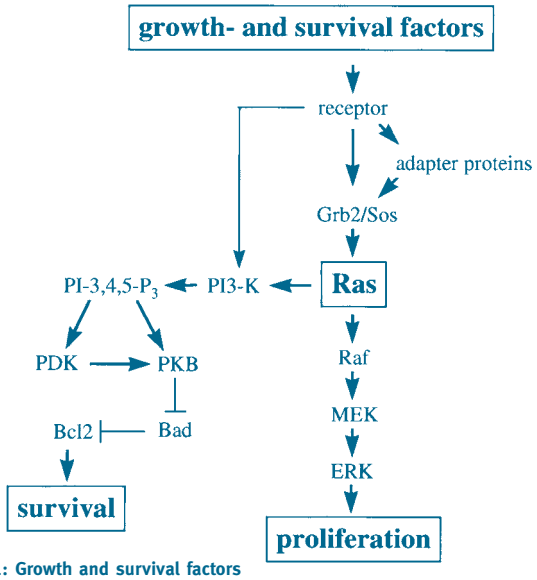

FIGURE 1: Growth and survival factors

The Small G-protein Ras is a Key Element in Regulating SMC Proliferation and Survival

The membrane associated small G-protein Ras is an essential component of all growth factor signaling pathways. Ras exists in an inactive, GDP-bound, and an active, GTP-bound state. Most growth factors that signal through receptor tyrosine kinases (RTKs) or heterotrimeric G-protein-coupled receptors, stimulate Ras by recruiting the guanine-nucleotide exchange factor Sos to the membrane. Sos exists in a complex with the adapter protein Grb2. Upon receptor activation, the Grb2/Sos complex is translocated to the membrane by binding of Grb2 to tyrosyl-phosphorylated residues in RTKs or additional adapter proteins. GTP-loaded Ras binds to Raf thereby initiating the activation of a protein kinase cascade consisting of Raf, MEK (ERK kinase), and ERK, (extracellular signal-regulated kinase).

The importance of the MEK/ERK signaling module for cell growth has been demonstrated by different approaches: the expression of ERK antisense RNA, or a dominant negative ERK mutant, inhibits proliferation in fibroblasts; similarly, when the activation of MEK/ERK is prevented by the specific inhibitor PD098059, cell growth is inhibited. On the other hand, the expression of a constitutively active MEK in fibroblasts is sufficient for cell transformation.

Ras dependent survival (or anti-apoptotic) signaling has been observed in many cell systems. The Ras effector involved is phosphatidylinositol-3-kinase (PI3K). Upon translocation to the membrane by binding to tyrosyl phosphorylated RTKs or to active Ras, PI3K phosphorylates the inositol moiety of phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$) on position 3 to generate $PI(3,4,5)P_3$. This lipid second messenger has two functions: it binds to PKB and activates the lipid dependent protein kinase PDK1. It has been suggested that $PI(3,4,5)P_3$ binding to PKB is required for its phosphorylation and activation by

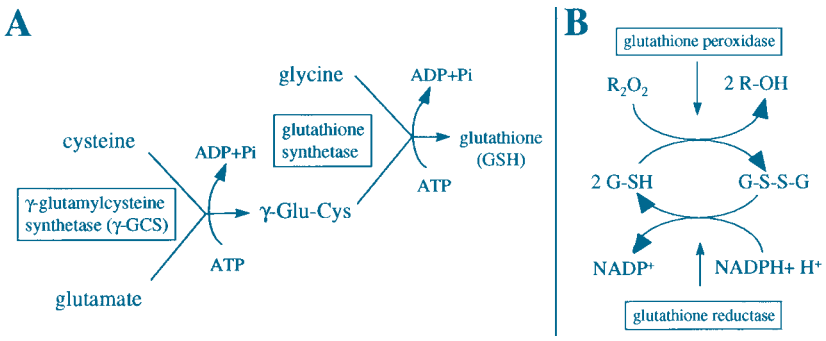

FIGURE 2: Glutathione synthesis (A) and role in peroxide detoxification (B)

PDK1. PKB appears to mediate survival by blocking Bad, an inhibitor of the anti-apoptotic proteins Bcl-2 and Bcl-x_L.

Oxidative Stress and Cellular Defense

Oxidative stress is a general term to describe reactions that lead to an increase of the oxidation potential in cells. Typically, oxidative stress is generated by free radicals, such as superoxide, that are deleterious to cells as they oxidize and damage biological molecules including DNA, proteins, and lipids. Critical for protein function is a reducing environment that prevents inter- and intramolecular formation of disulfide bonds by oxidation of cysteine residues. Compounds preferentially oxidizing free thiol groups include per(oxo)vanadate (a mixture of vanadate and hydrogen peroxide) and arsenite.

Cellular defense mechanisms against oxidative stress include the expression of specific enzymes to neutralize free radicals (e.g. superoxide dismutase that converts superoxide to peroxide). Most importantly, cells maintain high levels (~10 mM) of the tripeptide glutathione (g-glutamylcysteinylglycine, GSH). GSH is synthesized in two steps; first, γ-glutamylcysteine synthetase (γ-GCS) forms an unusual γ-amide bond between glutamate and cysteine. In the following reaction, glutathione is synthesized by glutathione synthetase that adds a glycine to the dipeptide (Fig. 2A).

GSH reduces disulfides and is used by the glutathione peroxidase to detoxify peroxides. GSH is regenerated by glutathione reductase that reduces GSSG utilizing NADPH as the reducing equivalent (Fig. 2B). The manipulation of GSH concentrations is a widely used experimental strategy to investigate effects of oxidative stress. GSH levels increase when cells are given N-acetyl cysteine (NAC) which serves as a cysteine donor in the γ-GCS reaction. They decrease when GSH synthesis is blocked by buthionine sulphoximine (BSO), an inhibitor of γ-GCS.

Oxidative Stress Inhibits Ras Activation in SMCs

Considering the pivotal role of Ras in proliferation and survival, we are exploring the influence of oxidative stress on the Ras signaling pathway. Using pervanadate and arsenite as model systems for thiol oxidants, we found that both prevent the activation of Ras by growth factors (shown for arsenite in Fig. 3). As expected, the signaling elements downstream of Ras, MEK and ERK, are also inhibited by the oxidants. Activation of PKB is partially blocked by arsenite consistent with the concept that the PKB activator, PI3K, can be activated by binding to both RTK and Ras (Fig. 1).

SAPKs have been known in various systems to mediate apoptotic cell death and it is an intriguing hypothesis that stress signaling interferes with growth factor signaling by blocking Ras activation.

To confirm that oxidative stress is involved in the arsenite-induced inhibition of Ras, we have treated SMCs with the anti-oxidant NAC before challenging with arsenite. With increasing concentrations of NAC, the inhibitory effect of arsenite is decreased. Notably, lowering glutathione levels by blocking glutathione synthesis with BSO kills SMCs suggesting that these cells are highly susceptible to oxidative stress.

From these observations, we conclude that oxidative stress inhibits Ras signaling which causes cell cycle arrest and possibly promotes apoptosis by decreasing survival signaling through PKB. Current experiments are being performed to identify how arsenite prevents Ras signaling. We are investigating whether arsenite or pervanadate form an inhibitory adduct with Ras or its activator Sos. Another possibility is that these compounds initiate a signaling pathway that involves the stimulation of stress-activated protein kinases (SAPKs). SAPKs have been known in various systems to mediate apoptotic cell death and it is an intriguing hypothesis that stress signaling interferes with growth factor signaling by blocking Ras activation. Overall, we hope to elucidate and eventually manipulate the molecular mechanisms by which oxidative stress in the vessel wall promotes SMC death.

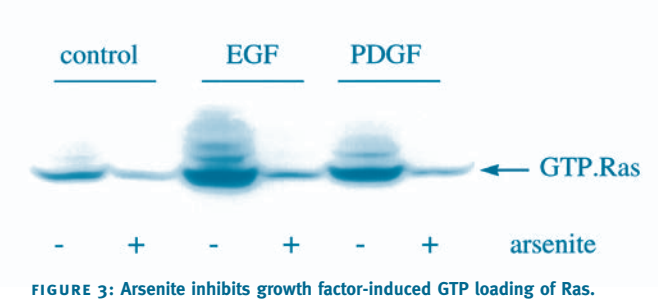

FIGURE 3: Arsenite inhibits growth factor-induced GTP loading of Ras.

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• MAGNETIC RESONANCE IMAGING OF THE HIGH-RISK ATHEROSCLEROTIC PLAQUE

FUNDING:
Astra-Zeneca Pharmaceutical
Esperion Pharmaceutical
Pharmacia Pharmaceutical
National Institutes of Health
VA Merit Review Grant

Our research work involves a collaborative effort between members of the Departments of Medicine, Pathology, Radiology, and the Division of Vascular Surgery. 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.

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).

	NC/IPH Present on Histology	NC/IPH Absent on Histology
NC/IPH Present on MRI	56	2
NC/IPH Absent on MRI	10	22

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± 0.06, specificity of 0.90± 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 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 neovasculature (Figures 3 and 4). In summary, MRI is capable of non-invasively 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.

MRI is capable of non-invasively 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.

	Rater 2			
Rater 1	**Thick**	**Thin**	**Ruptured**	**Row total**
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.

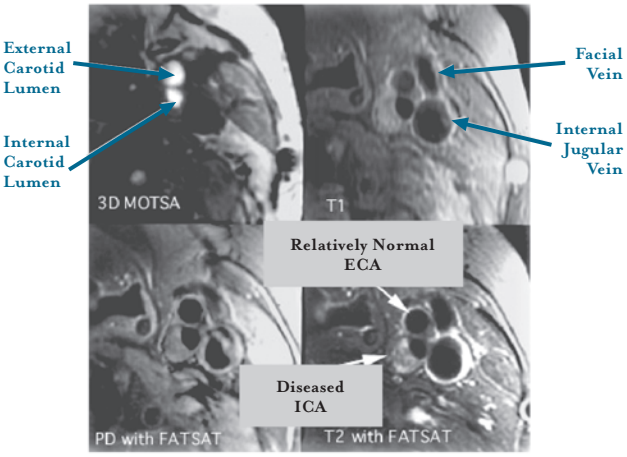

FIGURE 1: appearance of eccentirc plaque in the internal carotid artery on time-of-flight MRA (3D MOTSA), T1-, proton density (PD-), and T2- weighted MRI. The four cross-sectional views are taken at the same level of the carotid artery. MOTSA is an ME angio technique where the lumen is bright. T1, PD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

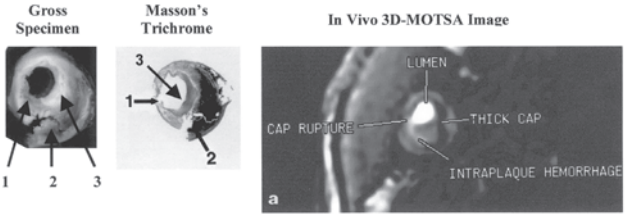

FIGURE 2: Appearance of a thick fibrous cap and adjacent region of fibrous cap rupture (at the 8:00 position) on MRI, with corresponding gross and histological cross sections.

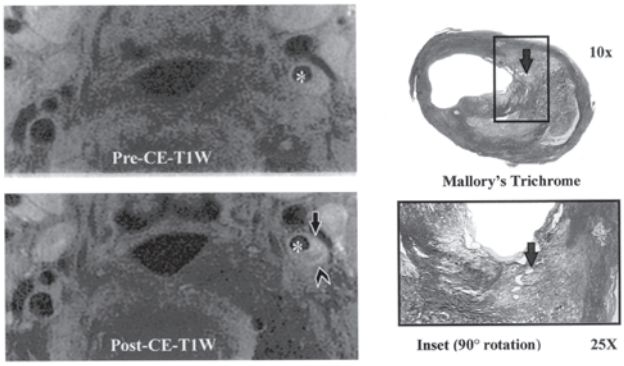

FIGURE 3: Enhancement in the common carotid artery (asterisk in lumen) at the 2:00 position seen after administration of gadolinium contrast. The enhanced region corresponds to an area rich in neovasculature seen on the histological section.

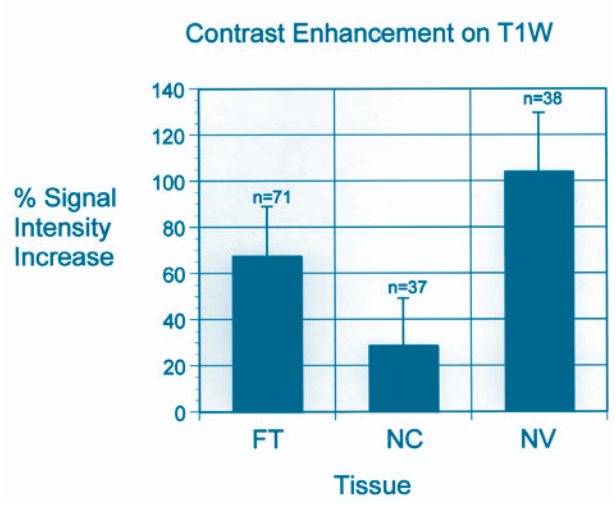

FIGURE 4: Percent signal intensity increase in post-contrast enhanced T1W MRI compared to pre-contrast, for fibrous tissue (FT), necrotic core (NC) and regions with neovasculature (NV). The differences between tissue types was statistically significant.

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Ted R. Kohler, M.D.

- ENDOVASCULAR THERAPY
- EFFECT OF BLOOD FLOW ON INTIMAL HYPERPLASIA AND ACCESS GRAFT FAILURE
- DIALYSIS ACCESS GRAFTS

FUNDING:
National Institutes of Health
Northwest Kidney Foundation

Endovascular Therapy

Endovascular therapy is an exciting new approach to aneurysm repair that uses a catheter-based delivery system rather than conventional open techniques. Patient morbidity and hospital stay are dramatically decreased. Endovascular grafts are held open and in proper position by attached metallic stents and are placed by a simple arterial cutdown or, in some cases, percutaneously. These devices have been very successful in early clinical trials and are soon to be approved for market release by the FDA. It remains to be seen, however, if these devices will perform as well over the long term (decades) as conventional grafts. The primary concern is whether or not the devices will remain well attached to the native artery at either end despite the native vessel's tendency to dilate over time.

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

Effect of Blood Flow on Intimal Hyperplasia and Access Graft Failure

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

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

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.

Dialysis Access Grafts

Effective renal dialysis requires several hundred cc's per minute of blood flow. To accomplish this, a fistula is created between an artery and vein, typically in the arm. This provides a high-flow conduit just under the skin surface where it can be accessed by needle puncture. Unfortunately, these fistulae have a high failure rate, even higher than other vascular grafts. Re-operation for failed access is a major cause of morbidity, prolonged hospital stay, and increased cost in the

treatment of renal failure. Most access failures are caused by intimal hyperplasia at the venous end of the graft. This is very surprising since in animal models we have found that increased blood flow reduces wall thickening after placement of prosthetic arterial grafts.

We are studying this problem in an animal model. Polytetrafluoroethylene (PTFE) grafts like those used in humans are placed in the neck of sheep, and measurements are made of the narrowing at the junction of the graft and native vessels. We have found that standard grafts fail within two to three months due to narrowing, which is much more pronounced at the venous end (see graph). Active thrombosis along the graft surface, particularly at the venous end, appears to be a major contributing factor. Thickening is greatly reduced if the grafts are sewn into an artery instead of a vein, even if blood flow is increased by creation of an artery-to-vein fistula beyond the graft. We have also found that special coating of the graft surface with phospholipids can stop this thickening process.

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

Like the clinical specimens, the sheep lesions have focal regions of prominent cellular proliferation, often adjacent to thrombus and in granulation tissue surrounding the graft. This can be seen in Figure 2, showing a proliferating-cell-nuclear-protein (PCNA)-positive nucleus marked by an arrow.

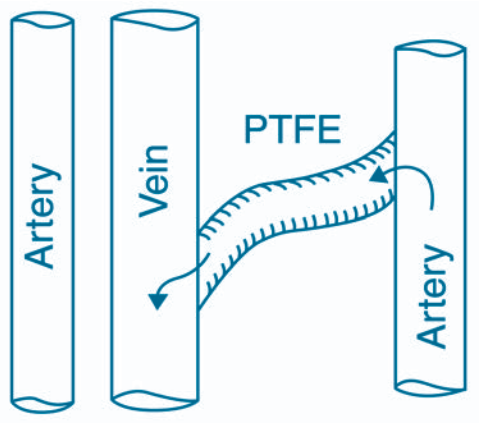
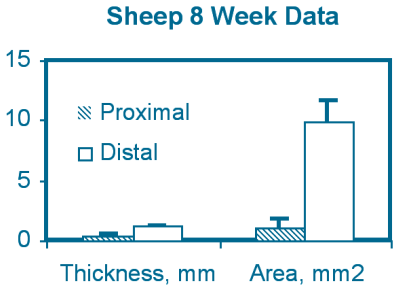


FIGURE 1: Fistula created between artery and vein to provide high-flow conduit.



GRAPH 1: Sheep Eight Week Data

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

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

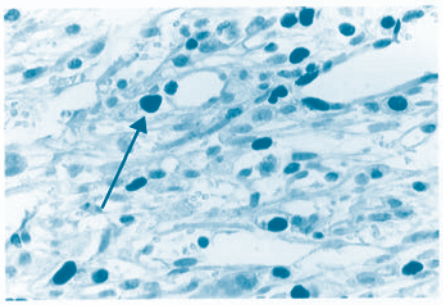


FIGURE 2: PCNA-positive nucleus.

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

PTFE grafts are seeded with smooth muscle cells that have been transduced with the erythropoietin (epo) gene (Dr. William Osborne, PI). Erythropoietin,

normally made by the kidney, stimulates production of red blood cells. Patients with renal failure do not make enough of this hormone and as a result are anemic. We will use a uremic sheep model to find out if epo made by cells placed in dialysis access grafts can reverse the anemia of chronic renal failure.

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 ANEURYSM WITH 3D ULTRASOUND
- TUMOR MEASUREMENT AND LOCALIZATION WITH 3D IMAGING
- AUTOMATED MEASUREMENT OF FLOW-MEDIATED VESSEL DILATION

FUNDING:
National Institutes of Health
National Cancer Institute

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.

Figure 1 shows 3D reconstructions of the proximal anastomosis of a femoral to popliteal saphenous vein bypass graft imaged at 2 months and 16 months after surgery. Surface reconstructions were generated for both the outer wall and the lumen of the vessel. Size changes are quantified as the mean percent change in

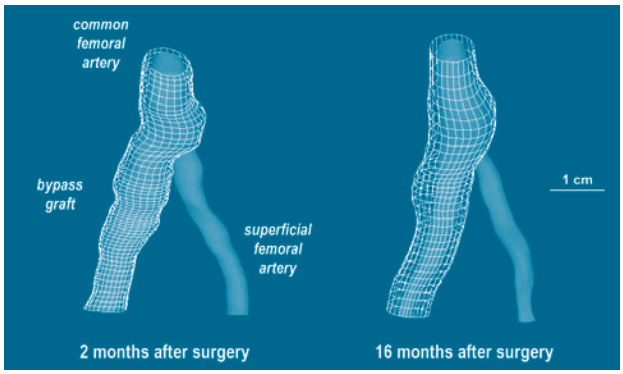

FIGURE 1: 3D surface reconstructions of the proximal anastomosis of a femoral to popliteal saphenous vein bypass graft imaged 2 months (left) and 16 months (right) after surgery. The outer wall of the graft is displayed as a mesh; the lumen is displayed as a surface.

cross-sectional area relative to the first post-operative study. The total vessel area decreased by 22%, the lumen area decreased by 45%, and the vessel wall area increased by 21%.

We have studied 10 sites of patch angioplasty revision over one year and compared the vessel remodeling patterns. Responses ranged from no appreciable change in dilation to normalization into a straight conduit (Figure 2). Lumenal narrowing documented by 3D scanning was not associated with consistent velocity changes on conventional duplex graft surveillance scans. Therefore, the 3D method provides documentation of anatomical changes in areas of complex geometry where velocity measurements are difficult to perform and interpret. Quantitative monitoring of vein graft morphology may provide a means to distinguish normal remodeling from pathologic changes that threaten vein graft patency.

A recent development in abdominal aortic aneurysm treatment is endovascular repair, which is a minimally-invasive procedure to exclude the aneurysm from the circulation.

Measurement of Abdominal Aortic Aneurysm with 3D Ultrasound

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

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

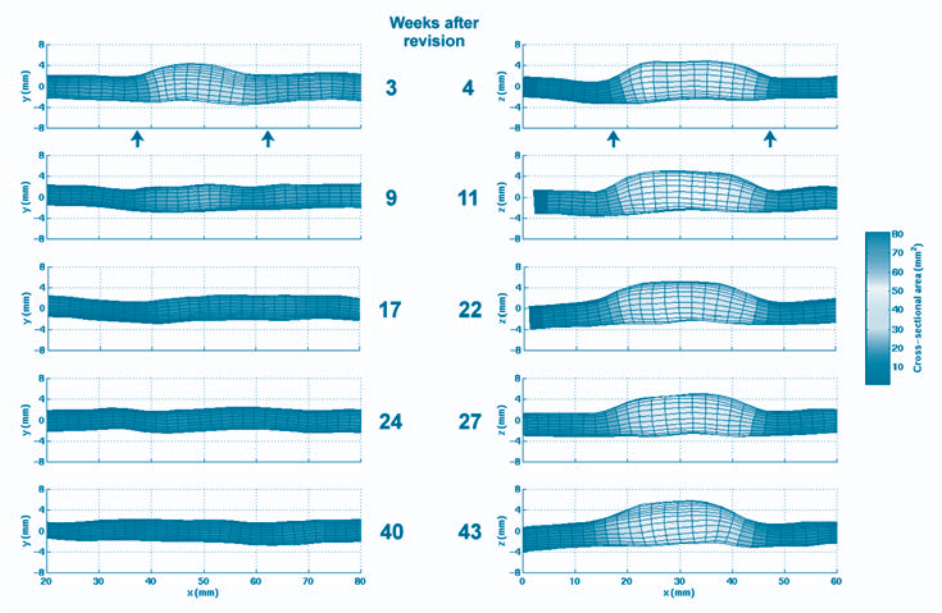

FIGURE 2. Serial 3D surface reconstructions of two cases of valve stenoses repaired by vein patch angioplasty. At 17 weeks the patch site on the left has narrowed to the point that the dilation is no longer evident. In contrast, the case displayed on the right demonstrated no significant change in size over the study period. Arrows beneath the top surface reconstructions indicate the start and end points of each patch; blood flow is from left to right.

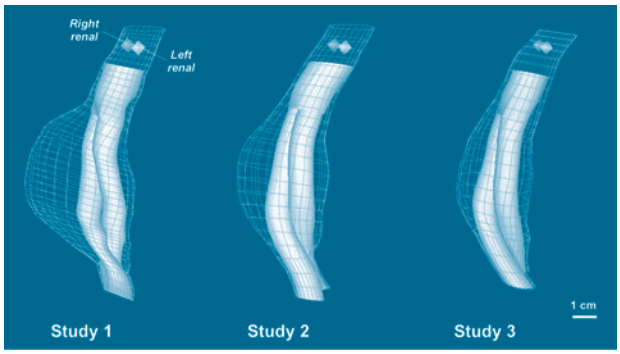

FIGURE 3: Serial study of an AAA repaired by an endovascular graft. The 3D reconstructions show both the aneurysm (outer mesh) and the graft (inner surface). The studies were performed 1) 2 weeks, 2) 6-months, and 3) 1 year after graft placement. Diamond markers show the origins of the renal arteries.

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 3 for a series of 3D ultrasound studies after endovascular repair, showing both the aneurysm sac and the graft. Figure 4 displays the changes in aneurysm cross-sectional area measurements over time. Measurement reproducibility was evaluated by repeated outlining of 10 sets of AAA ultrasound and CT scans. Measurements computed from the 3D surface reconstructions were overall maximum diameter, overall maximum cross-sectional area (CSA), and aneurysm volume. The intra-observer variability results, reported as the standard deviation of the differences between paired scans, are summarized in the table below. Three-dimensional surface reconstructions from ultrasound images yield intra-observer AAA measurement variability that is comparable to that for CT scans.

	RANGE OF MEASUREMENTS	3D ULTRASOUND VARIABILITY	3D CT VARIABILITY
MAXIMUM DIAMETER	43-68 mm	0.7 mm (1.3 %)	0.6 mm (1.3 %)
MAXIMUM CSA	1200-3355 mm2	37.7 mm2 (1.8 %)	26.5 mm2 (1.4 %)
VOLUME	51-222 ml	3.0 ml (3.3 %)	2.3 ml (1.9 %)

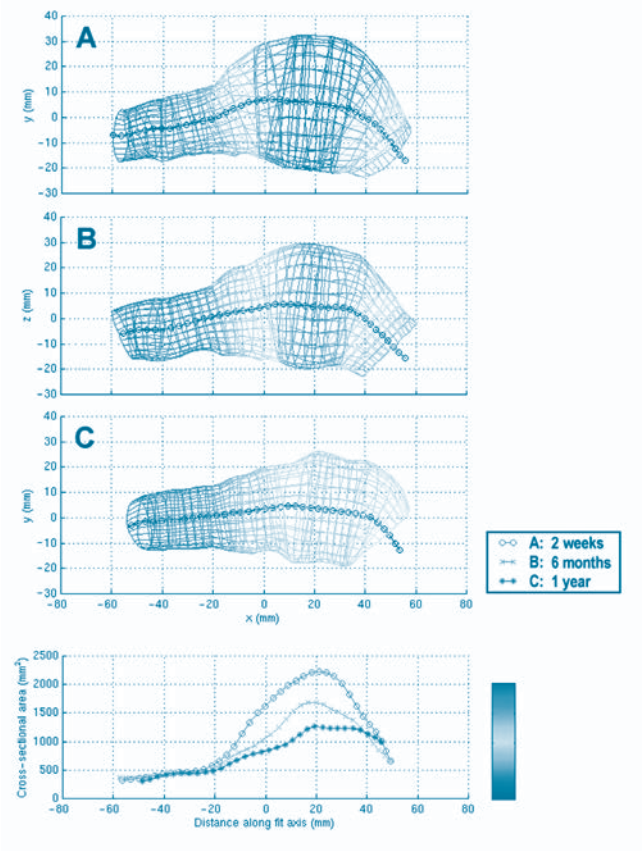

FIGURE 4: Cross-sectional area measurement of an AAA after endovascular repair. At the top are mesh representations of the aneurysm imaged at three time points, with shading corresponding to the cross-sectional area. Below are plots of the cross-sectional area measured at each sample point along the vessel center axis for each of the studies. The cross-sectional area measurements are mapped to the surfaces according to the gray scale at the right. A cross-sectional area of 2000 mm² is approximately equivalent to a diameter of 5 cm.

Tumor Measurement with 3D Imaging

A National Cancer Institute project under Dr. Kirk Beach in the Division of Vascular Surgery is investigating the use of ultrasound to detect small tumors by their pulsatility relative to surrounding tissues. As part of this effort, we are developing 3D displays of tumor location, and methods to quantitatively monitor tumor size and blood flow. Preliminary studies of 3D ultrasound scanning of tumors were performed in collaboration with Shahram Vaezy in the UW Center for Industrial and Medical Ultrasound, who is working on tumor treatment methods using high-intensity focused ultrasound (HIFU).

A study of a uterine fibroid was conducted in the power Doppler mode to assess tumor blood flow. The color (blood flow) and gray scale (anatomy) data were

reconstructed separately and displayed in a common 3D coordinate system (Figure 5). The structure of the vascular network associated with the tumor is well visualized in the 3D reconstruction. Our goal for future work is to track both the growth of tumors and their response to HIFU treatment using measurements of tumor volume, vascularity, and pulsatility.

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 pre-eclampsia conducted by Dr. Darcy Carr in the Department of Obstetrics and Gynecology, we have developed an automated image analysis method to measure the response of the vessel as a function of time after transient ischemia (Figure 6). 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.

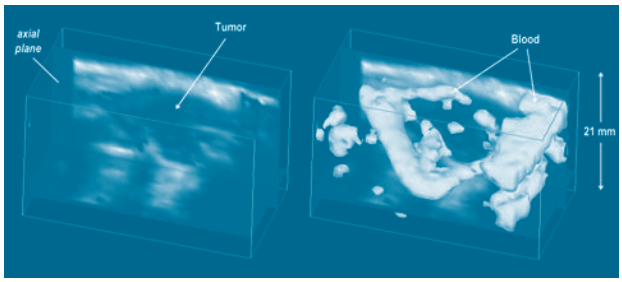

FIGURE 5: 3D volume reconstruction of a uterine fibroid imaged with power Doppler. Orthogonal planes through the gray scale data are shown on the left; the blood flow detected by power Doppler imaging is superimposed on the right. Voxel size = 0.6 mm. Volume size = 36 x 59 x 32 voxels = 21 x 35 x 19 mm.

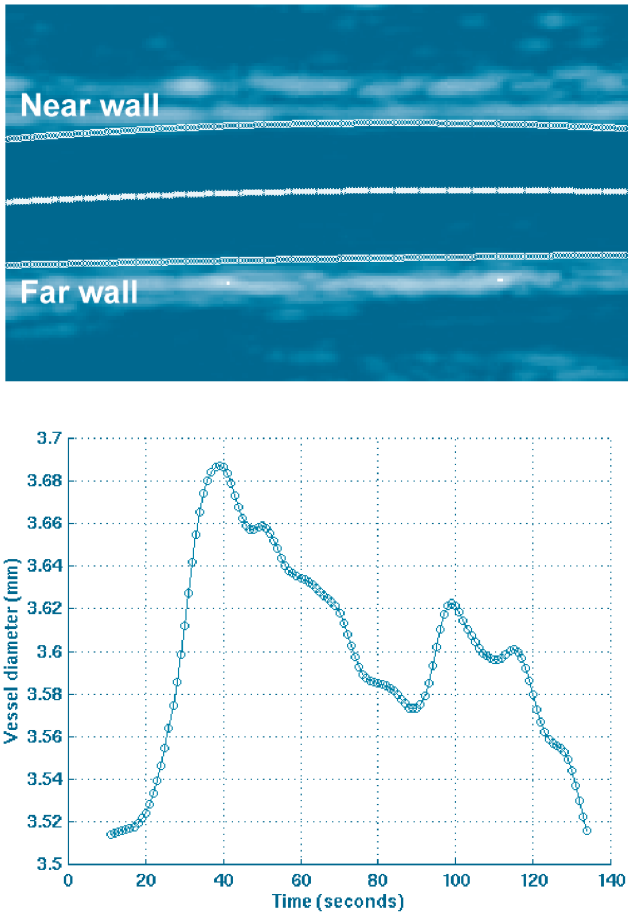

FIGURE 6. A longitudinal image of the brachial artery is shown at the top, with the locations of the vessel walls superimposed as detected by automated image processing. The diameter is measured along lines perpendicular to the vessel center axis; the measurements are averaged over the length of the 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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- IMMUNOMODULATION OF THE ALVEOLAR MACROPHAGE
- THE CYTOKINE PROFILE OF BURN PATIENTS RECEIVING PLASMAPHERESIS
- PRE-HOSPITAL MANAGEMENT OF THE DIFFICULT AIRWAY
- RIB FRACTURE MANAGEMENT
- VARIATIONS IN THE CARE OF HEAD INJURED PATIENTS
- THE USE OF ANABOLIC STEROIDS IN THE CHRONICALLY VENTILATED SURGICAL PATIENT

AWARDS:

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/ Wyeth-Ayerst Research Scholarship

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

Medic One Foundation

Northwest Burn Foundation

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 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. On the clinical front, I have been interested in the pre-hospital management of the difficult airway, impact of rib fractures in the elderly, variations in the care of head injured patients, the use of anabolic steroids in the ICU.

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. We are currently exploring the intracellular signaling pathways interrupted during this process and have extended these studies to animal models of ARDS. PAF AH has recently been studied in a phase II clinical trial for trauma and septic patients at risk for ARDS with encouraging results. The PAF AH treated group was found to have a significant decrease in 28 day mortality, the development of ARDS, and length of ICU stay compared to placebo controls.

The Cytokine Profile of Burn Patients Receiving Plasmapheresis

Burn mortality has dramatically decreased over the past twenty years due to improvements in ICU manage-

ment 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.

Pre-hospital Management of the Difficult Airway

The introduction of endotracheal intubation to the pre-hospital arena in the 1970s has resulted in definitive airway control for the majority of critically ill and injured patients, leading to a significant improvement in morbidity and mortality. There remain, however, patients who have a "difficult airway" in that they can not be successfully intubated by conventional techniques.

These include patients with prohibitive head and neck habitus or anatomy such as trauma patients with significant facial and neck injuries and medical patients with severe upper airway inflammation or obstruction. These are patients in whom orotracheal or nasotracheal intubation, with or without the use of chemical paralysis, is impossible to accomplish. Successful definitive airway control for these patients requires advanced surgical airway access techniques: cricothyroidotomy, tracheostomy, or retrograde intubation. There also remains considerable controversy in the literature concerning the pre-hospital use of paralytic agents to facilitate endotracheal intubation in the combative patient or one with significant muscular spasm.

The Seattle Medic One program has been on the forefront of advanced field care by providing training and access to the techniques of surgical airway access and extensive experience with the pre-hospital use of paralytic agents since 1970. This experience provides the opportunity for a population based study of the indications for pre-hospital intubation and the use of paralytic agents. We have recently completed an analysis of all pre-hospital intubations in Seattle over the past three years with detailed investigation regarding the management of the "difficult airway patients." In addition, we are collecting prospective data for every prehospital intubation event. Our goal is to optimize the field management of these complex patients.

Rib Fracture Management

Rib fractures are a common injury in the blunt trauma population and are often under appreciated in the setting of multiple injuries. The elderly are particularly susceptible to complications resulting from rib fractures and underlying pulmonary injury. We recently reviewed all patients > age 65 admitted to HMC with rib fractures over the past ten years and compared these to a cohort of younger patients. Of note, there was a nearly linear increase in mortality and complication rates associated with increasing rib fracture number in the elderly group. An elderly patient with only 3-4 rib fractures had a 19% mortality and a 31% rate of pneumonia. For an elderly patient with >6 rib fractures mortality was 33% with a pneumonia rate of 51%.

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. We hope to use this data to develop an optimal management strategy for these patients.

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.

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 lead 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 believe that post-surgical or trauma patients who require a prolonged period of mechanical ventilation (>7 days) may benefit from oxandrolone therapy. To test this hypothesis we are currently enrolling patients in prospective, randomized, blinded trial of oxandrolone vs. placebo in this patient population.

In addition to the clinical arm of this trial, we are simultaneously investigating the effect of anabolic agents on monocyte function, *in vitro*, and receiving circulating monocyte samples from the patients in this study to evaluate their activation status.

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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 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.

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.

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.

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

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9. Gibran NS, Tamura R. Tsou R, Isik FF, Human dermal microvascular endothelial cells produce nerve growth factor: Implications for wound repair (In Press, Shock)
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11. Gibran NS, Jang YC, Isik FF, Greenhalgh DG, Underwood R, Muffley LA, Usui ML, Larson G, Smith DG, Bunnett N, Ansel JC, Olerud JE. Diminished Neuropeptide Levels Contribute to the Impaired Cutaneous Healing Response Associated With Diabetes Mellitus (In Press, JSR)

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- NATIONAL STUDY ON COSTS AND EFFECTIVENESS OF TRAUMA CARE
- PROSPECTIVE, RANDOMIZED STUDY OF ELDERLY RIB FRACTURE MANAGEMENT
- IMPACT OF TRAUMA SYSTEM ON ACUTE CARE IN RURAL HOSPITALS
- RANDOMIZED COMPARISON OF ULTRASOUND WITH DPL IN THE INITIAL EVALUATION OF BLUNT TRAUMA
- 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 Prof. 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.

This is an ambitious project, which we hope will provide answers to numerous questions regarding the cost-effectiveness of trauma system design and implementation. To date, over 3,000 patients have been enrolled, and long-term follow-up has begun as enrollment has continued.

Prospective, Randomized Study of Rib Fracture Management

Rib fractures are common in blunt trauma victims, occurring in perhaps 10% of blunt trauma hospital admissions. In the elderly trauma victim, this seemingly minor injury can have a devastating outcome, with reported mortality rates of 12% and pulmonary complications of 33%. A key factor in the care of patients with rib fractures is felt to be adequate pain control. Several different strategies of pain relief have been employed, including intravenous narcotic, local rib block, and epidural analgesia. A recent retrospective cohort study was undertaken at Harborview Medical Center to examine the impact of rib fractures and morbidity and

mortality, particularly in the trauma patient over the age of 65.

Of the 277 elderly patients with rib fractures, 31% developed pneumonia compared to only 17% of the younger cohort. Additionally, mortality in the elderly patients with rib fractures was 22%, compared to only 10% in the younger patients. These differences are particularly noteworthy since the pattern of injury, the mean severity of chest injury, and the total injury score was nearly identical between elderly and young patients. Nonetheless, the elderly with rib fractures had more ventilator days, more ICU days, and longer hospital length of stay. As illustrated in the figures, a nearly linear relationship between the number of rib fractures and the development of pneumonia and mortality was observed in the elderly cohort, distinctly different from the morbidity and mortality pattern observed in younger patients.

This retrospective study could not convincingly demonstrate an advantage to one type of pain control, although epidural anesthesia patients had lower mortality, despite a greater number of rib fractures and more severe overall injuries. As a result, this study was expanded into its next phase. Currently underway is a joint Anesthesia Pain Service - Trauma Service prospective, randomized trial of epidural vs. no-epidural for the management of thoracic pain in patients with rib fractures. Enrollment of trauma patients at Harborview Medical Center is currently being conducted, and will continue throughout this year.

Impact of Trauma System on Acute Care in Rural Hospitals

Integrated statewide trauma systems are believed to improve the outcome of injured patients, but most supporting evidence for the effectiveness of trauma systems has come from urban populations. The goal of this study is to assess the impact of Oregon's statewide trauma system on the outcome of injured patients in the rural setting. The state legislatures of Oregon and Washington have established trauma systems that encompass large geographic regions with diverse needs. The trauma systems in these two states were implemented five years apart. This difference in timing and the natural similarities between these states make comparison of patient outcomes a valuable measure of the impact of statewide trauma systems on clinical practice and system effectiveness.

The specific aims are to:

- Determine if implementation of statewide trauma systems reduces the risk-adjusted death rates for hospitalized injured patients;
- Determine if trauma systems were associated with alterations in the statewide hospitalization patterns of severely injured patients; and
- Using data abstracted from rural hospital medical records and transferred into a unique Rural Trauma Registry, determine if trauma systems alter the acute care of head injured patients.

This project is nearing completion. A total of 2,458 rural trauma patients in Washington and Oregon have been enrolled in the study. Some of the highlighted findings of this study were that overall, an organized trauma system improved outcome, with some notable exceptions, notably severe head injured patients in the rural setting fared no better in Oregon than Washington. Advantages to the state with an organized system were most evident to those in the extremes of age: the pediatric and the elderly patients. Hospital discharge death rates were shown to be an incomplete measure of death frequency for injured patients, and 30-day or 60-day post-discharge mortality should be included in any outcome study.

This is an ambitious project, which we hope will provide answers to numerous questions regarding the cost-effectiveness of trauma system design and implementation.

Randomized Comparison of Ultrasound with DPL in the Initial Evaluation of Blunt Trauma

Abdominal trauma remains an important cause of morbidity and mortality in trauma, and unrecognized intraabdominal injury remains the primary cause of preventable trauma deaths. Diagnostic techniques used in abdominal trauma include diagnostic peritoneal lavage (DPL), ultrasound (US), and computed tomography (CT). Each diagnostic technique has advantages

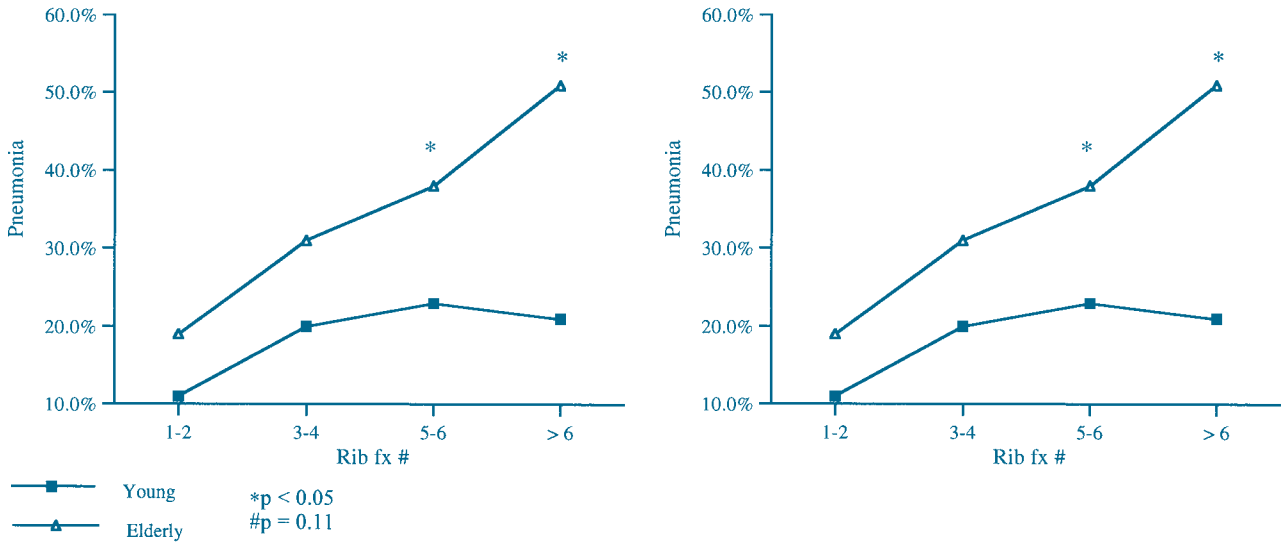

FIGURE 1: Pneumonia and mortality rate increases per occurrence of rib fractures in young and elderly patients.

and disadvantages. Ultrasound and DPL are most comparable, as both are done at the bedside, provide rapid interpretation with poor specificity but high sensitivity, and both are relatively inexpensive. While DPL has long been the standard diagnostic technique used in urban trauma centers in North America, ultrasound has been the standard in Europe and Japan. Although interest in US as an alternative to DPL has recently been evident in many North American trauma centers, to date no study has prospectively randomized patients to these two techniques. Furthermore, studies conducted to date have focused on diagnostic accuracy and effect, with no assessment of overall patient outcome or therapeutic effects in protocols comparing US, DPL and CT.

We are pursuing a prospective randomized study of adult blunt trauma patients who are in need of an adjunct to the physical examination of the abdomen. After the need for a diagnostic adjunct is determined by the surgery team, patients will be randomized into one of two arms: DPL and US. The primary endpoints of the study are therapeutic effects (length of stay, time to laparotomy, number of laparotomies, repeat or delayed laparotomy, findings at laparotomy, blood product utilization) and patient-oriented outcomes (morbidity and mortality, missed injuries, wound infections). Ultrasound is performed by ER radiology residents or staff; DPL by surgery residents or staff. Enrollment is currently complete, with approximately 1000 patients in each arm of the study, giving the study the power to detect at least a 5% difference in outcome. Analysis of data is underway.

A significant spin-off of this study is the training of UW Surgery residents in the performance of the Focused Assessment Ultrasound in Trauma (FAST exam). Currently the R3, R4, and R5 residents have been given the opportunity to participate in a FAST training course, which will be offered yearly to the R3 residents.

Washington State Trauma Registry and Central Region CQI

As Washington State continues to evolve and expand its statewide trauma system, hospitals and prehospital 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. A comparison of Central Region trauma patient outcomes to a national reference, the Major Trauma Outcome Study, reveals a significantly lower mortality for both adult blunt and penetrating trauma patients treated in the Central Region compared to this national norm.

Post-Traumatic Stress Disorder in Trauma Patients

A new faculty member in 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 PTSD in trauma patients. Posttraumatic stress disorder (PTSD) occurs in 20-40% of patients over the course of the year after physical injury. Youth admitted to the hospital for physical injury are at increased risk for recurrent traumatic life events; identifiable risk factors appear to be assault injury and history of injury prior to inpatient admission. Prospective cohort studies suggest that between 13-35% of injured children and adolescents ages 7-21 may go on to develop PTSD over the course of the year after injury . Other posttraumatic behavioral and emotional disturbances such as depression and substance abuse are also common occurrences . Furthermore, between 10-20% of the parents of injured youth may also develop posttraumatic distress .

A growing body of clinical trials research suggests that PTSD may be efficaciously treated with psychotherapeutic and psychopharmacological interventions. Also, there is now evidence that pediatricians can successfully detect and intervene with youth and their families who are suffering from psychosocial disturbances . The Surgeon General in a recent report on child mental health has recommended that primary care providers be educated regarding psychiatric disorders in children and adolescents . There is, however, no investigations that have systematically described the detection and treatment of posttraumatic behavioral and emotional disturbances in adolescents by surgical inpatient or pediatric outpatient providers. The Centers for Disease Control's Acute Care Division has made the development of screening and intervention protocols for patients with psychiatric and substance abuse disorders in the acute care setting a tier 1 research priority.

This investigation is a prospective cohort study of posttraumatic stress disorder (PTSD), substance use, functional impairment, and health service utilization among traumatically injured adolescents and their parents. The investigation will prospectively follow 110 randomly selected hospitalized injured adolescents ages 12-18. Adolescents and their parents will be interviewed during the inpatient surgical hospitalization and again at 1,4 and 12 months after the injury. We hypothesize that adolescents with PTSD will demonstrate clinically and statistically significant functional impairments even after adjusting for other injury and demographic characteristics. An additional aim of the investigation is to elucidate the clinical, family and community infrastructures available to support the implementation of psychosocial interventions for injured youth with PTSD. The overarching goal of the proposed investigation is to provide preliminary data that will inform the development of a larger scale R01 funded randomized intervention trial targeting PTSD and posttraumatic functional impairment among injured adolescents.

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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 ANDMODULATION OF THE TRAUMA-RELATED MACROPHAGE INFLAMMATORY RESPONSE TO PREVENT ARDS, MOFS, AND DEATH IN THE SEVERELY INJURED AND SEPTIC PATIENT

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- NIGMS Institutional NRSA

Shock Society
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American Trauma Society
- William S. Stone Award

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

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."

Relationship Between Trauma Center Volume and Outcome

The premise underlying regionalization of trauma care is that optimal outcomes can be achieved at greatest efficiency if care is restricted to relatively few dedicated trauma centers. Implicit in this premise is that higher patient volumes will lead to greater experience and this experience translates into better outcomes. This relationship appears to hold for other areas of surgical care involving complex procedures but, in contrast, there is no such relationship when less complex procedures are evaluated. Previous studies evaluating the relationship between institutional volume and outcomes in trauma patients are difficult to interpret because of multiple logistic issues. Two distinct cohorts of trauma patients are evaluated, including those with penetrating abdominal injury or those with multisystem blunt trauma with a minimum head injury and lower extremity, long bone fracture, treated at 31 academic Level I or Level II trauma centers across the United States, participating in the University Health System Consortium. Preliminary results indicate a strong association exists between trauma center volume and outcome, with significant improvements in mortality and length of stay, but only when the volume exceeds at least 600 cases

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 c oating of biomaterials with various molecules. These include ostepontin and various anti-inflammatory agents, such as anti-oxidants, including Vitamin E and components of the extracellular matrix, such as hyaluronic acid derivatives, to test the subsequent response of adherent macrophages to inflammatory stimuli, such as endotoxin.

In addition, we are studying materials of various selected pore sizes to minimize cell spreading and to test 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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- STRENGTHENING TRAUMA SYSTEMS IN DEVELOPING COUNTRIES
- CRASH INJURY RESEARCH AND ENGINEERING NETWORK (CIREN)

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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 continued high toll in younger children. However, even in these locations, injury is usually one of the leading causes of death among 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, economic consequences of injury, trauma treatment, and epidemiologic methodology. Further publications will hopefully include injury related disability and occupational injuries.

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 lies.

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

The main finding of this study was that with decreased socio-economic status, the overall rate of death among seriously injured patients increased, 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 1200 parents in the Monterrey area. We have received a grant from the American Trauma Society to further this work. In particular the grant is for research and development of injury prevention counseling materials oriented for the different socio-economic levels in Mexico.

Worldwide, injury prevention and control work has often been misconstrued as merely admonitions to be careful. However, it is a scientific field like any other. As such, it has a need for expertise in 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 two 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 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.

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.

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 are interviewing drivers 1 year after having taken the course. Thus far, we have obtained follow-up information on 71 drivers. 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 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 have 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 15% of the need for EMS in the city. The upcoming program, which is due to start in January 2002, is to provide upgraded training for prehospital trauma care for ambulance personnel in Hanoi. This will be done through exchanges of personnel between the two cities. Of special note is the fact that this is one of the first times that USAID has funded an EMS or trauma related project in a developing 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 five 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 lessons learned from this one country's experience as well as from similar programs in other countries need to be expanded internationally. In addition, other issues, such as staffing, equipment, supplies, organization, and administration need to be addressed. 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) this year created a "Task Force for Essential Trauma Care," with me as chair, to specifically address this issue.

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.

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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 OUTCOME
- TRAUMA PATIENT IN AN URBAN COUNTY HOSPITAL: BENEFIT OR BURDEN?
- EFFECT OF PRE-HOSPITAL TRIAGE TO A LEVEL I TRAUMA CENTER VS. A LEVEL III/IV TRAUMA CENTER
- PROSPECTIVE RANDOMIZED CONTROLLED TRIAL OF ANTIOXIDANT THERAPY IN CRITICALLY ILL SURGICAL PATIENTS

AWARDS:

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

American College of Surgeons, Committee on Trauma National
- Runner up paper competition, 1999
- Runner up paper competition, 2000

FUNDING:

Centers for Disease Control and Prevention
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 organized systems of trauma care. After controlling for secular trends in crash mortality and implementation of traffic safety laws (restraint laws, maximum posted speed limits, laws designed to limit drinking and driving), trauma systems had a significant impact on deaths due to traffic crashes. Eight years following initial trauma system implementation, mortality due to traffic crashes began to decline; about 15 years following trauma system implementation, mortality was reduced by 9% (95% CI, 2-15%) (Figure 1).

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

The high cost of uncompensated trauma care is the principal obstacle to trauma system development. Designation as a regional Level I trauma center may burden an institution with an unprofitable mix of uninsured patients with severe injuries.

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

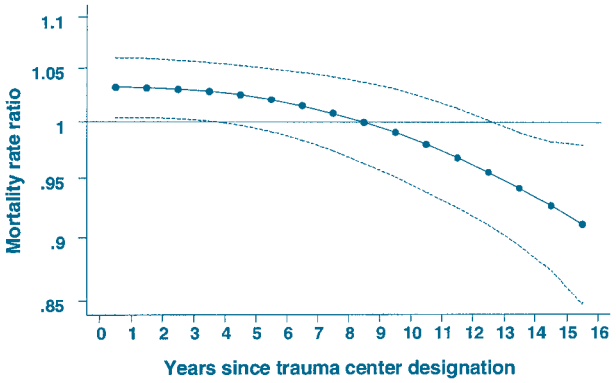

FIGURE 1: Adjusted mortality rate ratio attributable to a trauma system as a function of time from first trauma center designation. The dashed lines represent upper and lower bands of the 95% confidence interval.

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.

Trauma Patient in an Urban County Hospital: Benefit or Burden?

The high cost of uncompensated trauma care is the principal obstacle to trauma system development. Designation as a regional Level I trauma center may burden an institution with an unprofitable mix of uninsured patients with severe injuries. This burden may weigh heavily on an inner-city hospital already taxed by the payer mix of non-trauma patients and may undermine the sustainability of large urban trauma centers. To assess the potential burden of the trauma patient on an urban Level I trauma center, we evaluated the payer-mix of trauma patients relative to non-trauma patients at different levels of trauma care in a mature trauma system. Patients admitted to hospital in the state of Washington over a two year period were classified as either trauma (ISS>9) or non-trauma and by insurance status as either commercial insurance (CI) (e.g. managed care) or non-commercial insurance (e.g. Medicaid or self-pay). Medicare patients were excluded from analysis.

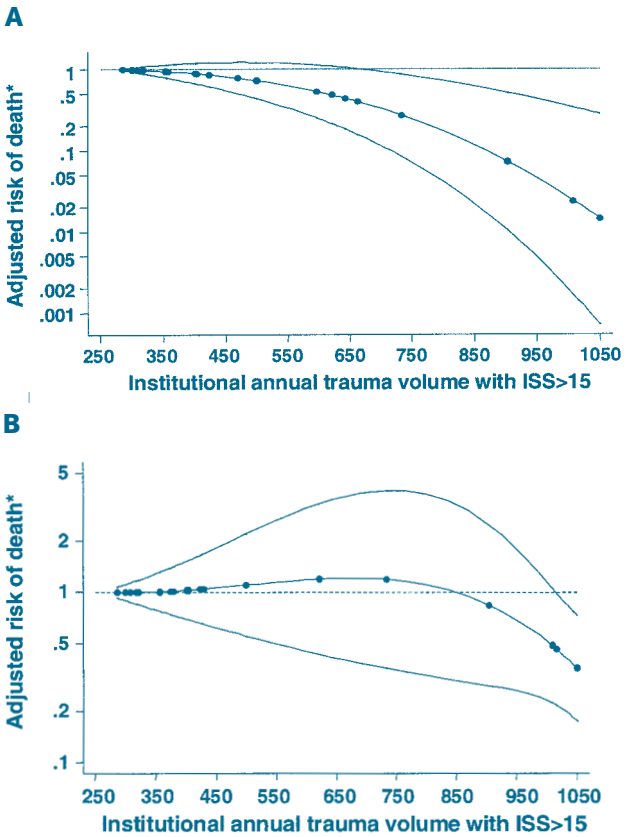

FIGURE 2: Relationship of the risk of death to trauma center volume in patients admitted in shock with penetrating abdominal injury (A) and in patients with coma and multisystem blunt trauma (B). Lines without • represent 95% confidence bands for the estimated odds ratio.

**Adjusted risk of death compared to the lowest volume institution.*

There were 10,386 trauma admissions and 474,944 non-trauma admissions to 87 centers. Trauma patients were less likely to have commercial insurance than non-trauma patients (69% vs. 74%, p<0.001). The proportion of trauma patients with commercial insurance treated at the urban Level I trauma center was significantly less than at other centers. However, trauma patients treated at the Level I trauma center were far more likely to have commercial insurance than non-trauma patients treated at this same center (52% vs. 30%, p<0.001). By contrast, there was no relationship between payer and trauma status at other levels of care.

These data suggest that referrals from across the state result in a disproportionate number of trauma patients with commercial insurance relative to non-trauma patients at this urban Level I trauma center. In this environment, designation as a Level I trauma center may actually improve care for inner city non-trauma patients by ensuring the ready availability of acute care services that follows designation as a trauma center and by means of cross subsidization of non-trauma care through trauma care reimbursement.

Effect of Pre-Hospital Triage to a Level I Trauma Center vs. a Level III/IV Trauma Center

Little is known about the effectiveness of regionalized, tiered trauma systems and whether clinical outcomes of trauma patients differ by the initial destination of the trauma patient. Level I trauma centers are designed to handle the most complicated and severe trauma patients, and Level III/IV facilities are designed to admit less severely injured patients and to stabilize severely injured patients before transfer to a Level I center.

The purpose of this population-based retrospective cohort study is to determine if injured patients who receive uniform care by pre-hospital advanced life support in the field, and who are transported directly from the field to a Level I trauma center, have better outcomes than those who are transported from the field to a Level III or IV center, and then transferred to the Level I center. The cohort will be restricted to all patients injured in King County transported by an Advanced Life Support crew to one of 8 trauma centers in the county (4 Level IV, 3 Level III, 1 Level I) during the years 1995 to 1998. It is anticipated that the results and conclusions derived from this analysis will identify subgroups of patients who are best served by direct transport to a Level I as well as those in whom optimal outcomes are achieved by triage at the Level III/IV prior to transfer.

Prospective Randomized Controlled Trial of Antioxidant Therapy 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 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. Primary clinical endpoints are the development of ARDS and pneumonia. A subset of these patients underwent bronchoalveolar lavage to assess the impact of antioxidant supplementation on markers of alveolar injury (alveolar fluid protein and neutrophil content, and F2 isoprostanes, a marker of oxidative tissue injury), the alveolar inflammatory response including alveolar cytokine levels, and markers of alveolar macrophage activation. Enrollment for this study has recently been completed after enrolling over 300 patients. Once available, the results should provide further insight into the role of oxidant-mediated tissue injury in the manifestations of critical illness.

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• PATHOPHYSIOLOGY OF POST-INJURY INFECTION AND ORGAN FAILURE

FUNDING:
National Institute of General Medical Sciences

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

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

We are interested in both of these related phenomena and our research program aims to characterize genetic influences on the risk for and outcome from injury-related nosocomial infection and organ failure and to better characterize the nature of the inflammatory response to tissue injury. In this report, we will demonstrate our findings regarding (1) the effect of genetic variations in the form of single nucleotide polymorphisms (SNPs) on cytokine production by whole blood leukocytes exposed to bacterial endotoxin, (2) relationships between SNPs and the severity of acute appendicitis and the associated cytokine production and, (3) the role of SNPs as markers for the development of severe sepsis and septic shock after trauma.

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.

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

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

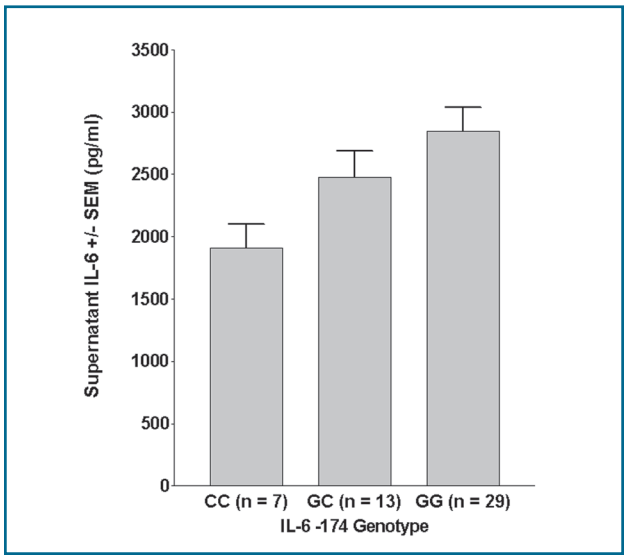

FIGURE 1

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 considered to possibly influence IL-6 gene transcription. We observed that the polymorphism at the –174 position was associated with IL-6 cytokine production. The number of C-alleles that were carried was associated with a progressive decrease in the IL-6 response to LPS (results shown in figure 1).

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

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

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

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

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

An additional advantage of appendicitis as a model of human inflammation is that it allows sampling of regional (peritoneal) and systemic (blood) compartments for cytokine measurements. We observed that both plasma and peritoneal fluid IL-6 concentrations were higher in the GG-homozygotes that the C-allele

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-a -308 A-allele	2.7	0.9 – 7.9	0.07

TABLE 1: Results of Multivariate Analysis

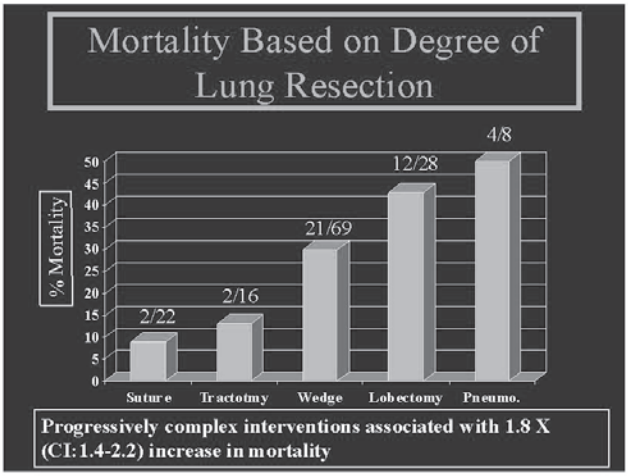

FIGURE 2: Plasma Concentrations

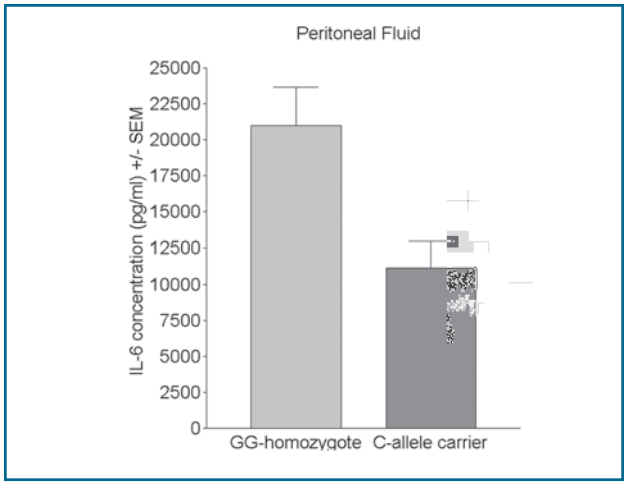

FIGURE 3: Peritoneal Concentrations

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.

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

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

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

In the study summarized here, we asked whether these naturally occurring genetic differences in the

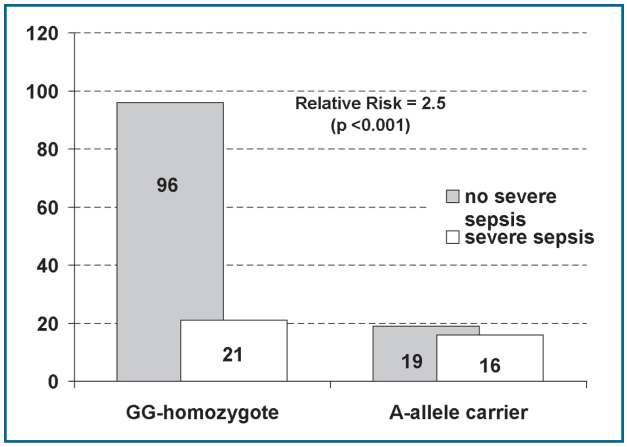

FIGURE 4: Sepsis Risk Factor

TNF-a promoter are markers for the development of complicated sepsis in severely injured patients. We hypothesized that carriage of the uncommon (A) allele at the -238, -308 or -376 position in the TNF-α promoter is associated with an increased risk for complicated sepsis.

Of the three SNPs in the TNF-α promoter that we studied, only the G⟶A transition at the -308 position was more frequent in patients with severe sepsis. The single patient who was homozygous for the A-allele developed severe sepsis, as did 15 of the 34 (44%) heterozygotes. Therefore, carriage of the A-allele was associated with a 46% risk (16/35) of severe sepsis See

figure 4). This is in contrast to the 18% risk (21/117) in patients homozygous for the G-allele (wild type).

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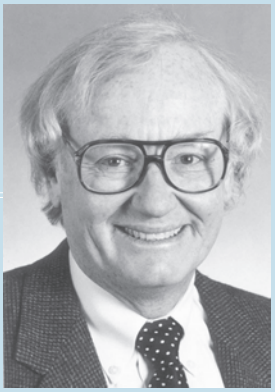
Robert K. Winn, Ph.D.



- ISCHEMIA-REPERFUSION INJURY
- SEPSIS (OVERWHELMING INFECTION)
- ADULT RESPIRATORY DISTRESS SYNDROME

FUNDING:
National Institutes of Health

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

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

Ischemia-Reperfusion Injury

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

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

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

Sepsis (overwhelming infection)

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

response to sepsis are under intense investigation in the hope of finding new approaches to therapy.

Signaling by bacterial products occurs through the recently described Toll-like receptors (TLRs) on the surface of cells. Intracellular pathways leading to 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 provide information that will allow the development of therapeutics for the treatment of this devastating syndrome.

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 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.

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Adult Respiratory Distress Syndrome

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

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

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

FUNDING:
CHRMC Craniofacial Endowment Fund
KLS Martin Ltd.

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

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

We have initiated a study to measure the metabolic rates of infants with cleft lip and/or cleft palate using indirect calorimetry, and to compare these with clinical measurements such as weight gain, growth, and diagnosis. The study is taking place at the Craniofacial Center at Children's Hospital and Regional Medical Center.

Since midface distraction osteogenesis is a relatively new technique in evolution, we are actively researching ways to improve the process at the Craniofacial Center of Children's Hospital.

Clinical Research

Cleft Lip and Palate

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

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

It will enroll 30 children a year in the study and follow them during the first year of their life, before and after each of their surgeries. The goal of the study is to create new guidelines for the nutritional care of infants with cleft lip and palate based on their individual needs.

Syndromic Severe Midface Hypoplasia

Children born with syndromes such as those described by Apert, Crouzon, and Pfeiffer can have such poor growth of their upper facial skeleton, or midface, that it compromises the closure of their eyelids and therefore the protection of their vision, the airway of their nose and therefore their ability to sleep, and the relationship between their upper and lower jaws and their ability to chew. The recognized surgical treatment of these children is to separate the upper facial skeleton from the rest of the skull, known as a LeFort III os-

teotomy, 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

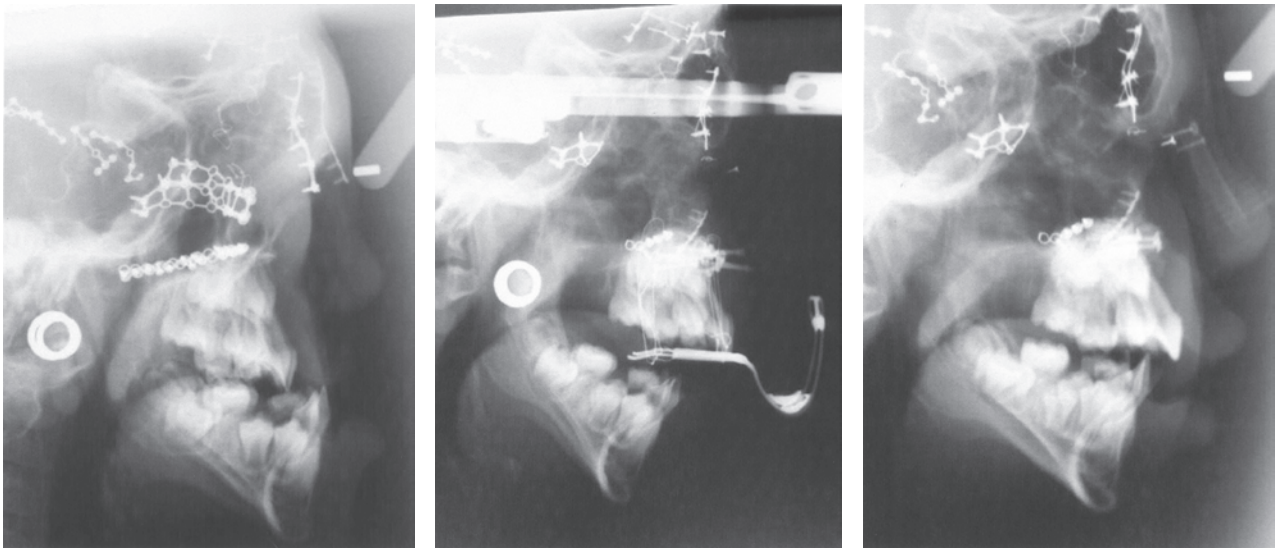

FIGURE 1: Lateral Cephalograms of a child undergoing midface distraction osteogenesis with an external device (Left) Before the operation, the child is having problems sleeping due to constriction of her nasopharynx, problems with dry eyes due to lack of cheek protection, and a problem chewing due to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

surgery operation with the need for blood transfusions and the risks of severe morbidity, or in rare cases, mortality. The ideal treatment of isolated craniosynostosis would be to prevent the suture fusion from occurring by blocking the responsible abnormal molecular pathway.

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

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

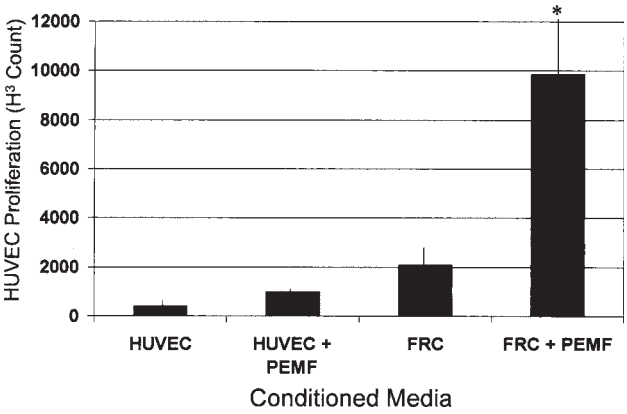

FIGURE 2: The effect of media from osteoblasts and endothelial cells stimulated with a pulsed electromagnetic field (PEMF) on endothelial cell proliferation. When human endothelial cells (HUVEC) were grown in media from fetal rat osteoblasts stimulated with PEMF (FRC+PEMF), their rate of proliferation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

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.

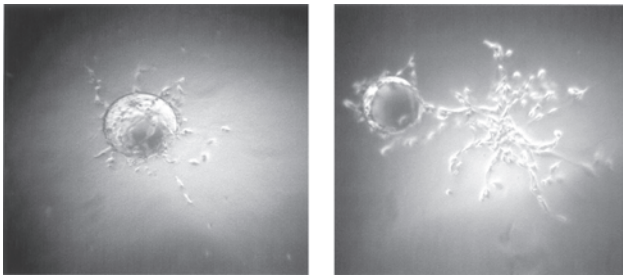

FIGURE 3: The effect of pulsed electromagnetic fields (PEMF) on endothelial cell tubulization. Endothelial cells attached to microspheres formed significantly more tubules when exposed to PEMF (left) than when not exposed the PEMF (right).

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• 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.

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

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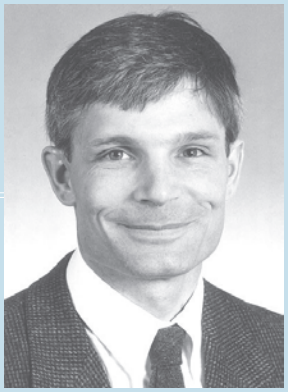

• SURGICAL TREATMENT REVIEW IMPROVES CHILDREN'S HEALING PROCESS

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

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.

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

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.

One of our recent submissions for publication detailed the technique and reported the results of our use of the transanal Swenson performed in the first several days of life. This technique, in which the Swenson is performed through the anus thus avoiding a large abdominal dissection, had not previously been described. There are several advantages of the one stage repair. Colostomy is avoided and its potential complications, which in the infant may approach a rate of 20%. The length of hospital stay is decreased and hospitalization for colostomy closure is avoided entirely. In theory long term function may be improved by earlier development of neural connections controlling anal sphincter function.

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

which some procedures, such as cholecystectomy, are performed. Few MIS procedures in children are encountered as often as some of those in adults, so that the ability for any one pediatric surgeon to become very 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 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 devise 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.

F. FRANK ISIK, M.D.

Loren H. Engrav, M.D.

- THE CONES OF SKIN
- THE FEMALE, RED DUROC PIG AS MODEL OF HYPERTROPHIC SCARRING
- UW BURN INJURY REHABILITATION MODEL SYSTEM
- HEALTH OUTCOME FOR BURN SURVIVORS
- DEPRESSION FOLLOWING BURNS
- TIME OFF WORK AFTER BURNS
- COMMUNITY INTEGRATION AFTER BURNS
- GRAFTING OVER INTEGRA
- EXCISION AND GRAFTING OF FACE BURNS

FUNDING:
Department of Education
- National Institute on Disability and Rehabilitation Research

Washington State Council of Firefighters

Laboratory Topics

The Cones of Skin

Hypertrophic scarring (ugly, raised, red, itchy scars), which follows injury to the deep layers of the skin, may be the worst outcome of burns. For decades researchers have tried to find a solution to this problem by studying the fibroblast and collagen of skin, without success. Clearly a new approach is necessary. We have determined that the cones of skin are dermal structures which are seldom described in textbooks and are located in the same anatomic areas where hypertrophic scarring occurs. They may be a clue to the cause of hypertrophic scarring. We have now clarified the histological anatomy of the cones in normal skin, burned skin, mature and hypertrophic scars, fetal skin, rats, rabbits, and pigs. It is our hope that understanding this histology will redirect research in hypertrophic scarring to a more productive path.

The Female, Red Duroc Pig as Model of Hypertrophic Scarring

Perhaps the main reason that hypertrophic scarring is so poorly understood is the lack of an animal model. In 1976, Silverstein, Goodwin, Raulston and Pruitt reported that the female, red Duroc pig develops hypertrophic scar. Nothing ever came of this report. Was it not reproducible? Missed? Not useful? We decided to clarify this matter by studying tangential wounds of varying depths allowed to heal spontaneously on these pigs. We found thick scar (up to 11 mm), which has many similarities to human hypertrophic scar including

Early excision of burns has become commonplace, but early excision of face burns has not. Many or most burn centers still permit face burns to heal over weeks, accept the result, and refer the patient for reconstruction.

the immunohistochemical localization of decorin, versican, TGFß1 and IGF-1. We plan to continue to study the usefulness of this model.

Model System Topics

UW Burn Injury Rehabilitation Model System

There is very little data available on the long-term outcome of burns. In 1993 and again in 1997, the National Institute on Disability and Rehabilitation Research (NIDRR) of the Department of Education funded model systems in burn care to obtain this data. The UW Burn Center was twice chosen to participate so now we have a nine-year history with burn model system research matched only by the burn center at UT Southwestern. Funding is $295,000 per year for five years. The vast majority of this money funds four personnel to 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.

In June 2002, we presented our progress to date at a summative review in Washington, DC. Our Model System includes 14 projects managed by four faculty. The UW program includes participation in the national burn outcome database as well as the individual and collaborative projects listed in the table below.

As is evident from the titles, this is an extensive study of burn outcomes. The UW has provided approximately 1000 or 40% of the patients enrolled in the national database.

UW RESEARCH PROJECTS
Scar Assessment and a Prospective Trial of Pressure Garment Therapy
Enhancing Adults' Compliance with Burn Therapies to Prevent Secondary Complications
Post-burn Itching
Continuous Scale Physical Functional Performance
Longitudinal Measurement of Recovery
Dexter Evaluation (a device to measure impairment)
Literature Review Meta-Analysis Time Off Work
Collaborative Research Activities:
Comparing Health Outcome Measures
Functional Outcomes
Longitudinal Study Post Traumatic Stress Disorder
Impact of Substance Abuse on Vocation Return
Time Off Work After Burns
UW Demonstration Projects:
Self-care System for Burn Survivors
Burn Rehabilitation Instruction System for Providers
Burn Telemedicine Program
Vocational Counseling and Case Management to Promote Independent Living and Return to Work
Community Reintegration
School Re-entry and Measurement of Post Burn School Performance
Burn Survivor Peer Support
Added UW Projects
Virtual Reality to Relieve Pain During Therapy

Health Outcome for Burn Survivors

Little is known concerning health outcome for patients who survive burn injuries, and how their health outcome compares with that of other medical populations. Such information is important given that the current direction of health care policy decision-making is toward outcomes-driven decision models. We compared the health status of 91 patients one month after severe burn injury with the published reports of the health status of 39 medical comparison sample, and two reports of health status for the general population. Additionally, we collected longitudinal data on a subsample of our surviving patients with burn injuries at 1 year. Our findings suggest that people who survive a severe burn experience a stable and relatively good health status after their injury compared with other medical samples. However, their health status remains worse than that of the general population over time. Further, people who survive a major burn indicate that the areas of vocational and psychosocial functioning are often the most troublesome for them.

Depression Following Burns

It is commonly assumed that patients hospitalized for burn treatment will experience some level of depression. However, little is known about the trends in severity of depression over time. The purpose of this study was to determine the rates and severity of depression over a two-year period. The Beck Depression Inventory (BDI) was administered at one month (n=151), one year (n=130), and two years (n=125)

after discharge. At one month, 54% of patients showed symptoms of moderate to severe depression, and at two years, 43% of the patients responding still report moderate to severe depression. The average correlation between scores over time was high. Women had higher depression scores than men at each time period. An interaction between gender and having a head or neck injury was also observed at one month and one year following discharge. Results suggest that routine outpatient screening for depression is warranted.

Time Off Work After Burns

The literature on time off work and return to work after burns is incomplete. This study addresses this and includes a systematic literature review and two-center series. The literature was searched from 1966-October 2000. Two-center data were collected on 363 adults employed outside of the home at injury. Data on employment, general demographics, and burn demographics were collected. The literature search found only 10 manuscripts with objective data, with a mean time off work of 10 weeks, and %Total Body Surface Area (TBSA) as the most important predictor of time off work. In the two-center study 66% and 90% of burn survivors had returned to work at 6 and 24 months post-burn. The mean time off work for those who had returned to work by 24 months was approximately 17 weeks and %TBSA was related. Psychiatric history, presence of extremity burns, and %TBSA were associated with returning to work. In the UW subset of the data, for patients with known outcomes, 58% had either not returned to work at two years, went on disability or found return to work disrupted. The impact of burns on work is an enormous cost to society.

Community Integration After Burns

Evaluation of community integration is a meaningful outcome criterion after major burn injury. The Community Integration Questionnaire (CIQ) was administered to 463 individuals with major burn injuries. The CIQ results in Total, Home Integration, Social Integration, and Productivity scores. The purposes of this study were to determine change in CIQ scores over time and what burn injury and demographic factors predict CIQ scores. The CIQ scores did not change significantly from 6 to 12 to 24 months post-burn injury. Home integration scores were best predicted by gender and living situation; Social Integration scores by marital status; and Productivity scores by functional outcome, burn severity, age, and pre-burn work factors. The data demonstrate that individuals with burn injuries have significant difficulties with community integration due to burn and non-burn related factors. CIQ scores did not improve over time but improvement may have occurred before the initial six-month post-burn injury follow-up in this study.

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

Clinical Topics

Grafting Over INTEGRA

INTEGRA® Dermal Regeneration Template is a "biodegradable template that induces organized regeneration of dermal tissue by the body", i.e. it is one of the new varieties of artificial skin. It is now a standard aspect of burn care at many burn centers for resurfacing large burns. The template is a bilayer membrane and the outer layer is silastic that must be removed and replaced with autograft after the template has become vascularized. The silastic must be replaced with a ultrathin, skin autograft, recommended to be .004". Prior to the release of INTEGRA, ultrathin autografts were never used. In fact, surgeons were not trained in methods to harvest such grafts and dermatomes were not designed to harvest such grafts. As a result, the early efforts resulted in grafts of poor quality.

We studied dermatomes for accuracy of settings and the microscopic thickness and histology of grafts harvested at the various dermatomes settings. We found the variability in thickness in autografts is large and that it is virtually impossible to harvest grafts thinner than .006". Further, grafts thinner than .006" may contain no epidermal stem cells. If the grafts contain no stem cells, it may initially take but then disappear over time as the cells contained in the graft die and are not replaced.

We now set the dermatome at .006" and make any adjustments necessary during the harvest to obtain the ultrathin grafts. Our results have improved with this method and it is now unusual for the harvesting of ultrathin grafts to yield grafts of poor quality.

Excision and Grafting of Face Burns

Early excision of burns has become commonplace, but early excision of face burns has not. Many or most burn centers still permit face burns to heal over weeks, accept the result, and refer the patient for reconstruction. A recent review of the English-language literature

revealed only eight manuscripts containing actual photographs of only 15 postoperative results. We began early excision of face burns in 1979 and have now accumulated a 20+ year experience with essentially one method, one surgeon and approximately 100 patients.

We reviewed all of the charts and slides files and contacted all patients for whom we had current phone numbers or addresses. We found approximately 50 patients with complete charts and photographic records. Since there is no objective measure of outcome for such a procedure, we published the actual photos of all patients, thinking that surgeons reading the manuscript could then form their own opinion from this "raw" data. The results demonstrated that the method yields facial appearance that permits these people to work and recreate in society. But it also demonstrated that many needed small, reconstructive procedures for scarring, contracture and pigmentation abnormalities. We need to able to control these three unfortunate events.

Like peritoneal lavage, which was accepted slowly, it is possible that excision of face burns will in time become the standard method.

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F. 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 represents a series of well-orchestrated events, including inflammation, angiogenesis, epithelialization and matrix remodeling. Our laboratory is interested in determining the cell-cell signaling and the changes in gene expression during normal wound healing. Each of the projects represents our efforts to understand the normal healing process in order to better understand and treat aberrant healing processes.

Angiogenesis: Role of Proteases

One of the cellular events of normal wound repair is angiogenesis, the formation of new blood vessels. Blood vessels are assembled by either vasculogenesis, in which a primitive vascular network is established from mesenchymal progenitor cells during development or angiogenesis, in which new blood vessels arise from preexisting vessels. Though vasculogenesis is limited to development, angiogenesis occurs both in the embryo and in adults. For angiogenesis to occur, endothelial cells must detach from adjacent endothelia, proliferate, migrate, and assemble into tubes.

Wound repair angiogenesis requires activation of fibrinolytic enzymes for cellular migration of microvascular endothelial cells through a fibrin matrix. These fibrinolytic enzymes include the plasminogen activator system, which consists of urokinase plasminogen activator (uPA) and tissue plasminogen activator (tPA), balanced by plasminogen activator inhibitor-1 (PAI-1) and vitronectin. It has been hypothesized that dysregulation of the plasminogen activator system can result in abnormal wound healing. We have studied the plasminogen activator system in models of excisional wound healing to clarify the role of the plasminogen activator system during normal healing.

Transgenic mice deficient for vitronectin, uPA, or uPAR (urokinase plasminogen activator receptor) genes appear phenotypically normal, but we have found marked disturbances in response to tissue injury. Our data indicate that vitronectin serves to limit fibrinolysis following injury and that elimination of vitronectin from the provisional wound matrix leads to microvascular hemorrhage and results in delayed wound healing.

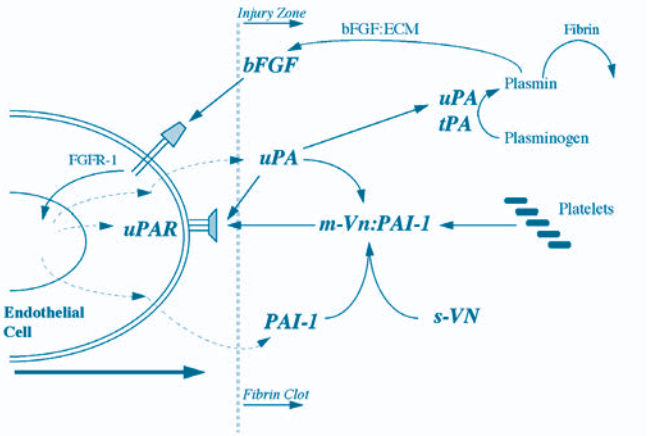

FIGURE 1: Urokinase plasminogen activator receptor.

In addition, limited function of uPA or uPAR genes disturbs cell migration This disturbance results in decreased wound angiogenesis and decreased wound epithelialization, leading to significant differences in the histologic appearance of the final wound. Interestingly, in spite of significant effects on wound microvasculature, deletion of uPA, or uPAR genes does not result in delayed healing. The murine wounds with uPA, or uPAR deletions compensate with increased wound contraction.

Angiogenesis: Role of Matrix

Tissue injury is immediately followed by coagulation, changing the extracellular matrix (ECM) environment of endothelial cells from a collagen and laminin rich matrix to a fibrin and vitronectin rich matrix. These newly deposited extracellular matrix molecules bind to integrins, a class of cell surface receptors that anchor the cell cytoskeleton to the surrounding extracellular matrix and provide cellular signals about the matrix environment to the endothelial cell.

Using cDNA microarrays, we have identified the changes in gene expression of endothelial cells exposed to different ECM, including vitronectin. Change in the matrix alone resulted in greater than a 3-fold difference in expression among 430 out of 4,400 genes examined. The quiescent state (characterized by laminin or collagen type IV) increased the expression of signaling molecules such as phospholipase C-alpha and PI 4,5 kinase, whereas the injury-associated matrices (characterized by vitronectin and fibrin) enhanced the signaling molecules PKC-alpha and multiple serine-threonine kinases. In addition, vitronectin increased the expression of DNA-damage repair proteins that protect cells from apoptosis. Finally, there were global increases in multiple transcription factors including POU-2, TF3, E2F-5, MEF2 and the transcription regulator, ISGF3.

Interestingly, we also found marked up-regulation of several growth factor receptors after exposure to an injury-associated matrix, such as vitronectin. These data show that changes in the matrix environment alone can result in large-scale gene expression changes that favor endothelial cell proliferation and survival. Together, these studies support evidence that manipulating the wound matrix environment alone may augment wound repair and have obvious implications for tissue bioengineering.

Angiogenesis: Role of Growth Factor Receptors

Angiogenesis is initiated by paracrine signals that stimulate the proliferation and migration of endothelial cells. Among the angiogenic ligands are fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF). A growth factor must bind to its specific endothelial cell surface receptor for a cellular response. FGF receptors on endothelium include FGFR-1 and FGFR-2; VEGF receptors include VEGFR-1 and VEGFR-2. Although normal adult dermal microvessels do not constitutively express these receptors, we have shown by our cDNA microarray studies that changes in the matrix environment following tissue injury induce endothelial cells to express these specific growth factor receptors.

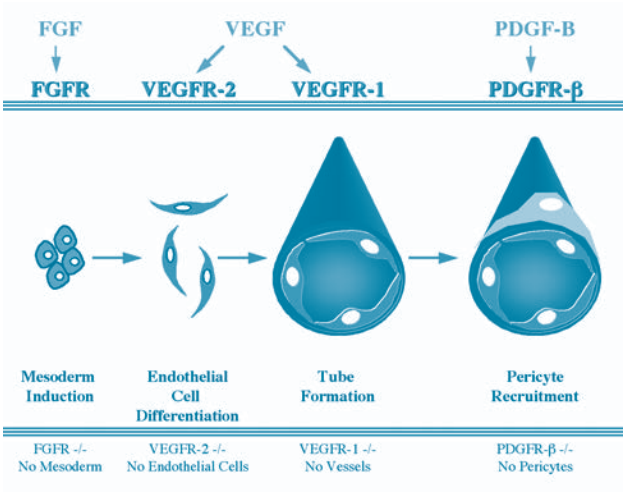

FIGURE 2: VEGFR-2 and FGFR-1 systems.

Mice carrying homozygous disruptions in FGFR-1, FGFR-2, VEGFR-1, or VEGFR-2 die in utero, highlighting the critical role of these receptors during vasculogenesis and developmental angiogenesis. To better understand the role of these receptors during repair, we are currently delivering truncation mutant growth factor receptors to normal mice wounds using a retroviral system. Current ongoing work suggests that the VEGFR-2 and FGFR-1 system appear to be critical for the proliferation of multiple cell types during wound repair, including microvascular endothelial cells.

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. In these studies, our aim is to analyze and catalogue the gene expression profile of human skin during the first few hours following cutaneous wounding.

We have found significant gene up-regulation 30 min. after wounding: expression of 334/4000 genes was increased >3 fold. Within the first 30 minutes, we found up-regulated expression of genes involved in cell signaling inhibition, including suppressor of cytokine signaling (SOCS) and suppressor of ras-1. In addition, there was silencing of cell cycle genes (e.g. Rb), proteases (e.g. uPA), and growth factor-related pathways. At 1 hour post wounding, 471/4000 genes were increased > 3 fold with a predominant reversal of the 30 min.

pattern: we found down-regulation of transcriptional and signaling inhibitors, and up-regulation of multiple transcriptional activators. A searchable web site is being constructed to disseminate this data.

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 in gene expression, new targets may emerge that could provide further insight into the study of normal response to injury.

genes that is activated belongs to the family of signaling molecules that are highly expressed during development but become dormant in adults.

We have found that Wnt genes are re-expressed in response to tissue injury. We are beginning to examine the role and contribution of HSC to murine wound repair and define the contribution that the Wnt signaling plays in the response to cutaneous injury. HSC may be a valuable source of regenerative capacity for a wound, especially when the local resident cells have been damaged by irradiation.

Together, these studies support evidence that manipulating the wound matrix environment alone may augment wound repair and have obvious implications for tissue bioengineering.

Hematopoietic stem cells in normal wound healing

Whereas morphogenesis in tissue repair has often been compared and contrasted to the morphogenesis during development, normal wound repair has always been thought to involve proliferation and migration of terminally differentiated cell types. Recent evidence suggests that normal cutaneous repair 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, capable of reconstituting multiple cell types in various tissues, including skin. This pool of cells may represent the opportunity to induce tissue regeneration in sites of injury similar to the morphogenesis seen in development. The ability to manipulate these cells may provide a previously unrecognized means of therapeutic intervention in patients with non-healing wounds

The most-studied progenitor cell type is the hematopoietic stem cell (HSC) from the bone marrow. We have recently found that HSCs migrate to sites of dermal injury, differentiate into several cell phenotypes and incorporate into the cutaneous wound long-term. These recent findings raise important and unanswered questions regarding the actual role of these progenitor cells in wound repair and the signaling pathways that regulate their path of differentiation. From our cDNA microarray studies of gene expression in normal healing human skin wounds we have determined that one of the

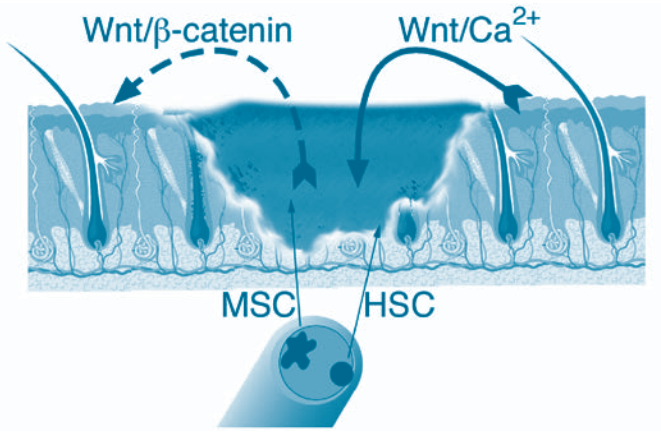

FIGURE 3: Graphic demonstration of our hypotheses. We think stem cells in the epidermis and stem cells from bone marrow can provide the missing cell types following loss due to injury. However, we think that the lack of morphogens in the wound may account for the lack of regeneration seen in wound repair.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

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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
- OBJECTIVE MEASUREMENTS OF SKILL IDENTIFY A LEARNING CURVE FOR ROBOTIC 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 introduction 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.

Our goal is to create a systematic approach for the clinical introduction of a surgical robotics system which involves the entire surgical team.

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.

Surgical robotics systems are sophisticated and unfamiliar to most hospitals. Nevertheless, 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.

Objective Measurements of Skill Identify a Learning Curve for Robotic Surgery

New devices and technology can improve the efficiency of our surgical procedures, but require adaptation to new techniques. Through the development of an objective scoring system of task goals and error analysis, we studied the learning curve for intracorporeal knot-tying in robotic surgery. (Figure 1)

Eight attending surgeons of varying laparoscopic expertise underwent at least five hours of individualized training in surgical robotics during a three-week period. Each surgeon performed an intracorporeal knot using both laparoscopic and robotic techniques prior to robotic skills training. Following training, robotic knot-tying was repeated. Each knot was evaluated using an objective scoring system based upon task goals and errors. This composite score as well as completion time were used to demonstrate improvement of skill.

Completion of an intracorporeal knot using standard laparoscopic techniques took 140 seconds with a mean composite score of 77 (100 total points possible) while average time for robotic knot-tying without any training took 393 seconds with a score of 40. After 4-6 hours of robotic training, average time decreased by 65% to 139 seconds and composite scores increased to 71. With more practice of 8-10 hours, mean scores further increased to 94 while completion time dropped to 105 seconds. This improvement continued after 14 hours to mean composite score of 99 and time to completion of 82 seconds.

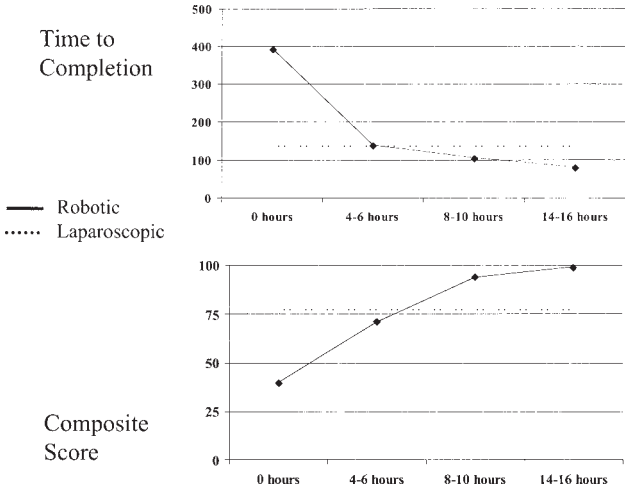

FIGURE 1: Learning curve for intracorporeal knot-tying in robotic surgery.

As with any new technology, surgical robotics requires dedicated training to achieve mastery. Even surgeons who routinely practice laparoscopy face an initial setback with robotics as demonstrated by ability to complete an intracorporeal knot, time to completion, and number of errors. However, after 4-6 hours of training, these surgeons are able to match their laparoscopic performance; and with even further practice, surgeons can complete intracorporeal knots better and faster using robotics than with laparoscopic technique.

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 & Material Command
• Telemedicine & Advanced Technology Research Center

Objective Assessment of Surgical Skills

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

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

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

Operating Room of the Future

Recent introduction of robotic systems into clinical surgery indicates a fundamental new direction for surgeons. Research will be conducted to integrate robotics into an entirely new concept for the operating 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.

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

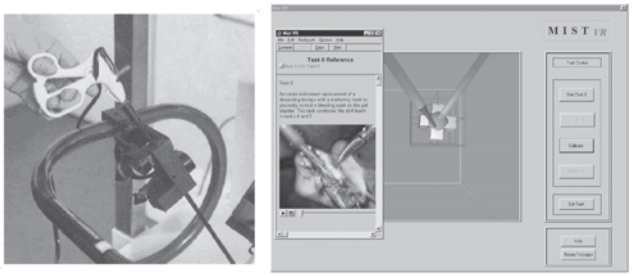

FIGURE 1: MIST-VR basic surgical skills simulator illustrating the image on the simulator screen, and the input handles for tracking motion.

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

FIGURE 3: Zeus surgical robotic system.

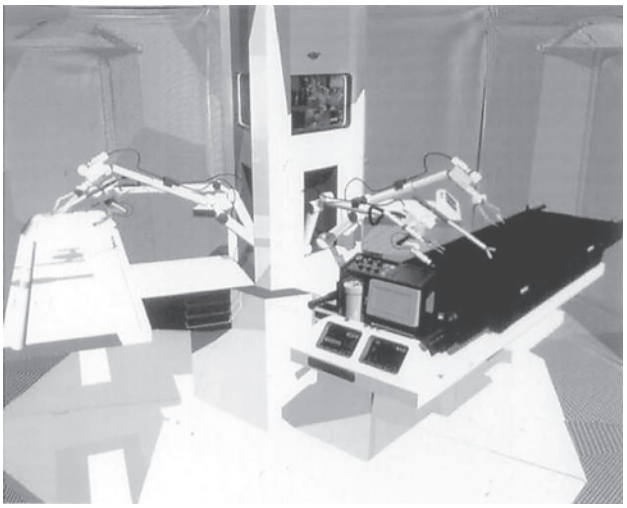

FIGURE 4: OR of the future – concept drawing from Integrated Medical Systems

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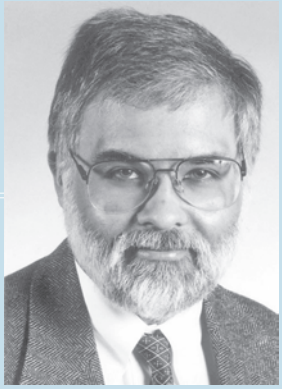

- **SURGICAL TELEMEDICINE: PROJECTION OF SURGICAL EXPERTISE IN THE WWAMI REGION**
  A COLLABORATIVE RESEARCH PROJECT WITH UW TELEMEDICINE, UW SURGERY AND BIOROBOTICS, UWMC, AND HARBOR SURGICAL ASSOCIATES, AND GRAY'S HARBOR COMMUNITY HOSPITAL IN ABERDEEN, WA.

Telemedicine 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.

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.

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.

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:

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™) to be cleared by the FDA in the newly created category of Robotic Telemedicine Devices. 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.

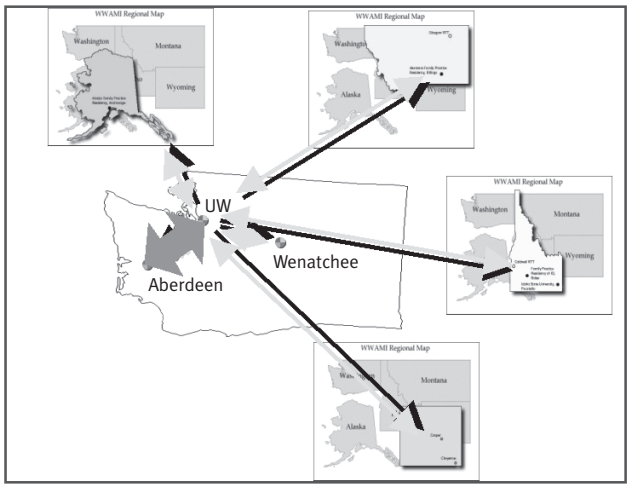

FIGURE 1: Proposed Phased Surgical Telemedicine Consortium

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 incorporated 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.

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 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.

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.

Identification of Genes Responsible for Immunologic Tolerance

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

Immunologic Tolerance in a Large Animal Model

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

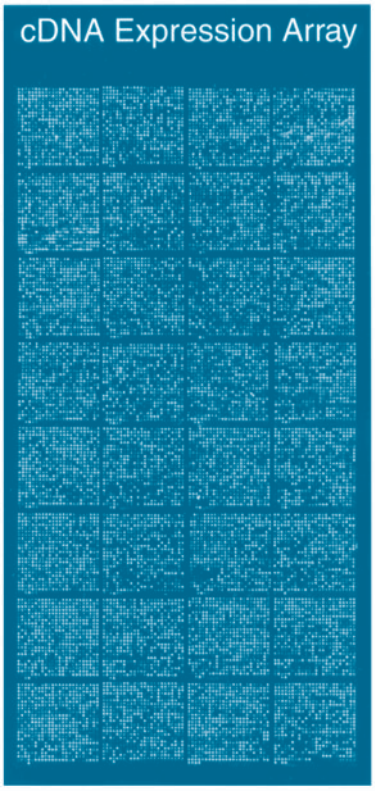

FIGURE 1

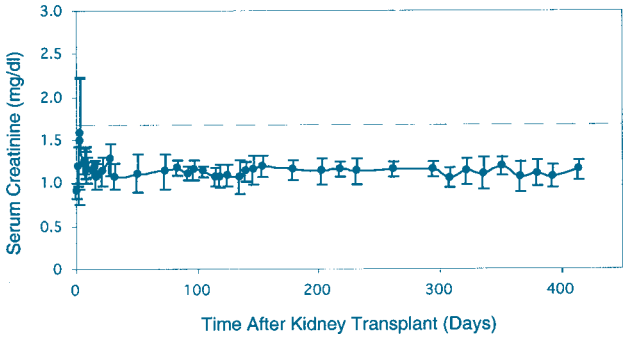

FIGURE 2

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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- ADVANCING THE CLINICAL SCIENCE OF SURGERY USING OUTCOMES RESEARCH TOOLS

FUNDING:
Purdue Pharma
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Over the last decade "outcomes" research became a catch phrase for healthcare administrators, providers and researchers. Outcomes research means different things to different people, however. For some it is viewed as a way to provide more services for fewer dollars; for others it means finding ways to regulate physician practice 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.

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 community standard, who are the outliers (both good and bad) and

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.*

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

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

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 community standard, who are the outliers (both good and bad) and what techniques work out of the research environment. In the absence of a tracking system for outcomes we often rely on estimates derived from randomized trials (which for most general surgical procedures have not been completed) or administrative data. Only by understanding the real level of risk can we determine the opportunities for improvement in the system.

Research I've been involved with during the last year has addressed this issue of community-level risk in commonly performed general surgical procedures by using administrative data. Determining population-level risk requires the analysis of large databases. For example, in evaluating rates of misdiagnosis in appendectomy we studied 80,000 patient records and found that the rate of misdiagnosis in appendicitis has not

improved in the past 13 years (~15% overall and ~25% in women of reproductive age) despite the growing availability of CT scanning. We studied over 30,000 patients undergoing cholecystectomy to describe the rates of major common bile duct (CBD) injury over time and found that rates of this outcome (0.025%) have not significantly improved with time.

To study outcomes from antireflux procedures we studied over 86,000 records 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. This population–based research is both the first step in assessing what needs to be fixed in the system and the last step in determining whether changes have had the expected effect.

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.

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

The next step is figuring out what avoidable factors contribute to the adverse outcome. 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 are important next steps 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

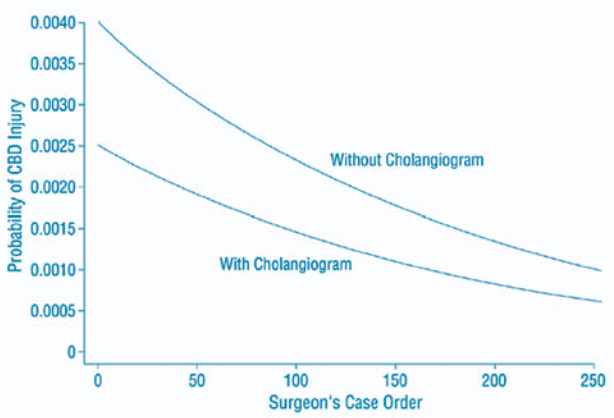

FIGURE 1: The predicted probability of CBD injury after laparoscopic cholecystectomy based on surgical experience and use of an intraoperative cholangiogram. As the level of surgical experience (case order) increases the predicted probability of CBD injury decreases. Failure to use an intraoperative cholangiogram is negatively associated with CBD injury at all levels of surgical experience but this effect is best demonstrated in the surgeon's first 20 procedures.

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 1.) This may be a more effective way of "informing" the informed consent process.

We recently completed a similar analysis looking at early experience and its association with adverse outcomes after anti-reflux surgery. We found the same relationship of surgeon experience to almost all adverse outcomes. Another study using statewide data from New York, Florida, New Jersey and South Carolina will address whether states that have effectively concentrated

antireflux procedures in the hands of the most experienced surgeons have better overall outcomes than states with more even distribution of procedures/surgeon.

In looking at the association of CT scan use on the adverse outcome of misdiagnosis in appendicitis we are currently studying over 5000 patients undergoing appendectomy at Group Health Cooperative over the last two decades to determine the relationship between CT scan use and misdiagnosis. This should help address the question, why has misdiagnosis remained stable all these years if we have such a reportedly good diagnostic test?

For incisional hernia surgery we have tried to link better outcomes (shortened length of hospitalization and lower reoperative rates) to recent developments in laparoscopic technology. We would like to determine whether or not laparoscopy should replace open hernia as the procedure of choice. Using available data we have been unable to identify differences in outcome when comparing the pre and post-laparoscopic eras. This information is helpful because it demonstrates the need for a randomized trial in assessing the importance of this new technology.

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

Once we have quantified the problem and determined the avoidable factors that influence these outcomes we can try to imagine what the practice of clinical surgery would be like with these factors controlled. For example, a recently completed cost and decision analysis demonstrated that if routine cholangiograms were required, the cost per CBD injury avoided would range between $50-86,000. The incremental cost per operation of adding the cholangiogram would be $100. When considering the overwhelming costs (both system wide and medicolegal) of a CBD injury, this may be considered a cost effective intervention. Another example is a cost analysis showing that nationwide, nearly $740 million is spent each year on misdiagnosed appendicitis. Modeling potential ways to improve care is also being applied in a theoretical decision and cost analysis for routine CT scanning of patients with presumed appendicitis and teleproctoring in antireflux surgery.

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

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 have reviewed the ~200,000 patients over age 65 undergoing cholecystectomy per year (n=1.6 million patients) to determine if our findings regarding cholangiogram and CBD injury are identified in this population. If so, these administrators would like 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.

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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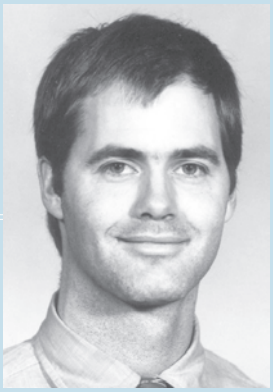

• THE SWALLOWING CENTER AT THE UNIVERSITY OF WASHINGTON

AWARD:
Society for Surgery of the Alimentary Tract
• Ross Resident/Fellow Research Award, 2001

The Department of Surgery, along with the University of Washington Medical Center, established the Swallowing Center in 1995. Under the direction of Dr. Carlos Pellegrini the center has grown to see over a 1000 patients a year, making it one of the largest esophageal labs in the country. It was established as a clinical lab, but generates a large amount of information for research purposes. Investigators in the departments of Gastroenterology, Pulmonology, and Otolaryngology have participated with us in many research endeavors.

The Swallowing Center went through major changes and advancements in 2002. We installed state of the art equipment for esophageal testing. We are not only able to perform standard esophageal function testing more precisely and efficiently, but we have added the ability to measure impedance: a novel, state of the art technique. We are one of a few centers in the world studying the measurement of impedance, which may revolutionize out ability to study esophageal function. We expect this to benefit not only our patients, but also eventually all patients with disorders of the esophagus.

We are one of a few centers in the world studying the measurement of impedance, which may revolutionize out ability to study esophageal function.

GERD and Airway Disease

One of the more perplexing medical problems today is the association between gastroesophageal reflux disease (GERD) and respiratory diseases (asthma, cough, laryngitis, etc.). It clearly exists and is very common, but we still lack the ability to prove its cause and effect relationship in individual patients. We have one of the world's largest experiences measuring acid in the pharynx with specialized pH monitoring. We have demonstrated that pharyngeal reflux (PR) provides evidence for microaspiration, and is a good predictor of airway symptom response to medical and surgical antireflux therapy. Most physicians rely on presence of typical symptoms (e.g. heartburn) and/or standard esophageal function testing (manometry and 24-pH monitoring). PR is rarely measured directly.

We recently studied a group of 518 patients with suspected reflux induced airway disease, of which 181 patients were PR + and 337 were PR -. Using established normal values of acid exposure at multiple levels of the esophagus, 49 (27%) PR+ patients had normal amounts of esophageal acid exposure. Therefore, relying on typical GERD symptoms and standard diagnostic testing may fail to identify patients with extraesophageal reflux. We have concluded that pharyngeal pH monitoring should be considered for patients with suspected reflux induced airway disease.

Repair of Paraesophageal Hernias with Small Intestinal Submucosa (SIS)

Laparoscopic techniques have been applied with increasing frequency to the repair of paraesophageal hernias, with the benefits to the patient being less pain,

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

We performed an initial pilot study that confirmed its safety with very few recurrences. We have developed a multi-center clinical trial with Oregon Health Sciences, Legacy Health System, University of California at San Francisco, and Washington University. This trial is now underway, and should answer whether this product has a positive impact on this difficult disease.

Extended Gastric Myotomy for Achalasia

Achalasia is a disease that affects the esophagus, and is characterized by a lack of peristalsis and a lower esophageal sphincter (LES) that does not relax. The result is significant difficulty swallowing. Surgical treatment entails cutting the muscle (myotomy) of the lower esophageal sphincter. There is, however, no agreement on how far the myotomy should extend onto the stomach to treat achalasia. Since its effectiveness likely relies on obliterating the (LES), we have investigated extending our myotomy further on the stomach.

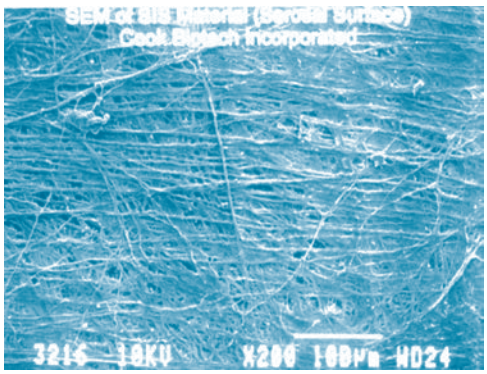

FIGURE 1: Small intestinal submucosa (SIS)

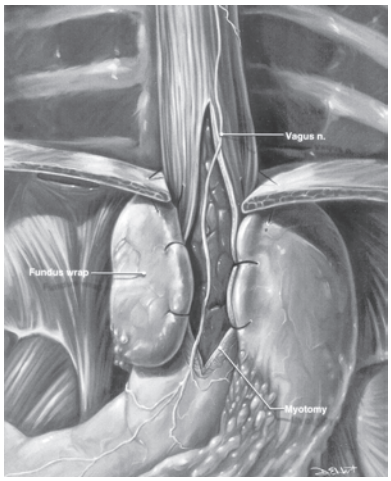

FIGURE 2: Extended Esophageal Myotomy with Toupet Fundoplication

Concurrently we converted from a Dor (anterior) to Toupet (posterior) fundoplication to prevent reflux. Postoperatively the LES pressure was significantly lower after extended myotomy and Toupet (9.5 vs. 15.8 mmHg). Dysphagia was both less frequent (1.1 vs. 2.1), and less severe (VAS 3.2 vs. 5.3) after this approach as well. Moreover, there was no significant difference in the amount of gastroesophageal reflux. We concluded that our most recent approach employing an extended gastric myotomy (3cm) more effectively disrupts the LES, improving of surgical therapy for achalasia.

Esophageal Impedance

As one of a few centers with the ability to measure esophageal impedance we will begin investigating the usefulness of this new approach in a variety of esophageal diseases.

Nonacid Pharyngeal Reflux in Normal Subjects

In order to use this tool as an indicator of reflux induced airway disease we need to know what is abnormal AND normal. We will simultaneously perform pH monitoring, for which we have standard values, further contributing to our knowledge of esophageal physiology.

Respiratory Symptoms and GERD

The measurement of acid reflux into the esophagus is currently the gold standard for the confirmation and quantification of gastroesophageal reflux disease (GERD). We have recently used the measurement of pharyngeal acid reflux to identify patients with reflux induced airway symptoms. This appears to be a better

predictor of response to therapy than standard pH monitoring. Still some patients without pharyngeal reflux respond to medical and surgical therapy and some patients in whom we have demonstrated pharyngeal reflux do not. It is possible that reflux of contents from the stomach that are not acid, or that have a neutral or alkaline pH, may cause airway injury, yet esophageal pH monitoring is not capable of identifying these patients.

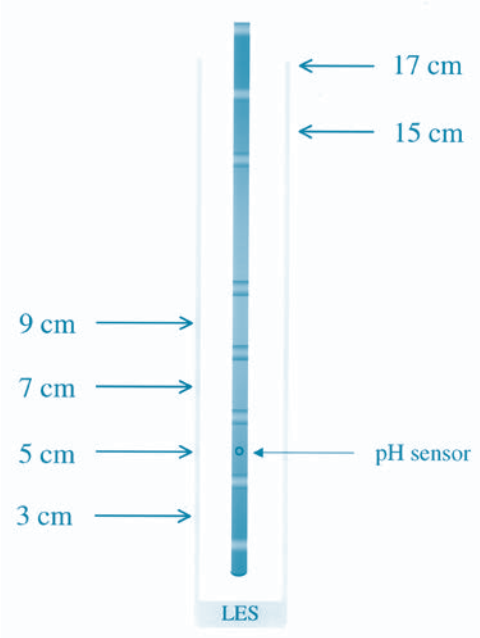

FIGURE 3: Impedance Catheter: Multiple electrodes measure electrical resistance in real time. Refluxate or swallowed material can be detected and measured at different levels of the esophagus.

The measurement of esophageal impedance detects a change in intra-luminal content. Gastroesophageal reflux can be detected regardless of the pH in the refluxate. Nonacid reflux into the airway may explain why some patients with airway symptoms and injury have little or no acid in the pharynx, but respond to antireflux operations. If detection of reflux by measuring impedance in the upper esophagus and pharynx is possible and reliable, then it may be a better predictor of which patients with respiratory symptoms will respond to antireflux operations.

Ineffective Peristalsis and Total Fundoplication

Patients with gastroesophageal reflux disease (GERD) and ineffective peristalsis present a challenge. Most operations that restore cardioesophageal competence increase the high-pressure zone and potentially the resistance to flow through the gastroesophageal junction. Until recently it was accepted that performing a total fundoplication in the presence of ineffective peristalsis would lead to dysphagia. Currently, the effectiveness of esophageal peristalsis is determined indirectly by manometry. This method does not record bolus transfer, a more direct measurement, which can be done with impedance calculations. If simultaneous manometry and impedance are performed, before and after Nissen fundoplication, it may improve our ability to detect patients with truly ineffective peristalsis. This may identify a subset of patients with an increased likelihood of developing dysphagia after Nissen fundoplication.

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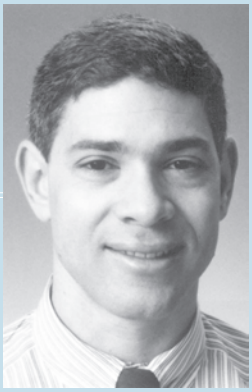


- SURGICAL ONCOLOGY AT VAPSHCS: OVERVIEW
- NORTHERN ALLIANCE CANCER CENTER AT VA PUGET SOUND HEALTH CARE SYSTEM
- OUTCOME STUDIES IN PANCREATIC CANCER
- CLINICAL STUDIES IN COLORECTAL CANCER
- COLORECTAL CANCER CARE AMONG VULNERABLE PATIENT GROUPS

FUNDING:
Department of Veterans Affairs New Clinical Initiatives Program
Pharmacia Upjohn
National Institutes of Health
National Cancer Institute

Surgical Oncology at VAPSHCS: Overview

The surgical oncology program at the VA Puget Sound Health Care System is focused on the delivery of optimal multidisciplinary cancer care to Veterans residing throughout the Pacific Northwest. The primary research goal of the program is to develop the use of telemedicine and other emerging technologies to facilitate patient access to multidisciplinary cancer care. Additional research projects are designed to study outcomes and quality measures in the multimodality treatment of gastrointestinal cancer, as well as racial and ethnic disparities in cancer care.

The telemedical outreach service is designed to allow patients with cancer to undergo initial evaluation at their closest VA facility. Local providers will present the relevant clinical information to a multidisciplinary tumor board at the Seattle VA. A comprehensive evaluation and treatment plan is developed. Patients either come to Seattle for further treatment, or they are treated at their home facility if feasible. We believe This system is designed to optimize patient care by providing consistent multidisciplinary consultation and eliminate unnecessary appointment scheduling and patient travel.

The telemedicine network also serves to link the facilities for educational purposes. A monthly

The telemedical outreach service is designed to allow patients with cancer to undergo initial evaluation at their closest VA facility.

Northern Alliance Cancer Center at VA Puget Sound Health Care System

The Northern Alliance Cancer Center at VA Puget Sound Health Care System is funded by the VA New Clinical Initiatives Program. This funding has allowed us to develop a regional telemedical cancer center that provides clinical oncology services to the veterans of the Pacific Northwest by utilizing telemedical technology. The center is located at VA Puget Sound Health Care System, Seattle Division. Contact is maintained with all satellite facilities via a telemedicine network.

multidisciplinary oncology conference provides a forum for continuing medical education for providers at all participating facilities. Providers currently receive CME credit for their participation.

Since the inception of the cancer center, 196 patients have received telemedical oncology consultation. Patients presented to the telemedicine tumor board had diagnoses that included the entire clinical spectrum of malignant disease. Our results indicate that referring providers have a high level of satisfaction with the use of telemedicine for multidisciplinary cancer

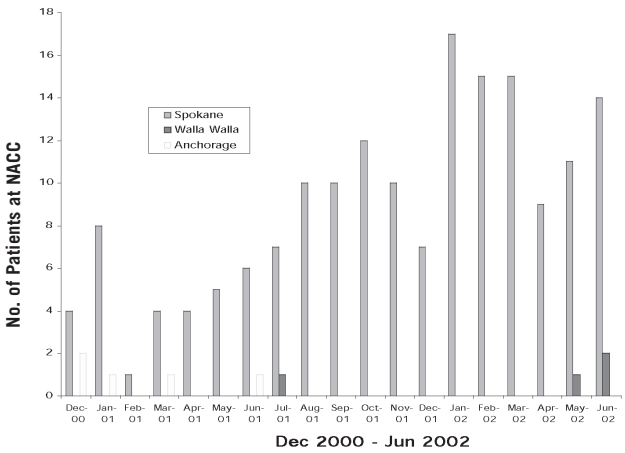
TABLE 1. Referring Provider Satisfaction with Telemedical Cancer Services

PERCEIVED BENEFITS	IMPROVES OR STRONGLY IMPROVES (%)	NEUTRAL (%)
Reduces Delays	100	0
Coordination of Care	83	17
Access to Multidisciplinary Care	100	0
Post Treatment Followup	100	0
Overall Quality of Care	100	0

evaluation and treatment planning (Table 1). Perhaps the most important indicator of the utility of this program is that the number of participating health care providers has continued to expand over time. (Figure 1)

Outcome Studies in Pancreatic Cancer

The National Surgical Quality Improvement Program (NSQIP) is a national VA database that prospectively collects data from all VA facilities regarding perioperative clinical variables and postoperative outcomes. This dataset serves as a rich resource for VA researchers who are investigating surgical outcomes. We have used this data to analyze risk factors for perioperative morbidity and mortality related to pancreaticoduodenectomy for periampullary cancer. Using logistic regression modeling, we have found that the most important predictive factors for postoperative death are low serum albumin, increasing American Society of Anesthesiologists (ASA) classification, preoperative bilirubin > 20 mg/dl, and lengthy operative procedures (Table 2). We found that the placement of a preoperative biliary stent did not increase the risk of mortality.



	DEC 2000 – JUN 2001	JUL 2001 – DEC 2001	JAN 2002 – JUN 2002
Mean	5.29	9.5	14
Std. Dev.	2.5	1.76	2.97

FIGURE 1: Number of healthcare providers participating each month in telemedical cancer care.

TABLE 2. Pancreaticoduodenectomy 30 day mortality: Logistic Regression Analysis Results

VARIABLE	PARAMETER ESTIMATE	P VALUE	ODDS RATIO
Intercept	-3.28	0.010	
Preoperative Albumin(g/dl)	-0.066	0.019	0.52
ASA* Class	0.684	0.029	1.98
Preoperative Bilirubin >20 mg/dl	1.13	0.010	3.09
Operative Time	0.18	0.003	1.207

C-index=0.734
R^2=0.07
Hosmer-Lemeshow goodness of fit = 9.66 (p=0.290)

Clinical Studies in Colorectal Cancer

We have established a prospective colorectal database in the department of surgery at the VA Puget Sound Health Care System. This data base is designed to serve as a resource to facilitate clinical as well as translational studies.

A study of the utility of preoperative CT scanning in patients undergoing surgery for colorectal cancer demonstrates that the clinical management of this group of patients was affected in 38% of the cases by the preoperative scan. Management was altered to include management of metastatic disease, treatment of coincident pathology, and, in some cases, *en bloc* resection of adjacent organs.

Colorectal Cancer Care Among Vulnerable Patient Groups

Our research group, which includes members from surgery, family medicine, medicine, radiation oncology, and health services research, has received funding to study disparities in colorectal cancer treatment among vulnerable patient populations. The analytic database for this study will be constructed by merging data from the SEER (Surveillance, Epidemiology, End Results) registry with medicare claims data.

The primary research question will focus on the documentation of usage of stage appropriate adjuvant therapy for colorectal cancer among ethnically and geographically vulnerable patient groups. If we document differential usage of adjuvant therapy among these groups of patients, the study will examine processes of care which may contribute to this disparity.

A supplemental study has also been funded which will focus specifically on the quality of surgical care for colorectal cancer among different patient groups. This study aims to use the SEER/Medicare database to identify differences in perioperative morbidity and mortality following colon and rectal resection. The goal of this investigation is to identify either provider or system features which may compromise the quality of surgical care that is delivered to rural or minority patient groups. Preliminary results from this effort demonstrate that it is possible to identify and track a broad range of surgical complications following colon and rectal surgery.

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Lorrie Langdale, M.D.

• MECHANISMS OF LIVER ISCHEMIA-REPERFUSION INJURY

FUNDING:
VA Merit Review Grant - submitted for funding

Hepatic failure following liver ischemia and reperfusion (IR) remains a challenge to all surgeons caring for trauma patients, performing liver resections, or liver transplantation. Unlike other organ systems which may be supported by aggressive critical care, the development of hepatic failure is uniformly fatal without liver transplantation. Strategies to disrupt isolated steps in the complex inflammatory cascade which mediates IR injury have met with variable degrees of laboratory and clinical success. Furthering our understanding of the mechanisms which normally regulate the components of the acute inflammatory response to injury is essential to effective control of injury, initiation of healing and a return to homeostasis.

Hepatic Ischemia-Reperfusion: Evolution of an Injury

The liver is particularly vulnerable to the effects of ischemia-reperfusion because of its "open architecture". The early phase of IR injury reflects the direct effects of local toxic reactive oxygen products that injure tissue and mediate neutrophil activation and chemoattraction. Hypoxia/reoxia also initiates the recruitment of leukocytes to the specific site of injury through the actions of a spectrum of cytokines and chemotactic proteins expressed by Kupffer cells and hepatocytes. Activated 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 becoming dominant between 8 and 24 hr of reperfusion.

Effects of Immunomodulation on Acute Injury

Recent evidence suggests that immunomodulating mediators, usually associated with chronic inflammatory states, play a role in the development and resolution of acute inflammation. Interferon gamma (IFNγ), a potent cytokine produced by T helper lymphocytes (Th1) and macrophages in response to stress and injury, is an accepted enhancer of cellular function. IFNγ modulates antigen presentation, cell differentiation, cytotoxicity, cytokine production, cellular adhesion, and oxidative metabolism as a result of its effects on leukocytes and endothelial cells. In laboratory studies, treatment with IFNγ improves survival after hemorrhagic shock or overwhelming sepsis through restoration of the animals' capacity to express major histocompatibility complex (MHC) class II (Ia) antigen, an important component of bacterial recognition and clearance. These findings have been further substantiated by clinical trials in which septic patients, whose monocytes have reduced MHC II antigen expression, were treated with IFNγ and showed improved survival with restoration of host defenses. Thus, the impaired response to injury and immunocompromise induced by hemorrhagic shock or systemic infection may be reversed with the administration of exogenous IFNγ.

In contrast to its beneficial immunomodulatory properties in the face of immunosuppression, pretreatment of normal, immunocompetent animals with IFNγ has been shown to potentiate the pathophysiologic

Expanding our understanding of normal negative control mechanisms of inflammation will potentially open new therapeutic strategies to the management of acute liver injury.

effects of subclinical doses of endotoxin. An exaggerated activation of the alveolar macrophage inflammatory response, induced in part by IFNγ, is postulated to be responsible for the increased mortality that accompanies an otherwise easily tolerated endotoxin challenge. In vitro data supports this "priming" hypothesis. IFNγ alone has a minimal but dose-dependent effect on isolated alveolar macrophage production of cytokines such as tumor necrosis factor (TNFα). However, pre-treatment with IFNγ enhances secretion of TNFα after stimulation with small doses of endotoxin (LPS)[8] Thus, as in the clinical arena, host immunocompetence as well as the sequence of events which precede injury (e.g. immune pre-conditioning or synergistic stimulation with endotoxin) are critical to the effects of IFNγ on the evolution of an acute inflammatory injury.

IFNγ may play a significant role in modulating the severity of acute liver injury. Mizuhara et al induced acute, liver specific, T cell activation-associated inflammatory liver injury by injecting two strains of mice with concavalin A (Con A). C57BL/6 mice, which express low levels of IFNγ in association with high levels of IFNγ-inducible nitric oxide synthase (iNOS) in response to Con A, developed a severe inflammatory hepatitis. Similarly treated BALB/c mice produced higher levels of IFNγ but less intrahepatic iNOS, and exhibited a delayed, more limited injury. Neutralization of IFNγ with monoclonal antibody completely prevented the effects of Con A. These data raise the question of a dose dependent immunomodulating role for IFNγ in the evolution of acute inflammatory liver injury.

We have previously shown that immunomodulation with IFNγ is protective in a model of liver IR when given in a dose known to restore. High dose IFNγ pre-treatment of normal, immunocompetent rabbits blunts progression of liver IR injury, as evidenced by transaminase concentrations, while lower dose IFNγ pre-treatment or saline control is associated with a significantly increased cellular injury 24 hr after liver IR (Fig 1: ALT data, mean ± SE shown). Histologic injury, characterized by midzonal and centrolobular necrosis, does not progress beyond the first phase of neutrophil-independent, oxygen free radical mediated injury when animals are pre-treated with immunomodulating (high) doses of IFNγ. Late neutrophil infiltration is virtually eliminated. Our data have been corroborated in a rat model of liver IR by other investigators and extended to secondary lung injury, which is also ameliorated. Proinflammatory cytokine and chemokine expression in both liver and lung is markedly attenuated by high dose IFNγ pre-treatment. These data strongly suggest a role for IFNγ in the immunomodulation of acute IR injury.

The mechanism(s) by which IFNγ influences the progression of acute inflammation, and thus the response to liver injury, have not been fully elucidated but may include direct and indirect effects on neutrophils, Kupffer cells, hepatocytes and endothelial cells. In order to exert regulatory effects on cells, however, the cytokine must first interact with a specific cell membrane receptor. The active IFNγ receptor, for example, is characterized by separate binding (α) and signaling (β) ligands, and the receptor complex is constitutively expressed on many cell types. In liver, hepatocyte expression of IFNγ receptor requires de novo protein production and occurs only in the face of acute or chronic inflammation. Once engaged with its cellular membrane receptor, IFNγ, like other cytokines, initiates a cascade of intra-cellular signaling events that inexorably link the inflammatory process with its control.

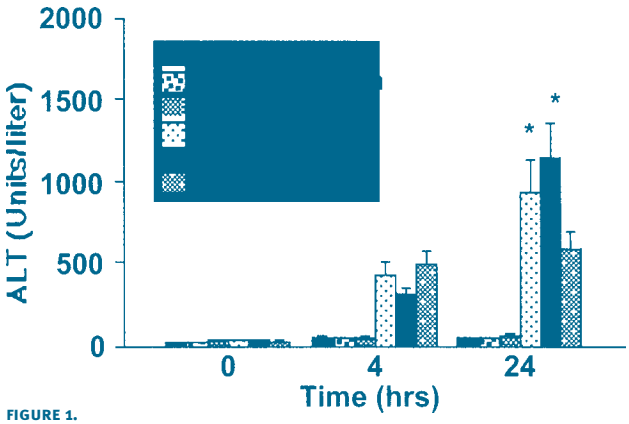

FIGURE 1.

JAK-STAT Cell Signaling Pathway and Suppressors of Cytokine Signaling (SOCS proteins)

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

complex allows tyrosine phosphorylation of the STAT with formation of an activated dimer or tetramer. The STAT dimer/tetramer translocates to the nucleus and binds with a specific DNA sequence and/or other transcription factors to effect target gene transcription.

JAK/STAT and SOCS in Inflammation

A regulated response to injury requires both active inflammation, with the expression of pro-inflammatory cytokine and chemokine mediators and neutrophil activation and trafficking, and active inflammation control. In addition to effecting cytokine signaling, STAT-mediated cell signaling induces the expression of Suppressors of Cytokine Signaling (SOCS) proteins that serve as classic negative feedback mechanisms for cytokine expression (Fig 2). Numerous cytokines important to acute inflammation activate cells through JAK-STAT, including TNFα, IFNγ, 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 IFNγ 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.

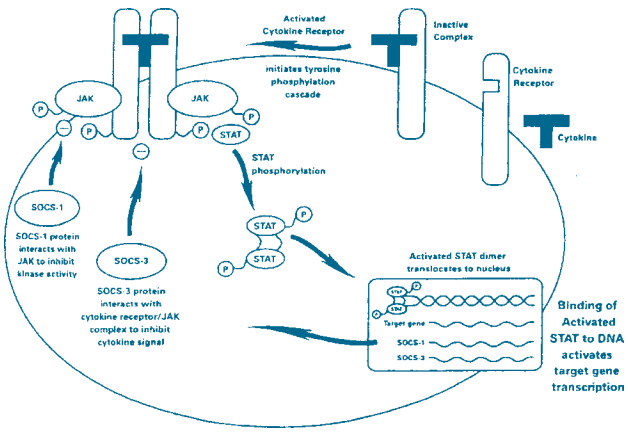

FIGURE 2.

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 IFNγ. Mice lacking both SOCS-1 and IFNγ however are viable and healthy. These data suggest that it is the loss of balance between the pro-inflammatory IFNγ 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.

The role of SOCS in ischemia-reperfusion has not been elucidated, but given our previous findings of protection from liver IR with high dose IFNγ treatment, early induction of negative regulatory mechanisms would potentially explain this phenomenon. Our current work is focusing on determining the role of JAK/STAT signaling and SOCS-mediated negative regulation on the evolution of liver injury severity and the effect of immunomodulation on injury resolution. Expanding our understanding of normal negative control mechanisms of inflammation will potentially open new therapeutic strategies to the management of acute liver injury.

Acute Primary Hepatic Injury and Secondary Endotoxin Injury: Tolerance versus Priming

The risk of multiple organ failure (MOF) has been postulated to significantly increase when pro-inflammatory cytokine and chemokine expression is sustained, as might be expected with serial inflammatory stimuli such as ischemia-reperfusion followed by a septic challenge. Since the liver serves as a primary clearance mechanism for circulating endotoxin, dysregulation of the hepatocellular response to LPS stimulation, as may occur after local or systemic ischemia-reperfusion, would likely contribute to the development of MOF with its attendant high mortality.

The normally functioning liver serves as an important barrier to an uncontrolled endotoxin-mediated inflammatory response. Normal intestinal mucosal turn-over and minor infectious events require the liver to regularly clear small amounts of endotoxin that collect in the portal vein after translocation of bacterial by-products from the gut. Hepatic ischemia followed by reperfusion might be expected to seriously alter the capacity of locally injured areas as well as any continuously perfused liver exposed to circulating inflammatory mediators to respond to a secondary endotoxic challenge.

Liver macrophages (Kupffer cells) are ideally situated to perform a pivotal role in the control or augmentation of primary and secondary host defense mechanisms. Studies comparing isolated Kupffer cells, cultured under normoxic or hypoxia/re-oxia conditions, have documented an augmented cellular responsiveness to stimulation with LPS, as manifested by de novo production of TNFα. Further, isolated Kupffer cells harvested within 4 hr of global hepatic ischemic-reperfusion express increased amounts of TNFα in response to subsequent in vitro LPS stimulation compared to cells harvested after IR alone. These data suggest that local ischemia-reperfusion primes the liver to elicit an exaggerated inflammatory response to early secondary endotoxin challenge.

When secondary LPS stimulation is delayed for at least 24 hr, however, animals subjected to hepatic ischemia followed by reperfusion show a relative "tolerance" to secondary injury, as manifested by decreased plasma cytokine levels and an abrogated remote pulmonary injury. Similarly, primary stimulation with LPS followed 24 hr later by a severe hepatic IR injury (90 min of ischemia) diminishes IR-associated pulmonary injury. Although pre-treatment with LPS augments TNFα serum levels after IR compared to IR alone, significantly less cytokine is expressed than with LPS stimulation alone. These findings suggest that, under certain conditions, the inflammatory response to an initial non-lethal injury may blunt rather than augment the severity of a secondary inflammatory response.

The mechanisms that differentiate the liver's response to endotoxic challenge and predispose to priming or tolerance after IR have not been elucidated. Since the same cytokines associated with IR are active in endotoxin-mediated injury, it is probable that priming and tolerance reflect the relative strength and duration of signaling through the JAK/STAT pathway as well as expression of negative regulatory mechanisms in control of the secondary insult. Using in vivo and in vitro liver slice culture models, we are working to elucidate the relationship between JAK/STAT signaling (STAT-1, STAT-3) and negative regulation (SOCS) in early priming or late tolerance to secondary LPS challenge after hepatic IR.

Summary of Significance

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

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• CLINICAL APPLICATION OF INTESTINAL MUCOSAL STEM CELL TRANSPLANTATION

FUNDING:
American College of Surgeons
• G.H.A. Clowes Research Career Development Award
VA Merit Review Grant

In the past two years, our laboratory has developed a novel method to transplant mucosal stem cells of the small intestine. This new methodology enables us to modify the mucosa of an intestinal segment. For example, we can now implant normal enterocytes into a host with mucosal dysfunction in order to correct the mucosal abnormality. We are using this principle currently in studies aimed at treating bile acid malabsorption. Beyond this narrower focus, successful stem cell transplantation provides us with a methodology to provide gene therapy using intestinal stem cells. Transplanted enterocytes can be used as production sites for certain proteins. They may act as therapeutic helper cells that synthesize peptides or proteins that the host is lacking, e.g. in the lumen of the gut to treat digestive disorders. They may also be used as a replacement for diseased cells. For example, an intact cystic fibrosis gene may be introduced into stem cells to prevent the intestinal symptoms of cystic fibrosis. In the future, transplanted stem cells might be used to secrete therapeutic peptide drugs into the bloodstream.

We are currently working on several projects that explore the possibilities of intestinal stem cell transplantation in two areas.

Generation of a "Neo- Ileum" for the Treatment of Bile Acid Malabsorption

Bile acids are the most important detergent substances in the body. They act as emulsifiers in the gut lumen and aid with fat absorption. After completing their task, over 90% of the bile acid molecules are reabsorbed in the terminal ileum by an active membrane transport process. They are then shuttled back to the liver via the portal vein and again excreted in the bile. This is called enterohepatic circulation of bile acids. The transport protein responsible for the ileal uptake process is called IBAT (i.e., ileal bile acid transporter). IBAT is expressed solely in the mucosa cells of the terminal ileum but not in the more proximal sections of the intestine. This has great clinical importance. Bile acid malabsorption is a major complication in patients undergoing ileal resection for Crohn's disease and in patients with ileal bladder reconstructions following bladder resections for cancer. Bile acid malabsorption leads to severe diarrhea and—in the long-term—to the formation of gallstones and kidney stones.

Our laboratory has studied intestinal bile acid transport for many years. Our research group has mapped the distribution of bile acid absorption in detail in different animal species as well as in humans for the first time, and studied the regulation of intestinal bile acid uptake.

Bile acid malabsorption is a major complication in patients undergoing ileal resection for Crohn's disease and in patients with ileal bladder reconstructions following bladder resections for cancer.

In recent years, we developed techniques to transplant ileal mucosa cells into the proximal jejunum. We prepared ileal stem cell clusters from neonatal donor intestines using gentle enzymatic digestions and gravity sedimentations. We tested different methods to strip the resident mucosa out of the recipient jejunal segment. Mechanical stripping did not yield uniform, reproducible results. Chemical stripping by perfusing with divalent ion chelators, however, was much more successful and resulted in good engraftment rates.

Following engraftment, a neo-ileal mucosa developed that resembled native ileal mucosa morphologically. Using reverse-transcription polymerase chain reactions and semiquantitative immunohistochemistry with anti-IBAT antibodies, we were able to demonstrate transcription of IBAT messenger-RNA and the expression on IBAT protein in this new epithelium. We were also able to detect active bile acid transport in this neo-mucosa, a feature that is normally unique to the terminal ileum.

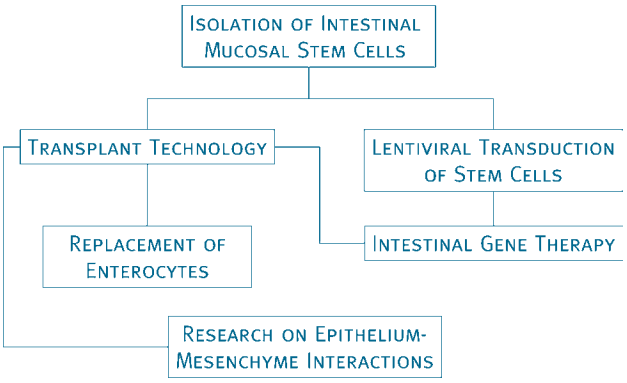
Our method of intestinal mucosal stem cell transplantation is now used experimentally to prevent bile acid malabsorption after ileal resection. In these studies, ileal stem cells are harvested from donor intestines and then transplanted in the proximal gut, i.e. the jejunum. Here they give rise to a "neo-ileal" mucosa. This neo-mucosa expresses ileal bile acid transport protein and is capable of active bile acid transport. Our current work focuses on the optimization of engraftment rates and on tests of the neo-ileal mucosa in different clinical settings.

Stem Cell Gene Therapy for Bilirubin - Glucuronosyltransferase Deficiency (Crigler-Najjar syndrome)

The aim of these studies is to demonstrate that intestinal stem cell gene therapy can lead to the production of therapeutic proteins to reverse a metabolically active enzymatic defect. Thus far, permanent incorporation and expression of a gene that is able to reverse an enzymatic defect has not been achieved in intestinal mucosa cells. Intestinal stem cell isolation and transplantation techniques that were recently developed in our laboratory are expected to provide a means of stable incorporation of such genes.

Crigler-Najjar syndrome is a congenital deficiency of bilirubin glucuronosyltransferase that causes jaundice caused by elevated blood levels of bilirubin. The disease can lead to severe brain damage in affected children. Crigler-Najjar syndrome has been repeatedly used as a model for gene therapy, since an animal model for this disease is available. So-called "Gunn" rats carry a congenital mutation of bilirubin glucuronosyltransferase and represent an animal model of Crigler-Najjar syndrome. They are unable to convert the bile pigment bilirubin into the less toxic bilirubin glucuronide that can be excreted by the body. As a result, these animals are permanently jaundiced.

The overall goal of this project is to transduce neonatal intestinal stem cells in vitro using a viral vector and graft the "repaired" cells into the gut of jaundiced Gunn rats. Intestinal stem cells are transduced using a special lentiviral vector that contains the intact bilirubin glucuronosyltransferase gene. The genetically altered cells are then transplanted back into segments of small intestine of other Gunn rats to produce a neo-mucosa. Following transplantation, the treated Gunn rats will undergo repeated measurements of their serum bilirubin to see if the implanted cells produce sufficient amounts of the bilirubin glucuronosyltransferase enzyme to reverse the jaundice.



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