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Research Conference

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Agenda

Introduction
Introduction by E. Christopher Ellison, MD, the Robert M. Zollinger Professor and Chairman of Surgery, Associate Vice President for Health Sciences, Vice Dean of Clinical Affairs, The Ohio State University College of Medicine

Moderator
Sessions Moderated by Ginny L. Bumgardner, MD, PhD, Professor of Surgery, Division of Transplantation, Associate Dean for Research Education, Director, Master’s of Medical Science Program, The Ohio State University College of Medicine

Oral Presentations, 8 to 9:15 a.m.
Inactivation of p16 in Patients with Esophageal Adenocarcinoma Portends Poor Prognosis in Esophageal Adenocarcinoma • Mary E. Dillhoff, MD • Discussant Peter Muscarella, II, MD ................................................ 3

Increased miR-21 Activity Enhances Invasion and Directly Targets TIMP3 Expression in Melanoma • Valerie P. Grignol, MD • Discussants Mark Bloomston, MD & Thomas Schmittgen, PhD .......... 4

Role of T Cell Subsets in Promoting Intestinal GVHD • Bryan Anthony • Discussant Charles Cook, MD ........ 5

Dicer Depletion in Adult Hearts Induces Mitochondrial Dysfunction, Oxidative Stress and Causes Rapid Loss of Cardiac Function • Jaideep Banerjee • Discussant Juan Crestanello, MD ................................. 6

Connective Tissue Growth Factor Production by Activated Pancreatic Stellate Cells in Mouse Alcoholic Chronic Pancreatitis • Alyssa Charrier • Discussant Ergun Kocak, MD .................................................................................. 7

Break and Poster Presentations, 9:15 to 10:45 a.m.
Acute and Chronic Response to Occluding Thrombi in a Thrombus-induced Sheep Model of Heart Failure • Chandrakala Aluganti ................................................................. 8

Lipid Mediators in Resolution of Wound Inflammation • Amitava Das .................................................................................................................. 9

Electrical Burns and the Development of Post Traumatic Stress Disorder and Associated Psychological Sequelae • Eric Luedke, MD .................................................................................. 10

Novel Reconstruction Technique of the Extrahepatic Biliary Trees with a Biosynthetic Absorbable Graft • Peter Nau, MD .................................................................................. 11

Glutathione Disulfide as a Cell Death Signal • Han-A Park ................................................................................. 12

Double-barreled Wet Colostomy in Patients Undergoing a Pelvic Exenteration Leaves Patients with a Single Stoma • Maureen E. Pons, MD ................................................................................. 13

Comparison of the Robotic-Assisted versus Video-Assisted versus Thoracotomy Approaches Toward Lung Cancer • Tyler C. Spata, MD ................................................................................. 14

The Impending Shortage and Implications of the Plastic Surgery Workforce • Jonathan C. Yang, MD ......... 15
Oral Presentations, 10:45 to 12 noon

Natural Killer Cells are Activated by IL-21 and Induce ADCC and IFN-Gamma Release in Pancreatic Cancer Cell Lines • Kristan D. Guenterberg, MD • Discussant Gregg Hadley, PhD ..................... 16

Surgical Management of Malignant Bowel Obstruction • Jon Henry, MD • Discussant Syed Husain, MD ..... 17

IL-4-dependent, IgG1-dominant Post-transplant Alloantibody Production is Regulated by CD8+ T Cells • Thomas A. Pham, MD • Discussant William Carson, III, MD .................................................. 18

The Role of Proton Leak, Membrane Potential and Reactive Oxygen Species Production in Ischemic Preconditioning • Ricardo Quarrie, MD • Discussant Sashwati Roy, PhD .................................................. 19

Development of a Novel Strain of Non-obese Diabetic (NOD) Mice with Targeted Disruption of the CD103 Gene • Elizabeth Stofko Barrie • Discussant Cameron Rink, PhD .................................................. 20
Inactivation of p16 in Patients with Esophageal Adenocarcinoma Potends Poor Prognosis in Esophageal Adenocarcinoma

Mary Dillhoff, MD, Wendy Frankel, MD, Sylvia Wojcik, MSc, Mark Bloomston, MD

Department of Surgery, The Ohio State University Medical Center, Columbus, Ohio

BACKGROUND: The p16 tumor supressor gene has been implicated as a critical step in the pathogenesis of esophageal adenocarcinoma. Epigenetic silencing and loss of genes such as p16 may determine tumor invasiveness, neovascularization, metastatic behavior and survival. We sought to determine the expression of p16 in resected esophageal adenocarcinomas as well as evaluate its correlation with survival.

METHODS: 60 resected esophageal cancer specimens were microdissected and tissue microarrays (TMA) created in duplicate. TMAs were also created for normal adjacent esophagus (N=50). In situ hybridization (ISH) was undertaken utilizing locked nucleic acid probes for p16. RNA U6 and scrambled RNA served as positive and negative control, respectively. ISH was scored as 0 (absent), 1+ (faint/focal expression), or 2+ (strong expression). Kaplan-Meier survival curves were constructed and compared by log-rank analysis.

RESULTS: p16 expression was demonstrated in 25 (42%) of esophageal cancers (1+ in 9, 2+ in 16) compared to 4/50 (8%, p<0.0001) in benign esophagus. P16 expression did not correlate with tumor size, differentiation, nodal status, or T stage. Loss of p16 expression was predictive of poorer outcome compared to strong expression of p16 in patients with node negative disease (p = 0.045, Figure)

![Figure 1](image)

CONCLUSIONS: Deletions and epigenetic changes leading to inactivation of p16 have been shown to occur in many malignancies including esophageal cancer. Loss of the tumor suppressor p16 leads to a significantly poorer survival in patients with node negative esophageal cancer. This may help stratify patients to receive further therapy and more aggressive treatment even in light of having no nodal disease.
Increased miR-21 Activity Enhances Invasion and Directly Targets TIMP3 Expression in Melanoma

Valeria P. Grignol, MD, Ene T. Fairchild, Volodomyr I. Karpa, Anthony Chan, Gregory B. Lesinski, William E. Carson, III, MD

Department of Surgery, Division of Surgical Oncology, The Ohio State University Medical Center, Columbus, Ohio

BACKGROUND: The median survival of patients with metastatic melanoma is about 6 months and currently there are no effective treatments for this condition. Understanding the pathogenesis of this disease is therefore critical to developing new therapeutic targets. MicroRNAs (miRs) are a class of small non-coding RNAs that exert their effects by binding to target mRNAs and inhibiting their translation into protein. miR-21 has previously been identified by our group as being over-expressed in cutaneous melanoma as compared to benign nevi. In this study we evaluated the effects of increased miR-21 expression on melanoma cell line behavior in vitro.

METHODS: Melanoma cell lines WM1552c, WM793b, MEL 39, and A375 were transfected with a pre-miR-21 expression vector and evaluated for proliferation, doubling time, migration, and invasion. Over-expression of mature miR-21 was validated in all transfection reactions by real-time PCR. Proliferation was measured using the 3-(4,5 Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) cell proliferation assay kit. Transfected cells were plated and evaluated for mitochondrial reduction of MTT after 72 hours. Doubling time was measured by counting total cell number on a hemocytometer at 72, 96, 120 and 144 hours after plating 5x10^3 transfected cells. Doubling time was determined to be equal to 1/slope of the line of best fit. Cell migration was determined using the scratch test. Transfected cell lines were plated and when they reached confluence a pipette tip was used to create a scratch in the monolayer. Percent wound closure was determined by calculating the ratio of the wound width at 18 hours to the width at 0 hours. Invasion was evaluated using a matrigel Boyden chamber invasion assay. Immunoblot analysis of miR-21-overexpressing cell lines was conducted for TIMP3, PDCD4, TM1 and PTEN. Luciferase assays were performed to determine targets of miR-21. Cell lines were co-transfected with the pre-miR-21 vector and a luciferase reporter construct under the control of the TIMP3 3’ untranslated region containing the miR-21 binding site (pmiR-Report-3’UTR-TIMP3-wt) or a luciferase reporter construct with a mutated miR-21 binding site (pmiR-Report-3’UTR-TIMP3-mut). As an additional control, the unmodified luciferase construct was employed (pmiR-Report). Luciferase activity was measured 24 hours post-transfection.

RESULTS: miR-21 over-expression in human melanoma cell lines WM1552c, WM793b, MEL 39 and A375 did not affect proliferation or doubling time. Migration at 18 hours was not different between controls and the pre-miR-21-transfected cell lines except for WM1552c (which was established from a primary superficial spreading melanoma). miR-21 over-expression led to a statistically significant increase in invasion potential between sham and pre-miR-21-transfected cell lines WM1552c, A375 and MEL 39 (p <0.05). Several mRNA targets of miR-21 have been identified. Tissue inhibitor of metalloproteinase-3 (TIMP3) has been identified as a putative target of miR21. Immunoblots of cell lysates 24 hours post-transfection with pre-miR21 revealed a decrease in TIMP3 protein compared to controls. Reduced expression of TIMP3 was achieved by siRNA knockdown and this manipulation significantly enhanced invasion of melanoma cell lines through matrigel, mimicking the effects of miR-21 over-expression in the cell lines. To determine if miR-21 repression of TIMP3 expression was mediated through direct binding of miR-21 to the 3’UTR of TIMP3, we obtained luciferase constructs under the control of wild-type TIMP3 3’UTR or a TIMP3 3’UTR mutant, where the miR-21 binding sites were mutated. Wild-type luciferase 3’UTR empty vector was used as a control. A 25% reduction in luciferase activity was seen in cells co-transfected with pre-miR-21 and TIMP3 3’UTR as compared to sham transfected cells or those expressing the control constructs (p < 0.05).

CONCLUSION: Increased expression of miR-21 enhances the invasive potential of melanoma cell lines and appears to mediate these effects through the inhibition of TIMP3. Efforts to target miR21 and inhibits its activity may result in a less invasive phenotype.

* Dr. Grignol is a second year resident in the Wright State University General Surgery program
Role of T Cell Subsets in Promoting Intestinal GVHD

Bryan Anthony, Jiao-Jing Wang, Alice A. Gaughan, Gregg A. Hadley, PhD

Comprehensive Transplant Center, The Ohio State University Medical Center, Columbus, Ohio

INTRODUCTION: Intestinal graft-versus-host disease (iGVHD) is a major obstacle to broader use of bone marrow transplantation (BMT) as a curative therapy for malignancy and genetic disorders. iGVHD is mediated by donor T cell contaminants carried in the bone marrow graft directed against host histocompatibility antigens expressed in the host gut. However, the relative contributions of CD4 vs. CD8 T cell subsets to this process remains poorly defined. Previous studies in this lab have shown that gut homing CD8 T cells express the integrin, CD103 during iGVHD. The ligand for CD103, E-cadherin, is highly expressed on intestinal epithelial cells, thus linking CD103 to the pathogenesis of GVHD. Our previous studies in an MHC-disparate mouse BMT model indicate that CD103 is critical for gut accumulation of T cells and development of iGVHD. The goal of the present study was to define the role of the CD103 pathway in promoting iGVHD in the more clinically relevant MHC-matched scenario where both CD4 and CD8 T cells contribute to the process.

METHODS: To assess the role of CD103 in MHC-matched transplant, lethally-irradiated BALB.B (H-2b) hosts were adoptively transferred with bone marrow and unseparated splenocytes from B6-WT or B6-CD103-/- donors (H-2b); cumulative disease indices and survival curves of mice in the two groups were then compared. For MHC-mismatch transplant, lethally-irradiated BALB/c hosts (H-2k) were transferred with cells from fully allogeneic B6 donors.

RESULTS: The initial goal of the present study was to assess the role of CD103 in promoting iGVHD following MHC-matched BMT, the most common clinical scenario. Our data showed no difference in the ability of WT or CD103-/- splenocytes to cause acute GVHD mortality in this transplant scenario. However, depletion of CD4 T cells on day 6 post transplant completely prevented acute GVHD mortality, despite a robust CD8 T cell response on day 6 post transplant. Although we cannot definitively rule out a role for acute CD8 T cell mediated mortality, these data are consistent with the hypothesis that acute gut injury and mortality elicited following BMT are mediated by CD4 T cells rather than CD8 T cells as is commonly assumed. Consistent with this hypothesis, CD4 depletion also prevented acute mortality following MHC-mismatched transplant. FACS analyses of long-surviving hosts in this model revealed that T cells infiltrating the host gut epithelium were initially CD103- but progressively acquired CD103 expression concomitant with progressive (subclinical) epithelial injury.

CONCLUSIONS: These data indicate that CD103 is preferentially expressed by CD8 T cells at late post-transplant intervals, and that effective blockade of GVHD pathology will likely require combined blockade of the CD4 and CD103 pathways.
**Dicer Depletion in Adult Hearts Induces Mitochondrial Dysfunction, Oxidative Stress and Causes Rapid Loss of Cardiac Function**

Jaideep Banerjee, Sashwati Roy, PhD, Surya C. Genyawali, Savita Khanna, PhD, Chandan K. Sen, PhD

Department of Surgery, The Ohio State University Medical Center, Columbus, Ohio

**INTRODUCTION:** *Dicer*, an RNase III endonuclease, plays a key role in the processing of miRNA into their functional mature form. A number of recent reports point towards a central role of miRNA in cardiac development and function. A decrease in *Dicer* is also a newly reported feature in heart failure patients with certain forms of cardiomyopathy. However, the short-term effects of dicer deficiency on the function of the adult heart and underlying mechanisms remain to be elucidated. Oxidative stress and dysregulated mitochondrial function are known to play a major role in acute cardiac pathologies.

**HYPOTHESIS:** Arrest of miRNA maturation and change in miRNA levels upon cardiac-specific *Dicer* ablation in adult animals result in rapid loss of cardiac function caused by mitochondrial dysfunction and oxidative stress.

**METHODS:** Mice homozygous for *Dicer*-floxed alleles (*Dicer<sup>f/f</sup>*) and transgenic Myh6-cre/Esr1 mice were crossed to generate double-transgenic (Myh6-cre/Esr1-*Dicer<sup>f/f</sup>*) mice. At 8 weeks of age Myh6-cre/Esr1-*Dicer<sup>f/f</sup>* mice were treated with vehicle or tamoxifen (20 mg/kg per day) daily intra-peritoneal injections for five consecutive days. To determine the acute functional consequences of *Dicer* depletion, heart function was determined using gated cardiac MRI and echocardiography. Myocardial miRNA profiling was performed using a bead array system. Candidate miRNAs were verified using QPCR. Mitochondrial function was assessed by oxygraph measurement of respiratory control ratio (RCR). Mitochondrial depolarization was measured by TMRM and JC-1 assays. Tissue redox status was assessed by EPR studies.

**RESULTS:** Tamoxifen injection for 5 days effectively depleted Dicer protein in myocardium. miRNA array data show significant (p<0.05, n=3) change in the levels of a number of miRNAs including miR-1, miR-133 a&b, miR-15b and miR-142-3p. A significant (p<0.05, n=5) decrease in ejection fraction (32±9%), stroke volume (23±7%) and cardiac output (28.3±6%) was noted in Dicer<sup>-/-</sup> compared to Dicer<sup>+/+</sup> mice. Studies with isolated mitochondria showed mitochondrial dysfunction indicated by a significant decrease in RCR (p<0.05, n=4). Among the miRNAs that showed significant variation, miR-15b is predicted by different algorithms to be targeting a number of proteins involved in mitochondrial and cardioprotective functions. Cardiomyocyte (HL-1) cells transfected with miR-15b mimic showed a significant (p<0.05, n=3) loss of mitochondrial membrane potential as assessed by JC-1 flow cytometry. Compromised mitochondrial membrane potential was also assessed by a significant (p<0.05, n=4) decrease in TMRM/PMPI ratio in mir-15b mimic transfected HL-1 cells. Such compromise in mitochondrial function was associated with increased tissue oxidative stress in Dicer<sup>-/-</sup> mice as assessed by a significant increase in TBARS (p<0.05, n=3) and GSSG/GSH ratio (p<0.05, n=4). EPR experiments show a significant (p<0.05, n=4) increase in the rate of decay of the nitroxide radical indicating a lower tissue oxygen status.

**CONCLUSIONS:** This study provides first evidence that depletion of *Dicer* in adult mice results in mitochondrial dysfunction and oxidative stress in the myocardium which is followed by acute loss of cardiac functions.
Connective Tissue Growth Factor Production by Activated Pancreatic Stellate Cells in Mouse Alcoholic Chronic Pancreatitis

Alyssa Charrier and David Brigstock, PhD
Nationwide Children’s Hospital Research Institute, Columbus, Ohio

INTRODUCTION: Alcoholic chronic pancreatitis (ACP) is characterized by pancreatic necrosis, inflammation, and scarring, the latter of which is due to excessive collagen deposition by activated pancreatic stellate cells (PSC).

PURPOSE: The aim of this study was to establish a model of ACP in mice, a species that is usually resistant to the toxic effects of alcohol, and to identify the cell type(s) responsible for production of connective tissue growth factor (CTGF), a pro-fibrotic molecule.

METHODS: C57Bl/6 male mice received intraperitoneal ethanol injections for three weeks against a background of cerulein-induced acute pancreatitis.

RESULTS: Peak blood alcohol levels remained consistently high in ethanol-treated mice as compared to control mice. In mice receiving ethanol plus cerulein, there was increased collagen deposition as compared to other treatment groups as well as increased frequency of α-smooth muscle actin-positive PSC which also demonstrated significantly enhanced CTGF protein production. Expression of mRNA for collagen α1(I), α-smooth muscle actin or CTGF were all increased and co-localized exclusively to activated PSC in ACP. Pancreatic expression of mRNA for key profibrotic markers were all increased in ACP.

CONCLUSIONS: A mouse model of ACP has been developed that mimics key pathophysiological features of the disease in humans and which shows that activated PSC are the principal producers of collagen and CTGF. PSC-derived CTGF is thus a candidate therapeutic target in anti-fibrotic strategies for ACP.
Acute and Chronic Response to Occluding Thrombi in a Thrombus-induced Sheep Model of Heart Failure

Chandrakala Aluganti, Chittoor B. Sai-Sudhakar, MBBS, Benjamin Sun, MD, Angela Philips, Pawel Kwiatkowski, MD, Sampath Parthasarathy, PhD, MBA

Department of Surgery, Division of Cardiothoracic Surgery, The Ohio State University Medical Center, Columbus, Ohio

BACKGROUND: The levels of many chemokines and cytokines are known to be increased in the sera of subjects with congestive heart failure (HF). These include MCP-1, TNFα, IL-6, IL-1β, IL-2 and IL-18. The causes and consequences of such induction of these cytokines are widely speculated. It is generally assumed that these increases reflect the remodeling that occurs in the heart as a result of tissue ischemia. In this study, we determined the plasma levels of over 80 different chemokines and cytokines in a new large animal model of thrombus-induced HF.

METHODS: Thrombus from autologous platelets was injected into the circumflex artery of the sheep. After 72 hrs and 90 days of embolization, animals were sacrificed and the blood samples were collected, processed for plasma, and used for cytokine array analysis.

RESULTS: The results showed that cytokines are maximally induced at both time intervals of HF; however, the induction was lower at 90 days after embolization as compared to those in 72 hrs. The induction followed a predictable pattern; angiogenesis cytokines were elevated suggesting potential neovascularization leading to collateral formation to compensate the loss of tissue perfusion. Cytokines relating to the digestion of the thrombus as well as inflammation and antibody production were also elevated, perhaps reflecting the “FOR-EIGN” nature of the thrombus as compared to the body’s own platelets. Finally, gene products related to apoptosis, cell proliferation, and remodeling were increased suggesting an active remodeling process. As HF progressed to 90 days after embolization, the levels of some of the cytokines decreased. The results showed temporal changes in cytokine levels may affect HF-associated factors.

CONCLUSIONS: As a result, the induction of cytokines during HF might suggest that, a) factors other than flow/volume changes affect cytokine levels, b) cytokines may be induced by stress due to an adaptive response to cardiac injury, and c) cytokines also switch during myocardial remodeling by effecting matrix metalloproteinases (MMPs) which play an important role in HF progression.

Key words: chemokines, ischemia, inflammation, matrix metalloproteinases.
Lipid Mediators in Resolution of Wound Inflammation

Amitava Das, Chandan K. Sen, PhD, Sashwati Roy, PhD

Department of Surgery, Comprehensive Wound Center, The Ohio State University Medical Center, Columbus, Ohio

BACKGROUND: Inflammatory response, the first crucial step in healing of wounds, provides signal, that coordinate tissue repair. In a healing wound, inflammation is usually followed by resolution where the affected tissues regain their normal structure and function. Persistent inflammation results in non-resolving chronic wounds. Macrophages at the wound site orchestrate both initiation and resolution phases of inflammation in wounds. ω-3 polyunsaturated fatty acids (PUFAs), found in high proportions primarily in fish oils, are reported to possess potent anti-inflammatory properties and are widely used as a dietary or nutritional supplement. Docosahexaenoic acid (DHA, C22:6n-3), is readily available in the diet from cold-water fish like salmon and tuna. Macrophages metabolize DHA to resolvins, protectins and maresins that are potent anti-inflammatory lipid mediators. LCMS studies in our lab demonstrated decreased levels of 17-HDHA, a metabolite of DHA in the day 3 wounds of diabetic mice compared to wound of non-diabetic normal mice.

HYPOTHESIS: DHA and its metabolites are potent anti-inflammatory mediators that attenuate inflammatory response in macrophages.

METHODOLOGY: To test the hypothesis we used human monocytic THP-1 cells. The cells were treated with increasing concentrations (0.1-100 μM) of DHA in RPMI-1640 media supplemented with serum for specified time (2h). Following pre-treatment with DHA, the cells were activated with a pro-inflammatory signal (lipopolysaccharide, LPS) for 24 or 48 h. The media and cells were collected and the levels of the pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) were assayed using ELISA.

RESULTS AND SIGNIFICANCE: LPS treatment to THP-1 cells resulted in potent induction of pro-inflammatory cytokine production. Such induction was significantly attenuated in the cells that were pre-treated with DHA. Significant decrease was noted at 0.1μM dose; however, the maximal inhibition was achieved by a 10 μM DHA treatment. Studies are currently ongoing to elucidate molecular mechanisms of such DHA mediated decrease in pro-inflammatory cytokine production by macrophages following LPS stimulation.

CONCLUSION: Data from this study demonstrates potential mechanisms underlying anti-inflammatory activity of fish oils. Use of such supplementation may prove to be beneficial for resolving chronic inflammation in non-healing wounds.
Electrical Burns and the Development of Post Traumatic Stress Disorder and Associated Psychological Sequelae

Eric Luedke, MD, Rebecca Coffey, NP, Sidney Miller, MD

Department of Surgery, The Ohio State University Medical Center, Columbus, Ohio

INTRODUCTION: Electrical injuries are the most common occupational related burn injury, resulting in 20,000 ED visits each year and are the fifth leading cause of occupational fatality. Psychological sequelae are common, however, many of these symptoms are nonspecific, and often appear months after the injury. Quantifying the incidence of these psychological effects as these injuries are frequently work related and establishing the relative incidence of psychological disorders may lead to better diagnosis and monitoring.

METHODS: An IRB approved retrospective chart review of patients who sustained an electrical injury between March 1, 2006 and February 28, 2009 was undertaken to quantify the development of Post Traumatic Stress Disorder (PTSD) and other psychological symptoms. The review included current medical history, previous mental health history, medication, psychological symptoms, and the induction of any forms of psychotherapy since the injury. Additional data collected included electrical source, degree of burn, length of hospital stay, and discharge disposition.

RESULTS: Charts of 30 patients who experienced electrical accidents were reviewed. The mean voltage of the electrical source was 2840V. Seventeen percent had previously diagnosed mental health conditions. Ten percent had antidepressant medication started while hospitalized. Forty-seven percent were evaluated by a mental health professional during hospitalization, and 13% developed eventual symptoms consistent with Post Traumatic Stress Disorder.

CONCLUSIONS: Future studies will be undertaken to establish whether there is an association between electrical injuries and anatomic changes to the central nervous system and the possible use of specific brain biomarkers that may aid in the diagnosis, estimation of prognosis, and in monitoring effective treatment in these patients.
Novel Reconstruction Techniques of the Extrahepatic Biliary Trees with a Biosynthetic Absorbable Graft

Peter Nau, MD, Christopher Ellison, MD, Jeffrey Hazey, MD, Matthew Henn, Vimal Narula, MD, W. Scott Melvin, MD

Department of Surgery, The Ohio State University Medical Center, Columbus, Ohio

BACKGROUND: The standard reconstruction of the extrahepatic biliary tree is considered a Roux-en-Y hepaticojejunostomy. This procedure is not without complications, both acute and long term, and may not be feasible in some patients.

OBJECTIVES: This project seeks to evaluate a novel approach for repairing common bile duct injuries with a biosynthetic graft, reconstructing the anatomy without an intestinal bypass.

METHODS: Utilizing an open approach, the common bile duct was isolated using minimal dissection and sharply transected in eleven mongrel hounds. A 1 cm tube graft of a synthetic bioabsorbable prosthetic was individually fashioned for biliary reconstruction. The graft was interposed over a 5 French pancreatic stent and secured with 6-0 absorbable suture. Intra-op cholangiograms and monthly liver function tests were completed.

RESULTS: The first five animals were removed from the protocol early and sacrificed for acute surgical complications during protocol development. Five animals are long-term survivors without evidence of graft stenosis. At six weeks, graft incorporation with acute inflammation is present on histologic analysis. At six months, reconstitution of the native common bile duct is present with evidence of chronic inflammation and re-epithelialization of the lumen. The mean alkaline phosphatase and total bilirubin at eight months are normal at 75 U/L and 0.1 mg/dl respectively. No evidence of cholestasis or intrahepatic biliary duct dilation is noted microscopically.

CONCLUSIONS: Biliary reconstruction using a synthetic bioabsorbable prosthetic as an interposition tube graft is feasible based on early initial results. Further study is needed.
Glutathione Disulfide as a Cell Death Signal

Han-A Park, Savita Khanna, PhD, Cameron Rink, PhD, Surya Gnyawali, Sashwati Roy, PhD, Chandan K. Sen, PhD

Department of Surgery, Laboratory of Molecular Medicine, The Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University Medical Center, Columbus, Ohio; 614-247-7840

PURPOSE/BACKGROUND: Reduced glutathione (GSH) is an ubiquitous low molecular weight intracellular thiol present in all aerobic cells in millimolar concentrations. Under conditions of oxidative stress, large amounts of GSH are rapidly oxidized to GSSG. Thus, elevated GSSG/GSH ratio is often used as a marker of oxidative stress. Over the years, GSSG has been viewed as a metabolic waste-product. Cell death is often associated with high GSSG levels. Such results are interpreted as evidence for oxidative stress without addressing any potential functional significance of GSSG in the death process. We hypothesized that under specific conditions, GSSG functionally participates in signaling for cell death.

METHODS: Mechanisms that trigger the oxidation of GSH to GSSG in a cell such as exposure to ROS or to ROS-generating cytokines also induce numerous other cellular responses. Thus, it is challenging to dissect which of those responses actually contributed to the death process. To address this complication, we raised cellular GSSG content by microinjection. Control cells were microinjected with either the corresponding reduced form GSH or the vehicle (PBS).

RESULTS: GSSG, but not GSH, caused cell death at pathophysiologically relevant concentrations. GSSG-induced death of the neural cells was protected in the presence of the 12-lipoxygenase inhibitor alpha-tocotrienol or BL15. Previous work from our laboratory has identified 12-lipoxygenase as a key executioner of neural cell death relevant to stroke. Results of this study indicate that GSSG induces 12-lipoxygenase dependent death of neural cells. Furthermore, GSSG-dependent glutathionylation of 12-lipoxygenase is a critical player in neural cell death. We tested our hypothesis by using glutaredoxin, a deglutathionylating enzyme in mammalian cells, and noted that glutaredoxin transfected neural cells were protected against glutamate challenge. To test the significance of our findings in vitro, GSSG was stereotaxically injected to the brain in vivo and MRI was performed to quantify tissue lesion.

CONCLUSIONS: Findings of this study lead to question the significance of GSSG in such processes. From the standpoint of novel therapeutic approaches, strategies directed at improving or arresting cellular GSSG clearance may be effective in minimizing oxidative stress related tissue injury or potentiating the killing of tumor cells, respectively.
Double-Barreled Wet Colostomy in Patients Undergoing a Pelvic Exenteration Leaves Patients with a Single Stoma

Maureen E. Pons, MD, Edward W. Martin, Jr., MD

Department of Surgery, The Ohio State University Medical Center, Columbus, Ohio

INTRODUCTION: Surgery for locally invasive pelvic malignancies focuses on complete resection of the tumor. The median overall survival (OS) for patients undergoing fecal and urinary bypass without resection is 4.14 months. Differing techniques for diversion have been developed since the initial description of the pelvic exenteration. These include the end colostomy and bilateral ureterosigmoidostomy; end colostomy and ureteroenterostomy; and double-barreled wet colostomy (DBWC).

METHODS: In an IRB-approved retrospective review, the charts of 34 patients who underwent a pelvic exenteration and DBWC between January 2005 and August 2009 were examined. Wound infections, anastomotic leaks, length of stay (LOS), and OS were analyzed.

RESULTS: Of the thirty-four patients, twenty-one (61.8%) were female and thirteen (38.2%) were male. The median age of the patients was 56 years old, with a range of 38 to 79 years old. These patients underwent a pelvic exenteration with DBWC for anorectal (25), gynecologic (6), urologic (2), and urologic or colorectal (1) malignancies. Five patients had primary malignancies, 24 had recurrent malignancies, and 5 had no malignancy upon pathologic examination. The median LOS was 13 days, with a range of 6 to 57 days. Twenty-one patients (61.8%) developed wound infections and ten (29.4%) developed anastomotic leaks from bowel resections and urinary diversion anastomoses. The median OS was 13.9 months. No thirty day operative mortality was seen within this group.

CONCLUSIONS: Invasive pelvic malignancies are difficult problems due to the complexity of the surgical resection. Historically, the success of urinary and fecal diversion was based on the absence of ascending pyelonephritis, electrolyte abnormalities, and odorous watery stools. These problems are rarely seen with more recent forms of fecal and urinary diversion. DBWC is a feasible technique for diversion and allows patients the advantage of having only one stoma to maintain.
Comparison of the Robotic-Assisted versus Video-Assisted versus Thoracotomy Approaches Toward Lung Cancer

Tyler C. Spata, MD, Paul A. Vesco, MD, Patrick Ross, Jr, MD, PhD

Department of Surgery, Division of Cardiothoracic Surgery, The Ohio State University Medical Center, Columbus, Ohio

PURPOSE/BACKGROUND: Robotic-Assisted Thoracoscopic Surgery (RATS) is a relatively new area of thoracic surgery. Robotic surgery has become a more accepted in other surgical fields (urology, cardiac, neurosurgery, orthopedics, gynecology, etc.) The Video-Assisted Thoracoscopic Surgery (VATS) has become a standard of practice for the majority of thoracic surgery centers due to the affordability of the equipment as well as the amount of thoracic surgeons trained in its use. Open thoracotomy is the more traditional procedure for lung carcinoma prior to video-assisted thoracoscopy. VATS was created to become a new option for patients in order to have better precision, miniaturization, smaller incisions, decreased blood loss, less pain, and quicker healing time. Further advantages are articulation beyond normal manipulation and three-dimensional magnification, resulting in improved ergonomics for thoracic surgeons. Due to the size, expense, and lack of properly-trained thoracic surgeons, RATS has not been openly used in thoracic surgery centers. The purpose of the study is to compare the clinical outcomes of RATS to VATS to open thoracotomy for patients with lung cancer.

METHODS: A retrospective analysis was performed of 66 patients who had a lobectomy for lesions suspicious/diagnosed with lung carcinoma from April 2008 to April 2010 at The Ohio State University Medical Center. These patients were divided into the categories of RATS (n=25), VATS (n=27), and Open Thoracotomy (OT) (n=14). Comparison was then performed by averaging the 1) length of surgery, 2) amount of blood loss, 3) chest tube duration, and 4) length of hospital stay between the categories.

RESULTS: For average length of surgery: RATS: 6 hours 52 minutes, VATS: 4 hours, 31 minutes, OT: 5 hours, 21 minutes. For average amount of blood loss: RATS: 200 mL, VATS: 217 mL, OT: 364 mL. For average chest tube duration: RATS: 6 days, VATS: 7 days, OT: 13 days. For average length of hospital stay: RATS: 7 days, VATS: 8 days, OT: 11 days.

CONCLUSIONS: RATS has the longest length of surgery while VATS was the shortest length of surgery. RATS had similar estimated blood loss, chest tube duration, and hospital length of stay compared to VATS. The open thoracotomy patients had larger, estimated blood loss, chest tube duration, and hospital length of stay compared to the other two categories.
The Impending Shortage and Implications of the Plastic Surgery Workforce

Jonathan C. Yang, MD, Pankaj Tiwari, MD, Michael Miller, MD, Thomas E. Williams, Jr, MD, Bhagwan Satiani, MD, MBA, Andrew Thomas, MD, E. Christopher Ellison, MD, Anne Taylor, MD

Department of Surgery, The Ohio State University Medical Center, Columbus, Ohio

OBJECTIVES: To estimate the workforce needed by 2030 in plastic and reconstructive surgery to serve a population of 364 million people and to quantify the cost associated with training additional plastic and reconstructive surgeons.

MATERIALS AND METHODS: A review of the certificates granted in plastic and reconstructive surgery was conducted. Using a population-based algorithm, we extended the results of Richard Cooper’s pioneering work to these fields of surgery. The assumptions were unchanged physician to population ratio, 30 years in practice from completion of residency to retirement, and no revision of the Balanced Budget Act of 1997, and therefore no additional residency positions offered. Per resident expenses were estimated annually at $80,000, including salaries, benefits, and other direct medical education costs.

RESULTS AND CONCLUSIONS: (1) There will not be enough surgeons in plastic and reconstructive surgery as studied. (2) We will have to train more than 2000 surgeons by 2030 to maintain access for our citizens at an annual cost of almost $40 million and total cost of about $740 million. (3) To train the extra needed surgical workforce will cost an additional $200 million. (4) To do this, the Balanced Budget Act of 1997 must be revised to permit more residents to be trained in the United States or other alternatives explored.
Natural Killer Cells are Activated by IL-21 and Induce ADCC and IFN-Gamma Release in Pancreatic Cancer Cell Lines

Kristan D. Guenterberg, MD1, Alena C Jaime-Ramirez2, Armika S. Tatum1, Sri Vidya Kondadasula1, Xuelian Pan1, Susheela Tridandapani, PhD3, William E. Carson, MD1,5

1Department of Surgery, Division of Surgical Oncology, 2Integrated Biomedical Science Graduate Program, 3Center for Biostatistics, 4Division of Pulmonary Medicine, Department of Medicine, 5Department of Molecular Virology, Immunology, and Medical Genetics, The Ohio State University, Columbus, OH USA

INTRODUCTION: Natural killer (NK) cells express an activating receptor for IgG (FcgRIIIa) and mediate antibody dependent cellular cytotoxicity (ADCC) and produce interferon-gamma (IFN-γ) in response to antibody-coated targets. Cetuximab is a humanized monoclonal antibody that targets the epidermal growth factor receptor (EGFR) which is present on the majority of pancreatic cancers. Interleukin-21 (IL-21) is an immunomodulatory cytokine that activates NK cells. We hypothesized that IL-21 treatment of NK cells would enhance their response to cetuximab-coated tumor cells.

METHODS: Expression of EGFR was measured on six human pancreatic cancer cell lines (AsPc1, BxPc3, MiaPaCa, Panc1, HPAF, and HPAC) via flow cytometry and immunoblot analysis. NK cells were treated overnight with IL-21 (10 ng/ml) and tested for ADCC against cetuximab-coated pancreatic cancer cells in a standard four hour 51Cr assay. Release of IFN-γ, IL-8, MIP-1α/β, and RANTES by IL-21-treated NK cells in response to cetuximab-coated pancreatic cancer cell lines was measured by ELISA. NK cells from patients with pancreatic cancer were stimulated overnight with IL-21 and ADCC was measured using a standard four hour 51Cr assay. The murine pancreatic cancer cell line Panc02 was transfected with the human EGFR and implanted subcutaneously in mice. Mice were treated with PBS, mIL-21, cetuximab, or the combination I.P. 3x/week. Two-tailed student t tests were used to compare means between groups.

RESULTS: All six cell lines showed >85% expression of EGFR by flow cytometry except HPAC (76%) and HPAF (54%), which was confirmed by immunoblot. There was 64.1 ± 3.5% lysis of cetuximab-coated AsPc1 cells by IL-21-treated NK cells as compared to 48.4 ± 5.3% lysis for unstimulated NK cells (p<0.001). Similar results were obtained in the Panc1 cell line (p<0.001) and the BxPc3 cell line (p=0.021). IL-21 treated NK cells from patients with pancreatic cancer mediated lysis of cetuximab-coated tumor cells at all effector:target ratios tested (p<0.0001). Culture of purified human NK cells with cetuximab coated pancreatic cancer cells in the presence of IL-21 led to the secretion of large amounts of IFN-γ (>9,000 pg/ml) as compared to the control conditions (cetuximab, IL-21, or normal IgG, all <1,800 pg/ml). Release of IFN-γ by NK cells began at 6 hours and peaked at 72 hours (p=0.006 vs. control conditions). Additionally NK cells released the chemokines IL-8, MIP-1α/β, and RANTES were released in response to IL-21 and cetuximab-coated cells. There was a significant decrease in tumor volumes of mice treated with IL-21 at 10 ug/mouse (558 mm³ ±128 mm³) as compared to PBS treatment (894 mm³ ± 146 mm³) (p=0.007). A similar decrease was seen in tumor bearing mice treated with cetuximab (1 mg/kg) (p=0.018) and in mice treated with the combination of cetuximab and IL-21 (p=0.018).

DISCUSSION: IL-21 enhanced NK cell lytic activity against cetuximab-coated pancreatic cancer cell lines. Importantly the NK cells of patients with pancreatic cancer were still able to mount an ADCC response following IL-21 treatment. IL-21 activation of NK cells in the presence of antibody coated tumor cells also led to enhanced release of IFN-γ (5 fold) as compared to control conditions. IL-21 and cetuximab in combination inhibited tumor growth in mice containing EGFR positive cells.

CONCLUSION: Stimulation of NK cells in the presence of cetuximab-coated pancreatic cancer cells leads to enhanced NK cell ADCC and cytokine secretion and tumor regression in mice. Cytokines may be a useful adjuvant to monoclonal antibody therapy in patients with Her1 positive pancreatic cancer.
Surgical Management of Malignant Bowel Obstruction

Jon Henry MD, Rachel Sullivan BS, Carl Schmidt MD, Edward Martin MD, Mark Bloomston MD

Department of Surgery, The Ohio State University Medical Center, Columbus, Ohio

INTRODUCTION: Bowel obstruction secondary to cancer is often a pre-terminal event with median survival of only 1-3 months. While the goal of therapy is to provide the longest and best quality of life the impact of surgical intervention for bowel obstruction in surgical patients is largely unknown. This study explores the outcomes following surgical intervention in patients with malignant bowel obstruction in comparison to cancer patients who had operations for bowel obstruction caused by other means than malignancy.

METHODS: A retrospective review of all patients admitted between 2000 and 2007 with the diagnoses of bowel obstruction and a solid tumor malignancy that underwent a surgical intervention for the bowel obstruction during that hospitalization. Data on demographics, specifics about the surgical procedure, specific cancer diagnosis, radiological findings, length of stay in the hospital, time to surgery from admission, discharge disposition, discharge diet, rate or re-obstruction, time to re-obstruction, and time to death were recorded. The individuals were then split into two groups based on the intra-operative findings for cause of the bowel obstruction creating a malignant bowel obstruction group (MBO) and a non-malignant bowel obstruction group (nMBO).

RESULTS: A total of 298 diagnosed cancer patients were operated upon for bowel obstruction at the above hospitals during 2000-2007. The malignant bowel obstruction group is 217 patients with 47% male and an average age of 60 years. The non-malignant bowel obstruction group is 81 patients with 49% male and an average of 61 years. Colorectal cancer (n=90), ovarian cancer (n=32), and carcinoid/neuroendocrine tumor (n=17) were the most common causes of malignant bowel obstructions. Colorectal (n=31), ovarian (n=5), and gastric/bladder cancers (n=4) were the most common cancer diagnosis in the nMBO. The length of hospital stay was 14.7 days in the MBO and 18.9 in the nMBO, which was significant. The time to surgery was 4.5 versus 6.2 days in the MBO and nMBO respectively. Disposition to home was 68.7%, 17.5%, and 4.1% to home, a medical institution or hospice respectively in the MBO. The nMBO disposition was very similar with 74.1%, 18.5%, and 2.5% going to the same entities respectively. Discharge diets were similar between the two groups with 69.6% and 70.3% of the MBO and NMBO groups being discharged on regular diets. The rate of re-obstruction in the MBO was 18.9% and 13.5% in the nMBO. The time to re-obstruction in the two groups was significant in the MBO it was 154 days compared to 837 days in the nMBO. Death rate during that hospital admission was 6.5% for MBO and 1.2% for nMBO. Finally the time to death for the two groups was significant with MBO average time to death of 239 days versus 361 days for the nMBO.

CONCLUSION: The two groups compared in this study did differ in their total numbers but were very similar in their age, sex distributions, and cancer diagnoses allowing reasonable conclusions to be drawn between the two groups. The length to surgery from admission while not significant was surprising in that MBO are taken to the operating room almost two days quicker and they are discharged on average 4 days quicker from the hospital. The disposition location and discharge diet were very similar to the two groups showing that surgery on malignant bowel obstructions does not severely alter their lifestyle compared to nMBO receiving surgery. The rate of re-obstruction was similar to the two groups, but it is not surprising that the time to obstructing was quicker in the MBO. The rate of deaths in that hospitalization was not significant, but note worthy in that a far great percentage of individuals in the MBO died in that hospitalization. Finally the time to death being significant between the groups is not surprising in that it may be presumed that the MBO group has a more aggressive disease. Overall this study shows it is safe to operate on MBO patient and expect them to return acceptable lifestyle most of the time, but we must suspect that they will have a short life expectancy and a quicker time to re-obstruction than our nMBO patient population.
**IL-4-dependent, IgG1-dominant Post-transplant Alloantibody Production is Regulated by CD8\(^+\) T Cells**

Thomas A. Pham, MD, Jason M. Zimmerer, Virginia M. Sanders, PhD, Ginny L. Bumgardner, MD, PhD

Department of Surgery, The Ohio State University Medical Center, Columbus, Ohio

**PURPOSE/BACKGROUND:** In the absence of CD8\(^+\) T cells, post-transplant alloantibody production in murine hepatocyte recipients is enhanced. The mechanism(s) contributing to enhanced alloantibody production in the absence of CD8\(^+\) T cells remains unclear. IFN-g-deficient recipients also manifest enhanced posttransplant alloantibody production that is downregulated with the reconstitution of wild type CD8\(^+\) T cells. We hypothesized that IFN--producing CD8\(^+\) T cells may inhibit antibody production by skewing towards a pro-inflammatory cytokine profile; whereas, when these cells are absent, an antiinflammatory cytokine profile shifts the alloimmune response towards alloantibody production.

**METHODS:** Wild type, CD8 KO, IFN-g, and IL-4 KO mice were transplanted with allogeneic hepatocytes. Serum alloantibody was quantified by flow cytometry. Cytokine mRNA was isolated from CD4\(^+\) and CD8\(^+\) T cells for analysis by Real Time PCR.

**RESULTS:** We found that IgG1 is the dominant alloantibody isotype in CD8-deficient recipients, as well as in wild-type recipients, although the amount of alloantibody in the latter group was substantially lower. Utilizing real-time PCR we found that CD4\(^+\) T cells from wildtype recipients demonstrated upregulation of IFN-g mRNA (1.47±0.12 fold; \(p=0.002\)) but no significant upregulation of IL-4 mRNA. In contrast, CD4\(^+\) T cells isolated from CD8-deficient recipients significantly upregulated IL-4 mRNA (1.91±0.14 fold; \(p=0.0005\)), while IFN-g was downregulated (0.29±0.1 fold; \(p=0.0004\)). Upon examining alloantibody profiles in IL-4 KO recipients, we found that IgG1 alloantibody production was significantly inhibited compared to wild-type recipients, indicating that IL-4 is essential for alloantibody production in this *in vivo* model. In contrast, IFN-g KO recipients have an enhanced alloantibody response that is downregulated by the adoptive transfer of wild-type CD8\(^+\) T cells.

**CONCLUSIONS:** Following hepatocellular transplant in wild type recipients, the cytokine milieu is IFN\(_g\)-dominant, whereas in the absence of CD8\(^+\) T cells, CD4\(^+\) T cells switch to an IL4-dominant profile. Thus, CD8\(^+\) T cells influence the cytokine balance which correlates with the subsequent dominant alloantibody isotype. Elucidation of the mechanisms by which CD8\(^+\) T cells regulate post-transplant alloantibody production will contribute to the development of immunotherapeutic strategies.
The Role of Proton Leak, Membrane Potential and Reactive Oxygen Species Production in Ischemic Preconditioning

Ricardo Quarrie, MD1, Daniel S. Lee1, Brandon Cramer1, Gregory E. Steinbaugh2, Warren Erdahl3, Douglas R. Pfeiffer1, Jay L. Zweier2, Juan Crestanello, MD2

1Division of Cardiothoracic Surgery, The Ohio State University Medical Center; 2The Dorothy M. Davis Heart and Lung Research Institute; 3Department of Molecular and Cellular Biochemistry, The Ohio State University, Columbus Ohio

BACKGROUND: Myocardial ischemia-reperfusion (IR) increases mitochondrial reactive oxygen species (ROS) production. ROS damage the mitochondrial and cell membranes leading to cardiac dysfunction. ROS are generated from the mitochondrial electron transport chain (ETC). Changes in mitochondrial membrane potential (mΔΨ) may regulate ROS production by changing the redox status of the ETC. Ischemic preconditioning (IPC) describes the phenomenon where short episodes of ischemia-reperfusion protect the heart from the damaging effects of a prolonged period of IR. The mechanisms responsible for the protective effects of IPC are not completely known but may involve the inhibition of ROS production. Proton (H+) leak is the reentry of protons into the mitochondrial matrix independent of ATP production. H+ decreases mΔΨ and may lead to decreased ROS production. The role of H+ leak during IPC is unknown. These experiments examined the relationship between mitochondrial H+ leak, mΔΨ and ROS production in IPC and Control mitochondria.

METHODS: Excised rat hearts (n=6/group) were subjected in a Langendorff apparatus to either Control (30 minutes of equilibration, 30 minutes of global normothermic ischemia, then 30 minutes of reperfusion) or IPC (10 minutes of equilibration, followed by induction of preconditioning by two 5 minute episodes of ischemia each followed by 5 minutes of reperfusion, then 30 minutes of global normothermic ischemia and 30 minutes of reperfusion). Rate pressure product (RPP) was recorded as an index of myocardial function. Mitochondria were isolated at end-equilibration, end-ischemia and end-reperfusion. Mitochondrial respiration was measured by polarography and titrated with increasing concentrations of malonate (0.5-2mM). mΔΨ was measured using a tetraphenylphosphonium electrode. H+ leak was measured as the respiratory rate required to maintain membrane potential at -145mV in the presence of oligomycin. Mitochondrial complex III ROS production was measured by fluorometry using Amplex-Red.

RESULTS: At end-reperfusion, IPC hearts recovered 63±4% of their end-equilibration RPP vs. 21±2% for Control (p<0.05). IPC preserved mΔΨ at end-ischemia compared to Control (-152±5 vs. -127±7 mV, p<0.05). At end-reperfusion, IPC had greater recovery of mΔΨ, compared to Control (-160±2 vs. -150±2 mV, p<0.05). H+ leak was lower in IP at end-reperfusion compared to Control (58±9 vs. 97±13 nanomoles O/mg protein/min, p<0.05). IPC attenuated complex III ROS production at end-reperfusion compared to Control (31±2 vs. 40±3 nanomoles O/mg protein/min, p<0.05).

CONCLUSIONS: IPC attenuates mitochondrial H+ leak, preserves mΔΨ, and decreases ROS production at end-reperfusion. Increased H+ leak does not appear to be responsible for the decrease in ROS production seen in IPC. H+ leak appears to be a marker of mitochondrial inner membrane dysfunction. Higher H+ leak is associated with lower mΔΨ, increased ROS production and worse myocardial tolerance to ischemia-reperfusion.
Development of a Novel Strain of Non-obese Diabetic (NOD) Mice with Targeted Disruption of the CD103 Gene

Elizabeth Stofko Barrie\textsuperscript{1,2}, Jiao-Jing Wang\textsuperscript{2}, Mark Lafferty\textsuperscript{3}, Gregg A. Hadley, PhD\textsuperscript{2}

\textsuperscript{1}Integrated Biomedical Sciences Graduate Program, The Ohio State University, \textsuperscript{2}Department of Surgery, College of Medicine, The Ohio State University, \textsuperscript{3}University of Maryland Baltimore

BACKGROUND: Transplantation of pancreatic islet allografts now offers a cure for Type I diabetes (T1D) but this therapeutic modality is limited by the poorly characterized immune response as islet allografts transplanted into diabetic hosts are potentially subject to both alloimmunity and recurrent autoimmunity. Our lab is focused on the role of the integrin CD103 in these two processes. This integrin is directed to an epithelial cell-specific ligand, E-cadherin, which is highly expressed on islets. In previous studies, our lab has found that islets transplanted into the renal subcapsule of CD103\textsuperscript{-/-} hosts survive indefinitely and CD103 is required for the rejection of islet allografts in a streptozotocin (chemically induced) model of diabetes. In contrast, recurrent autoimmunity is thought to be mediated by autoreactive CD4 T cells which fail to express significant levels of CD103. Based on these data, I postulate that CD103 is required for alloimmunity but not recurrent autoimmunity to islet allografts.

METHODS: To test this hypothesis, we backcrossed BALB/c CD103\textsuperscript{-/-} mice with wild-type (WT) NOD (non-obese diabetic) mice for 12 generations to yield a NODCD103\textsuperscript{-/-} strain. The WT NOD mice were ordered from Jackson Laboratories, while the NODCD103\textsuperscript{-/-} were bred in house. NOD mice spontaneously develop diabetes, with 90\% of females becoming diabetic by 30 weeks of age. The marker Mit 320, on chromosome 11 closely flanks the mouse CD103 locus (Itgae). Using primers for this marker, PCR amplification shows bands of either 124 bp for mice on the NOD background, or 140 bp for BALB/c mice. Blood glucose levels in the mice were measured weekly, using an Ascensia Elite Glucometer (Bayer) and diabetes was confirmed upon measurement of persistent hyperglycemia (blood glucose > 250 mg/dL). Blood glucose levels of less than 200 mg/dL, by day 3 after transplantation, defined primary graft function whereas blood glucose levels of >250 mg/dL indicated allograft dysfunction and loss. To isolate donor islets, the common bile duct was injected with 3 ml cold HBSS collagenase P solution (2 mg/ml) and the pancreas was excised. Islet purification was then performed ex vivo. The islet graft was injected under the kidney capsule.

RESULTS: Genomic DNA PCR amplified with Mit320 primers show the BALB/c control had a band at 140 bp, while the NOD control had a band at 124 bp. The NODCD103\textsuperscript{-/-} backcrossed mice had PCR products aligned with the WT NOD at 124 bp. In order to determine if the CD103 deficiency had phenotypic effects, we measured the incidence of diabetes in the two colonies: WT NOD and NODCD103\textsuperscript{-/-}. We found that CD103 is not required for the development of diabetes, as the NODCD103\textsuperscript{-/-} mice still acquired the disease. Also, there is no significant difference between the two groups in terms of the age of diabetes onset between NODCD103\textsuperscript{-/-} mice (25.4 ± 5.9 weeks, N=17) and WT NOD (21.5 ± 8.5 weeks, N=16) with a t-test or an ANOVA (p<0.05). Next we performed islet allografts and found no significant difference in graft survival time between WT NOD (9.5± 0.7 days) or NODCD103\textsuperscript{-/-} (9.5 ± 0.5 days) recipients.

CONCLUSIONS: We have documented that the strain is on a NOD background but completely lacks Itgae. Critically, NODCD103\textsuperscript{-/-} mice develop diabetes, demonstrating that CD103 is not necessary for the autoimmune destruction of the endogenous islets. Also, the timing of diabetes development is similar to that seen in the WT NOD. Our strain has a similar response to islet allografts, and both strains reject the grafts at the same time. This novel mouse strain offers a powerful tool with which to dissect the role of CD103 in autoimmune and alloimmune responses to islet transplants.
Previous Award Winners

2009

**Intern Award**
Meghan Forster, MD - Optimal ganciclovir prophylaxis to prevent cytomegalovirus reactivation in immuno-competent hosts.

**Poster Award**
Alicia Thomas, MD - Occult cytomegalovirus infection in vivarium housed mice.

**Best Post Doctoral Presentation Award**
Cameron Rink, PhD - Characterization of the therapeutic potential for supplemental oxygen therapy in acute ischemic stroke.

**1st Place Resident Award**
Kristan Guenterberg, MD, MS - Enhanced anti-tumor activity of interferon-alpha in SOCS1-deficient mice is mediated by CD4\(^+\) and CD8\(^+\) T cells.

**2nd Place Resident Award**
Peter Nau, MD - Safe alternative transgastric peritoneal access in humans: NOTES.