1

PRE-EXISTING IMMUNE PHENOTYPE IMPACTS THE HOST RESPONSE AND SURVIVAL DURING SEPSIS

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Several non-mutually exclusive factors can influence the clinical outcome in septic patients including the severity and nature of infectious agent, genetic pre-disposition, as well as the vigor and/or phenotype of the host response. The latter factor suggests that prior unrelated infections could influence a subsequent septic event. To examine this, we tested two polarizing immune responses for their effects using a mouse model of sepsis (CLP). Mice were infected with either lymphocytic choriomeningitis virus (LCMV, induces a strong Th1 response) or Nippostrongylus brasiliensis (Nippo, induces a strong Th2 response). Three months after their prior infection (infectious agents are cleared within ten days); previously infected mice were subjected to CLP. Surprisingly, mice with a prior LCMV infection had increased mortality and bacterial load after CLP. Macrophages from prior LCMV infected mice had decreased phagocytic activity, but increased expression of MHC II and CD86. Conversely, mice with a prior Nippo infection were protected from CLP and had less bacterial load compared to controls. Macrophages from prior-Nippo infected mice had increased phagocytic activity, but had decreased expression of MHC II and CD86. While it is possible that sepsis drives activation of polarized memory T cell populations that could impact subsequent sepsis, it is unlikely because neither Nippo nor LCMV share antigens with bacteria. Further, there was no difference in serum levels of IFN-γ between prior LCMV infected and uninfected mice, arguing against “bystander” activation. Combined, our data suggest that prior infections can cause long-lasting effects that may involve polarization of innate immune cells.

2

IMPAIRED INNATE AND ADAPTIVE IMMUNITY OF ACCELERATED-AGING KLOTHO MICE WITH SEPSIS

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Background: Sepsis is primarily a disease of the aged, and 60% of cases involve patients older than 65 years; 80% of deaths due to sepsis occur in this age group. Klotho knockout mice (Klotho mice) develop a syndrome resembling human aging and exhibit shortened life-spans in almost 8 weeks; however, the immunity of and immunological changes in Klotho mice after sepsis are still unclear.

Methods: A survival study involving CLP was performed on Klotho and wild-type (WT) mice and their 4-day survival were compared. In a cell analysis study, mice were sacrificed at 8 hours after CLP or sham surgery. The spleen, thymus, and serum were harvested for FACS analysis with aspase-3 as an apoptosis marker; blood was collected for cytokine assay. The bacterial colony count for peritoneal lavage was also analyzed.

Results: The Klotho septic mice started dying from 8 to 12 hours after CLP, and their final survival was significantly lower than that of WT-CLP mice (0% vs. 100%, p < 0.01). Klotho-CLP mice had more than 30-fold higher bacterial count in the peritoneal cavity and 50% lower neutrophil and macrophage recruitment to the peripheral cavity than WT mice (p < 0.01). The IL-6, TNF, and IL-10 serum levels in Klotho-CLP mice were significantly higher than those in WT-CLP mice (serum IL-6 level, 25,076 vs 1,100 pg/ml; p < 0.01). Flow cytometric and immunohistological analyses showed a considerable increase in the proportion of caspase-3-positive splenocytes and thymocytes in Klotho-CLP mice (p < 0.01).

Conclusion: Impaired innate and adaptive immunity may cause considerably poor survival in Klotho septic mice, which is characterized by impaired bacterial clearance with decreased recruitment of neutrophils/macrophages to the peritoneal cavity, elevated serum cytokine levels, and increased apoptosis in the thymus and spleen.

3

A NOVEL ROLE FOR TUMOR-INDUCED EXPANSION OF MYELOID DERIVED CELLS IN HOST RESISTANCE AND CANCER CACHEXIA

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Cancer progression is associated with inflammation, increased metabolic demand, infection, cachexia, and eventually, death. Myeloid-derived suppressor cells (MDSCs) expand during cancer and are associated with adaptive immune suppression and inflammatory metabolite production. We hypothesize that expansion of MDSCs may also contribute to cancer-induced cachexia. To study this, 6-8 week old BALB/c mice were inoculated with either 4T1 mammary carcinoma, shown to induce the expansion of MDSCs, or its subclone 66C4, shown to NOT induce the expansion of MDSCs. Animals were either studied at an early to intermediate time point (21 days post 4T1 inoculation) or at a later time point (28 days post 4T1 inoculation). We demonstrate that early expansion of MDSCs in 4T1 mammary carcinoma-bearing hosts actually contributes to host defense against polymicrobial sepsis and listeriosis, despite T cell suppression, but the late expansion of these cells during progressive tumor growth also leads to sequelae consistent with cachexia. MDSC expansion in 4T1-bearing hosts is associated with a hepatic acute phase response and altered host energy and fat metabolism, and eventually, reduced survival to polymicrobial sepsis and endotoxemia. Similar results are not seen with equivalent growth of the 66C4 subclone, without MDSC expansion. Importantly, reducing MDSC numbers in 4T1-bearing animals can ameliorate some of these late responses, and reduce susceptibility to inflammation-induced organ injury and death. Thus, we propose a previously undescribed role for tumor-associated MDSC expansion, whereby early it may contribute to protection, but during advanced disease, progressive MDSC expansion is associated with changes in host protein and energy metabolism consistent with cancer-associated cachexia, and reduced resistance to sepsis.

4

CIRCADIAN RHYTHMS INFLUENCE SEPSIS SEVERITY DEPENDING ON THE TIMING OF CLP SURGERY

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Several studies have associated surgery at night with worse patient outcomes. Circadian rhythms orchestrate many aspects of mammalian physiology, including inflammatory responses. We investigated how circadian rhythms affect the cecal ligation and puncture (CLP) mouse model of sepsis, depending on the timing of surgery. We found that C57BL/6 mice developed a worse sepsis phenotype when CLP was performed at zeitgeber time (ZT) 19 (2 am) vs. ZT 7 (2 pm): mice
exhibited earlier mortality, more severe hypothermia, worse disease score, and higher elevations in serum proinflammatory cytokines and creatine kinase. In contrast, Per2Brdm mice, which have a mutation in the Per2 circadian clock gene, exhibited a similar sepsis phenotype when CLP was performed at ZT19 vs. ZT7. We used an in vitro model to investigate if circadian rhythms could modulate the inflammatory response in a cell-intrinsic manner. Thioglycollate elicited macrophages were exposed to commensal bacteria derived from the mouse cecum at various time points after serum shock: a treatment that induces cycling of circadian genes. We observed an oscillating pattern of cytokine production, which mirrored the oscillations in Per2 gene expression. Our data indicate that in the CLP model, circadian rhythms influence the severity of the inflammatory response and the morbidity and mortality that ensue. Our in vitro data indicate that cell-intrinsic circadian oscillations are correlated with an oscillating inflammatory response to commensal bacteria. Our findings imply that it may be important to take circadian rhythms into account when operating on patients who are at risk of developing sepsis.

5

EARLY STABILIZING ALVEOLAR VENTILATION PREVENTS ARDS S.K. Roy1, B. Sadowitz1, N. Habashi1, P. Andrews1, L. Ge1, G. Wang1, L. Gatto2, D.J. Carpenter3, D. Dean3, J. Sataila1, Y. Vodovotz1, K. Snyder4, and G.F. Nieman1.

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ARDS afflicts 200,000 patients annually with 30-60% mortality. Low tidal volume (LTV) ventilation, the standard of care, is a supportive intervention applied at ARDS onset and does not alter ARDS pathophysiology.

Hypothesis: Early application of a mechanical ventilation strategy designed to stabilize alveoli & reduce permeability (Airway Pressure Release Ventilation APRV) before clinical signs of lung injury will prevent development of ARDS.

Methods: Yorkshire pigs (30-40 kg) were anesthetized & subjected to a 2-hit injury: 1) Ischemia-Reperfusion (IR)- superior mesenteric artery was clamped for 30 min and 2) Peritoneal Sepsis (PS)- a fecal cecum at various time points after serum shock: a treatment that induces cycling of circadian genes. We observed an oscillating pattern of cytokine production, which mirrored the oscillations in Per2 gene expression. Our data indicate that in the CLP model, circadian rhythms influence the severity of the inflammatory response and the morbidity and mortality that ensue. Our in vitro data indicate that cell-intrinsic circadian oscillations are correlated with an oscillating inflammatory response to commensal bacteria. Our findings imply that it may be important to take circadian rhythms into account when operating on patients who are at risk of developing sepsis.

Results: APRV animals did not develop ARDS over 48 hrs, whereas all ARDSnet animals did as shown by PaO2/FiO2 ratio: (APRV: Baseline 471±16; 48 hrs 392±8 vs ARDSnet: Baseline 551±28; 48 hrs 138±88 p<0.001.) Histology showed severe ARDS in ARDSnet group vs. normal lungs in APRV group. Gross lung pathology corroborated histology (Fig 1). Surfactant protein A was significantly higher in BALF of APRV (p<0.05 vs ARDSnet) suggesting APRV preserved alveolar stability.

Conclusion: Application of APRV immediately following injury prevented the development of ARDS; the mechanism may be reduced surfactant dysfunction. This ventilation strategy could change the clinical paradigm from treating ARDS to preventing the development of ARDS.
PARASYMPATHETIC STIMULATION PREVENTS SYSTEMIC ORGAN DYSFUNCTION BY ABROGATING GUT INJURY AND LYMPH TOXICITY IN TRAUMA AND HEMORRHAGIC SHOCK. G. Levy, J. Fishman, E. Feketova, D.A. Xu, L. Ulloa*, and E. Deitch*. UMDNJ-NJMS, Newark, NJ

Objectives: We tested if vagal nerve stimulation (VNS) of the parasympathetic nervous system would prevent gut injury, lymph toxicity and systemic MODS following trauma/hemorrhagic shock (THS).

Methods: In experiment 1, male Sprague-Dawley rats underwent actual or sham VNS (5V for 10 minutes) following which they underwent THS or trauma/sham shock (TSS). Gut permeability was assessed with an in-vivo plasma FD4 assay. Lung permeability was measured with % Evans Blue dye in BALF. Systemic factors were assessed by measuring neutrophil (PMN) priming and red blood cell (RBC) deformability via elongation index (EI) (lower number = less deformable RBC). In experiment 2, similar groups of rats had lymph collected. The biologic activity of lymph was tested by injecting it into naïve mice following which lung injury, RBC elongation index and PMN priming was measured as above. We tested four lymph groups THS +/-VNS, TSS +/-VNS.

Results: In experiment 1, VNS decreased THS induced gut injury, (THS-VNS vs THS 0.53±0.4 vs 4.3±2.8 ng/mL, p=0.05) following ethanol and burn injury. Lung permeability was measured with % Evans Blue dye in BALF. Systemic factors were assessed by measuring neutrophil (PMN) priming and red blood cell (RBC) deformability via elongation index (EI) (lower number = less deformable RBC). In experiment 2, similar groups of rats had lymph collected. The biologic activity of lymph was tested by injecting it into naïve mice following which lung injury, RBC elongation index and PMN priming was measured as above. We tested four lymph groups THS +/-VNS, TSS +/-VNS.

Results presented in mean +/- SD. *p<0.05 vs all groups

VNS abrogates organ injury

| GUT INJURY (FD4 ng/mL) | LUNG INJURY (%EBD) | PMN PRIMING (MEAN FLOURESCENCE UNITS) |
|------------------------|---------------------|---------------------------------------|
| THS 4.3±2.8 (n=4)      | 8.5±0.4 (n=4)       | 396±69 (n=4)                          |
| THS+VNS 0.53±0.4 (n=6) | 4.9±0.8 (n=4)       | 282±38 (n=4)                          |
| TSS 0.33±0.2 (n=6)     | 2.8±0.8 (n=4)       | 210±31 (n=4)                          |
| TSS+VNS 0.3±0.1 (n=6)  | 2.2±0.6 (n=4)       | 221±13 (n=4)                          |

VNS abrogates lymph toxicity in vivo

| RBC PRIMING INDEX | PMN PRIMING (MEAN FLOURESCENCE UNITS) | LUNG INJURY (%EBD) |
|------------------|---------------------------------------|---------------------|
| THS 0.050±0.002  | 0.378±57                              | 9.8±1.5             |
| THS + VNS 0.057±0.003 | 327±54                              | 4.3±2.4             |
| TSS 0.063±0.006 | 278±39                                | 2.5±1.8             |
| TSS + VNS 0.060±0.005 | 252±19                                | 3.2±1               |

Results presented in mean +/- SD. *p<0.05 vs all groups, **p<0.05 vs TSS, TSS + VNS

IL-22 MODULATES GUT EPITHELIAL AND IMMUNE BARRIER FUNCTIONS FOLLOWING ACUTE ALCOHOL INTOXICATION AND BURN INJURY. J.L. Rendon*, X. Li, S. Akhtar, and M.A. Choudhry*. Loyola University Chicago Health Sciences Campus, Maywood, IL

Interleukin (IL)-22, a cytokine released by Th17 cells, maintains gut epithelial integrity and expression of antimicrobial peptides (AMPs) Reg3β and Reg3γ. Burn patients with a measurable blood ethanol level at the time of hospital admission have an increased risk of morbidity. Our lab has shown that acute alcohol (ethanol) exposure prior to burn injury results in increased gut permeability, intestinal T cell suppression and enhanced bacterial translocation. Furthermore, we also found a decrease in intestinal IL-22 levels and Reg3β and Reg3γ expression, which were not noted following ethanol or burn alone.

Study Objective: To examine whether in vivo restitution of IL-22 modulates gut permeability, Reg3β and Reg3γ and bacterial load within the intestine following ethanol and burn injury.

Methods: Male mice, ~25g, were gavaged with ethanol (2.9mg/kg) prior to receiving a ~12.5% TBSA full thickness burn. Mice were immediately treated with saline control or IL-22 (1mg/kg) by I.P. injection. One day post injury animals were sacrificed and intestinal permeability, Reg3β and Reg3γ expression and bacterial load measured.

Results: Ethanol combined with burn injury resulted in decreased expression of Reg3β and Reg3γ (65% and 70%, p<0.05 vs sham), which was attenuated following treatment with IL-22 (p<0.05). Qualitatively, combined insult resulted in increased bacterial load in intestinal luminal content, which was decreased in half of IL-22 treated animals. IL-22 treatment also prevented increased gut permeability (p<0.05) following ethanol and burn injury.

Conclusion: Treatment with IL-22 maintains gut epithelial and immune barrier integrity following ethanol and burn injury; thus, the IL-22/AMP pathway may provide a novel therapeutic target for the treatment of patients who sustain burn injury under the influence of ethanol.
SILENCING OF FAS, FADD OR CASPASE-3 (C-3) DIFFERENTIALLY AFFECTS LUNG INFLAMMATION, APOPTOSIS AND DEVELOPMENT OF SEPTIC ACUTE LUNG INJURY (ALI) M. Messer, S.J. Weber, P. Kellermann, C. Hohmann, and M. Perl*. Ulm University, Ulm, Germany

The activation of Fas signaling is an important pathophysiological mechanism in the development of septic ALI by regulating both lung apoptosis and inflammation. We still don’t know of any optimal targets in this signaling cascade that can positively affect the development of ALI. Therefore, we tested the hypothesis that in vivo gene silencing of Fas, FADD or C-3 by intratracheal administration of small interfering RNA (siRNA) would ameliorate ALI in a clinical relevant mouse model of chest trauma induced septic ALI.

Male C57Bl6 mice (n=6/group) received 100μg of inhibitory (Fas, FADD, C-3) or scrambled RNA 12 hours before and after blunt chest trauma induced by a single blast wave centered on the thorax. Polymicrobial sepsis was induced by cecal ligation and puncture 24 hours after chest trauma. 12 or 24 hours later lung tissue, plasma, and bronchoalveolar lavage fluid (BALF) were harvested.

Silencing of C-3 or FADD both markedly reduced pulmonary apoptosis (assessed by active C-3 western blotting and TUNEL staining), however this was not the case after Fas silencing. While C-3 silencing did not affect lung inflammation (assessed by ELISA of BALF), FADD (MCPI, IL-6, TNFα, MIP2, FasL) or Fas siRNA (MCPI, IL-10, KC, FasL) administration substantially decreased lung cytokine concentrations and subsequently decreased lung myeloperoxidase activity. It is notable that, only following C-3 silencing, ALI induced epithelial dysfunction (assessed by protein quantification in BALF) was mitigated.

Taken together, the downstream inhibition of lung apoptosis via C-3 silencing proved to be superior in attenuating ALI when compared to upstream inhibition of apoptosis via FADD silencing, even in the presence of additional anti-inflammatory effects of the latter, indicating a predominant pathophysiologic role of apoptosis.

THE IMPACT OF CARDIAC ARREST DURATION ON EXTRAVASCULAR LUNG WATER AND PULMONARY VASCULAR PERMEABILITY IN POST-CARDIAC ARREST PATIENTS T. Tagami†,2 R. Tosa2, M. Omura2, and H. Yokota1. 1Nippon Medical School, Tokyo, Japan, and 2Aidu Chuo Hospital, Aizu-wakamatsu, Japan

Introduction: Pulmonary dysfunction after cardiac arrest is a common phenomenon. Evidence appears to support the usefulness of quantitative assessment of pulmonary dysfunction using extravascular lung water (EVLW) and the pulmonary vascular permeability index (PVPI). We hypothesized that the duration of cardiac arrest (CPA TIME) would impact on pulmonary dysfunction in patients with post-cardiac arrest syndrome. The aim of the present study was to investigate lung dysfunction quantitatively by using EVLW and PVPI in successfully resuscitated patients after cardiac arrest (CPA).

Methods: This was a 2-year prospective observational study of post-cardiac arrest syndrome patients. Eligible patients included all those who were in CPA upon arrival at the hospital and experienced effective resuscitation resulting in return of spontaneous circulation. All patients were resuscitated as per the therapeutic protocol in our hospital. The CPA TIME from the scene was recorded. Thermodilution EVLW and PVPI measurements were performed using the PiCCO monitoring system (Pulsion Medical Systems, Munich, Germany) as soon as patients were admitted to the Intensive Care Unit.

Results: Among 106 (59 male, 47 female) patients, a moderate positive correlation was documented between the CPA TIME, EVLW (r = 0.36, p < 0.001) and PVPI (r = 0.43, p < 0.001).

Conclusions: Although the cause-effect relationship should be confirmed by future studies, the duration of cardiac arrest was found to correlate strongly with EVLW and PVPI. Clinical trial registration information: UMIN-CTR: UMIN00000 3224.

This paper won the Best Presentation Award in the 26th Japan Shock Society (2011, Japan).

FIBRINOGEN GAMMA-CHAIN PEPTIDE-COATED, ADENOSINE DIPHOSPHATE-ENCAPSULATED LIPOSOMES RESCUE THROMBOCYTOPENIC RABBITS FROM NON-COMPRESSIBLE LIVER HEMORRHAGE K. Nishikawa*, K. Hagisawa, T. Sakamoto, and M. Kinoshita*. National Defense Medical College, Tokorozawa, Japan

Objective: Dodecapeptide HHLGGAQAGDV (H12), a fibrinogen gamma-chain carboxy-terminal sequence, is the primary recognition site of the ligand for GPIIIa/IIa on activated platelets. We developed the H12-coated, adenosine-diphosphate (ADP)-encapsulated liposomes [H12-(ADP)-liposomes] as a platelet function-supporting synthetic product. H12-(ADP)-liposomes accumulate at sites of vessel injury via selective interaction with activated platelets and augment agonist-induced platelet aggregation by releasing ADP. We evaluated the efficacy of the H12-(ADP)-liposomes for non-compressible liver hemorrhage in acute thrombocytopenic rabbits.

Methods: Thrombocytopenia was induced in rabbits by repeated blood withdrawal and isovolemic transfusion of autologous washed red blood cells. H12-(ADP)-liposomes with platelet-poor plasma (PPP), platelet-rich plasma (PRP), PPP alone, or ADP liposomes with PPP was administered to the thrombocytopenic rabbits, and liver hemorrhage was induced by hollowing out the liver tissue.

Main Results: After 72-hour observation, administration of H12-(ADP)-liposomes, as well as PRP, rescued all thrombocytopenic rabbits from liver hemorrhage (p<0.01, vs PPP or ADP liposomes), although rabbits treated with PPP or ADP liposomes showed 20% survival in the first 24 hours. Administration with H12-(ADP)-liposome as well as PRP also suppressed both bleeding volume and time from the site of liver injury. Histological examination revealed that H12-(ADP)-liposomes accumulated at the bleeding site in the liver, and neither macro- nor micro-thrombi were detected in the lung, kidney or liver in rabbits.

Conclusions: H12-(ADP)-liposomes may be a safe and effective therapeutic tool for acute thrombocytopenic trauma patients with massive bleeding.

INCREASED FUNCTIONAL ACTIVITY OF ENDOGENOUS ACETYLCHOLINE ON BRAIN M1 MUSCARINIC RECEPTORS SUPPRESSES LETHAL PERIPHERAL INFLAMMATION V. Pavlov*, 1 M. Ochani*, M. Dancho†, Y. Al-Abed†, N.M. Nathanson*, and K.J. Tracey**. 1The Feinstein Institute for Medical Research, Manhasset, NY. *University of Washington, Seattle, WA

Brain muscarinic acetylcholine receptors play a role in controlling peripheral inflammation through neural vagus nerve-based signaling (Proc Natl Acad Sci USA, 2006, 5219; Brain Behav Immun, 2009, 23:41). Recently, very selective muscarinic receptor agonists were
THE IMMUNE RESPONSE TO BACTERIAL PNEUMONIA FOLLOWING TRAUMATIC BRAIN INJURY IS ENHANCED IN THE PERIPHERY BUT DECREASED IN THE LUNG D.M. Stepken, E.L. Chiswick, D.R. Beal, K. Iskander, E. Duffy, and D. Remick* Boston University School of Medicine, Boston, MA

Traumatic brain injury (TBI) is an important clinical problem affecting 1.7 million Americans annually. Following injury, the function of immune cells is significantly impaired leaving TBI patients at an increased risk for infection, particularly pneumonia.

**Methods:** This study used a controlled impact model of TBI using 186 male rats. Injured animals were given a subcutaneous injection of 5×10⁷ CFU of Pseudomonas aeruginosa. Respiratory function was analyzed using whole body plethysmography. Blood and bronchoalveolar lavage fluid (BALF) were collected for cytokine concentrations and differential cell counts.

**Results:** Injured mice with pneumonia were found to have increased plasma levels of IL-6, MIP-2, and IL-1ra compared to sham infected mice with pneumonia but decreased levels of the same cytokines in BALF when compared to sham infected mice with pneumonia. Blood and BAL cell counts showed similar total and differential cell counts in both injured and sham injury mice with pneumonia but the injured mice showed significantly increased bacterial colony-forming units recovered from the lung. There were no differences in respiratory function between injured and sham injured animals with pneumonia but interestingly the injured animals had increased plasma levels of IL-6, MIP-2, and IL-1ra compared to sham infected animals with pneumonia. Blood and BAL cell counts showed similar total and differential cell counts in both injured and sham injury mice with pneumonia but the injured mice showed significantly increased bacterial colony-forming units recovered from the lung. There were no differences in respiratory function between injured and sham injured animals with pneumonia but interestingly the injured animals had improved survival over sham after bacterial challenge (ps<0.0249).

**Conclusions:** These results indicate that while TBI may induce an inflammatory reaction in the periphery, there is a simultaneous decrease in pulmonary inflammation that may enhance survival following pneumonia challenge.

INDIRECT BRAIN INJURY RESULTING FROM SEVERE BURNS RESULTS IN ESTROGEN REVERSIBLE ELEVATIONS OF MARKERS OF ALZHEIMER’S DISEASE (AD) OVER THE FIRST 45 DAYS POST-BURN J. Wigginton1, P. Pepe2, K.R. AbdelFattah1, D. Maass1, J. Gatson1, J. Minei1, K.G. Wigginton1, and J. Simpkins1, 2UT Southwestern Medical Center Dallas, Dallas, TX, 3University of North Texas Health Science Center, Fort Worth, TX, 4Texas A&M University, College Station, TX

**Background:** Prior studies have found that patients with severe burns may suffer neurocognitive changes. While frequently attributed to psycho-social issues, recent data from our lab revealed a substantial, rapid and sustained (30 min - 45 day) increase in rat brain inflammation following remote burns that is blunted by a single post-burn dose of estrogen. Other brain injury processes, such as TBI and stroke have shown an accelerated accumulation of Aβ40, Aβ42, and Tau, leading to a clinical picture of early Alzheimer’s disease (AD). We hypothesized that similar AD processes may be triggered in severe burn injury, and altered by post-burn estrogen.

**Methods:** In this study, 186 male rats received a 3° 40% TBSA burn (divided into sham, burn+estradiol, or burn+placebo). Fifteen minutes following burn injury, the animals received a subcutaneous injection of either placebo (vehicle/corn oil) or 17 β-estradiol...
S100B AND NSE AS PREDICTIVE BIOMARKERS OF OUTCOME IN RESUSCITATED TBI PATIENTS
A.J. Baker¹, S.G. Rhind², S.B. Rizoli³, W. Junger*, J. Cuschiari**, P.N. Shek, A.D. Romaschin¹, and E.M. Bulger*. ¹Brain Injury Laboratory, Cara Phelan Centre for Trauma Research Keenan Research Centre, St. Michael’s Hospital, University of Toronto, Toronto, ON, Canada; ²Defence Research & Development Canada (DRDC) Toronto, Toronto, ON, Canada; ³Department of Surgery & Critical Care Medicine, Sunnybrook Health Sciences Centre, Toronto, ON, Canada; ⁴Department of Surgery, Beth Israel Deaconess Medical Center & Harvard Medical School, Boston, MA; ⁵Department of Surgery, University of Washington, Harborview Medical Center, Seattle, WA

Background: Traumatic brain injury (TBI) is a leading cause of death and disability in military and civilian trauma. Predictive biomarkers of TBI severity could facilitate triage and early intervention. S100B and neuron specific enolase (NSE), markers of astroglial and neuronal damage, are reported to reflect TBI severity. Hypertonic saline is an effective osmotherapeutic agent for cerebral edema with potential to limit secondary injury.

Objectives: To evaluate biomarkers of TBI in relation to 6-month neurologic outcome (GOS-E; dichotomized as >4 or ≤4) and hypertonic resuscitation.

Design: Prospective randomized controlled trial conducted within the Resuscitation Outcomes Consortium, with assessment of biomarkers obtained on a subset of 82 patients with severe TBI (GCS≤ 8) and 20 healthy controls.

Intervention: A single, prehospital bolus (250mL) of 7.5% hypertonic saline (HS;n=21), 7.5% HS plus 6% dextran-70 (HSD;n=22) or 0.9% saline (NS;n=39).

Measurements & Results: Blood samples were obtained on admission, and 24 h. Serum concentrations (µg/L) of S100B and NSE were determined by electrochemiluminometric immunoassay (Elecsys, Roche). On admission, >90% of patients had levels of S100B (2.4±0.4) and NSE (23.4±3.3) that were significantly (P<0.05) above control values. S100B and NSE levels in patients with poor outcome (GOS-E≤4 or death) were up to twice as high as those with good outcome. Highest levels of both markers were found in patients with poor outcome who were resuscitated with HS. By comparison, patients treated with HS or HSD had significantly lower S100B and NSE.

Conclusions: These findings demonstrate high levels of S100B and NSE are indicative of poor neurological outcome. Hypertonic fluids may reduce cellular injury after TBI as evidenced by lower biomarker levels. Defence R&D Canada; NIH R01GM076101.
OVEREXPRESSION OF CARDIAC MICRONRNA-146A ATTENUATES MYOCARDIAL DYSFUNCTION IN POLYMICROBIAL SEPSIS

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Toll-like receptor (TLR)-mediated NFκB signaling plays a central role in cardiac dysfunction in polymicrobial sepsis. MicroRNAs (miRs) regulate endogenous gene expression via degradation or translational inhibition of their target mRNAs. MicroRNA-146a (miR-146a) has been reported to prevent MyD88-mediated NFκB activation by targeting IRAK and TRAF6. We hypothesized that overexpression of miR-146a will attenuate cardiac dysfunction induced by polymicrobial sepsis. To critically evaluate our hypothesis, lentivirus expressing miR-146a (LmiR-146a) was transfected into mouse hearts (n=6). Untreated mice were also subjected to CLP (n=6). Sham surgical operation served as sham control (n=6). Cardiac function was examined by echocardiography before and 6 hrs after CLP. CLP sepsis induced significant cardiac dysfunction as evidenced by decreases in ejection fraction (EF) by 37.5% and fractional shortening (FS) by 47.2%, respectively. In contrast, overexpression of miR-146a significantly attenuated CLP induced cardiac dysfunction. The %EF (56.7±2.0% vs. 40.1±2.57%) and %FS (30.6±1.3% vs. 19.0±1.26%) values were significantly higher in LmiR-146a treated septic mice than the untreated CLP group. Overexpression of LmiR-146a prevented CLP induction of NFκB in the myocardium. The levels of IRAK and TRAF6 in LmiR-146a transfected hearts were markedly reduced compared with the untreated CLP controls. Overexpression of miR146a also significantly attenuated CLP induced expression of IL-1β and IL-6 in both plasma and peritoneal fluid. We conclude that miR-146a attenuates sepsis induced NFκB activation, cytokine expression and cardiac dysfunction by targeting IRAK and TRAF6.

A SMALL MOLECULE SIRTUNI ACTIVATOR REDUCES CYTOKINE RELEASE AND COAGULATION ACTIVATION IN HUMAN ENDOTOXEMIA

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Silent information regulator transcript-1 (SIRT1) is a member of the sirtuin family that influences gene expression through deacetylation of histone and non-histone proteins. We here describe SRT2104 as a highly selective small molecule activator of SIRT1. To determine the effect of SRT2104 on the inflammatory and coagulation responses in normal healthy male subjects after exposure to LPS, we performed a randomized, double-blind, placebo-controlled study consisting of three treatment arms (N = 8 per arm): (1) Oral SRT2104 for seven consecutive days; (2) Placebo on Days 1-6 and SRT2104 on Day 7; (3) Placebo for seven consecutive days. On Day 7, all subjects received intravenous LPS (4 ng/kg) 3 hours after dosing with SRT2104/placebo. SRT2104 significantly attenuated LPS-induced release of IL-6 and IL-8 and activation of coagulation, as measured by the plasma levels of the prothrombin fragment F1+2. C-reactive protein levels measured 24 hours post-LPS were significantly lower in subjects treated with a single SRT2104 dose. This is the first human study to demonstrate biological anti-inflammatory responses consistent with the activation of SIRT1 by a small molecule.
21

ESTROGEN PROTECTS AGAINST OXIDANT-INDUCED ENDOTHELIAL DAMAGE BY RESTORING THE CORTICAL ACTIN CYTOSKELETON THROUGH A NON-GENOMIC PIP2-DEPENDENT PATHWAY

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A single dose of 17β-estradiol (E2) significantly reduces burn-induced systemic inflammation in rodents, and E2 promotes membrane ruffling, reinforces focal adhesions, and increases PIP2 production in vitro. PIP2 regulates the actin cytoskeleton and is an obligatory precursor for PIP3 in E2-induced Akt activation.

Objective: To determine if E2 increases PIP2 production in endothelial cells through the nongenomic pathway to protect against oxidant endothelial damage.

Methods: HUVEC endothelial cells were treated with E2 (10 nM, 10 min) or the membrane impermeable E2-dendrimer (EDC) that activates a nongenomic pathway exclusively, and the effects on PIP2 generation, cytoskeletal and signaling responses were compared. PIP5Kβ was silenced by RNA interference (RNAi) to evaluate its contribution of the E2 responses.

Results: EDC increased PIP2 generation and actin polymerization to a similar extent as E2, and these effects were abrogated by pretreatment of cells with ICI to inhibit the estrogen receptor α. E2 partially protected against H2O2 induced disruption of cell: cell junctions and cell: substrate adhesions, and restored paxillin phosphorylation. PIP5Kβ RNAi slowed cell migration into scratch wounds, decreased cytoskeletal responses to E2 or EDC, and attenuated E2-induced Akt and FAK activation.

Conclusions: E2 protects against oxidative stress through a non-genomic ERα dependent pathway, and PIP5Kβ is a key E2 effector that acts upstream of cytoskeleton remodeling and generation of pro-survival signals. Our findings provide mechanistic insight into how a single dose of E2 may protect against burn induced injuries in vivo. Supported by the NIH P50 GM02168 Burn Center Grant.

22

OPPOSITE ROLES OF ASC AND NLRP3 INFLAMMASOMES IN HOST DEFENSE DURING INFECTION WITH SEROTYPE 2 AND 3 STREPTOCOCCUS PNEUMONIAE

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Background & Objective: Streptococcus pneumoniae (Spneu) is a frequent causative agent of community acquired pneumonia and sepsis. Defined by capsular antigenicity, 90 Spneu serotypes exist. Previous studies, all using the common Spneu strain D39, have indicated that the ASC and NLRP3 inflammasomes are important components of protective immunity during pneumococcal pneumonia. Aim of the current study was to obtain insight into the roles of ASC and NLRP3 in pneumonia caused by different Spneu serotypes.

Methods: ASC knock out (KO), NLRP3 KO and wildtype (WT) mice were intranasally infected with one of two different Spneu strains: D39 (serotype 2, St2) or ATCC 6303 (serotype 3, St3). Low (5 × 104 CFU) or high (2 × 107 CFU) infectious doses were used.

Results: In accordance with literature, after infection with Spneu St2 both ASC KO and NLRP3 KO mice showed a reduced capacity to mount an early inflammatory response in the lungs accompanied by enhanced bacterial growth; ASC KO, but not NLRP3 KO mice showed enhanced lethality. However, much to our surprise, ASC KO and NLRP3 KO mice were strongly protected against lethality after infection with Spneu St3 (both P<0.001 vs WT mice), which was associated with an enhanced early inflammatory response in the lungs and reduced bacterial growth and dissemination.

Conclusion: The current paradigm that the ASC and NLRP3 inflammasomes are essential for protective immunity during pneumococcal pneumonia needs to be modified. The roles of ASC and NLRP3 depend on the Spneu serotype; these inflammasomes impair host defense during pneumonia caused by Spneu St3.

23

POST-BURN, SINGLE-DOSE ESTROGEN DECREASES INFLAMMATION IN THE HEART FOR 45 DAYS FOLLOWING SEVERE BURN INJURY

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Background: Patients with severe burn injury experience a rapid elevation in multiple circulating pro-inflammatory cytokines, with the levels correlating with both injury severity and outcome. Accumulations of these cytokines in animal models have been observed in remote organs, however data are lacking regarding long-term serial heart cytokine levels following burn injury, and the therapeutic effects of estrogen on these levels. Using a rat model, we studied the effects of a full-thickness 3º burn on cardiac levels of IL-6 and TNF-α. Here, we hypothesized that single-dose acute estrogen treatment decreases inflammation in the heart up to 45 days.

Methods: In this study, 186 male rats received a 3º 40% TBSA burn (divided into sham, burn+estradiol, or burn+placebo). Fifteen minutes following burn injury, the animals received a subcutaneous injection of either placebo or 17β-estradiol (0.5 mg/kg). Hearts were harvested at 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 24 hours, 7 days and 45 days after injury, and the cardiac cytokine levels (IL-6 and TNF-α) were measured by ELISA method.

Results: In the burned rats, 17β-estradiol significantly decreased the cardiac levels of TNF-α through 45 days post-burn, with the sham animal levels (30 pg/mg) more comparable to the estradiol group (70 pg/mg), and significantly less than the placebo animals (332 pg/mg).
Similarly, IL-6 levels in the sham animals (70 pg/mg) were comparable to the estradiol group (86.5 pg/mg), and significantly less than the placebo group (730 pg/mg) at 45 days post-burn.

**Conclusion:** Following severe burn injury, estrogens decrease cardiac inflammation. Importantly, estrogen treatment following burn injury significantly blocks the early and late burn-induced increase in TNF-α and IL-6 in the heart, which has previously been linked to a poor outcome.

24

**β-ARRESTIN1: AN IMPORTANT REGULATOR OF INFLAMMATION AND BACTERIAL CLEARANCE IN POLYMICROBIAL SEPSIS**

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β-Arrestins, initially identified as scaffolding proteins in GPCR signaling, have been shown to be important mediators of inflammation following endotoxemia. The aim of the current study is to delineate the role of β-arrestin1 in polymicrobial sepsis via cecal ligation and puncture (CLP). We performed CLP on WT, A2−/− and A2+/− mice and examined progression of sepsis including, inflammatory response, resolution of infection and mortality. At 6 hrs post-CLP, serum and peritoneum fluid levels of IL-6 and IL-10 were significantly enhanced in A2−/− mice compared to wild type mice. Interestingly, A2−/− mice also had enhanced cytokine production in serum and peritoneum fluid suggesting that β-arrestin1 likely regulates inflammation in a gene-dose dependent fashion. The number of infiltrating monocytes, neutrophils and macrophages in the peritoneum were comparable in all three genotypes demonstrating that the enhanced inflammatory response is not due to differential infiltration of immune cells. We also observed exaggerated production of inflammatory mediators in heart (iNOS, TNFα) tissue of septic A2−/− mice in comparison to septic WT mice, possibly exposing the tissues to greater extent of collateral damage and resulting MODS. Contrary to enhanced production of inflammatory mediators; bacterial clearance was impaired in the A2−/− mice, with higher bacterial load in blood and peritoneum at 32 hrs post CLP, compared to the wild type mice. The A2+/− mice, however, had bacterial load comparable to the wild type. In accordance with enhanced inflammatory mediators and increased bacterial load, the A2−/− mice also had higher

![Graph showing TNF-α levels in heart tissue over time for different groups](image1.png)

**One Dose of E2 Delivered Post-Burn Significantly Reduces Cardiac TNF-α for 45 Days.**

![Graph showing IL-6 levels in heart tissue over time for different groups](image2.png)

**One Dose of E2 Delivered Post-Burn Significantly Reduces Cardiac IL-6 for 45 Days.**

![Diagram illustrating the role of β-arrestin1 in mediating progression of polymicrobial sepsis](image3.png)

**Fig. 2. Schematic representation of proposed role of β-Arrestin1 in mediating progression of polymicrobial sepsis.**
mortality as compared to WT and A2<sup>−/−</sup> mice. Together, these data suggest that β-arrestin1 is an important regulator of inflammation and bacterial clearance, thereby affecting the outcome in septic peritonitis.

SYNDECAN-1 MEDIATES GUT PROTECTION BY ENTERAL GLUTAMINE IN A RODENT MODEL OF GUT ISCHEMIA/REPERFUSION

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**Objective:** Syndecan-1 is the predominant heparan sulfate proteoglycan on epithelial cells, and like glutamine, is essential in maintaining the intestinal epithelial barrier. We therefore hypothesized that syndecan-1 contributed to enteral glutamine’s protection of the postischemic gut.

**Methods** Wild type (WT) and syndecan-1 knockout (KO) mice were administered enteral glutamine followed by 60 min of gut ischemia/reperfusion (IR). Intestinal injury was assessed by fluorescent dye clearance from an intestinal sac using IVIS Imaging System (IVIS); permeability as mucosal to serosal clearance by ex vivo everted sacs, and inflammation by myeloperoxidase (MPO) activity. Results were analyzed by ANOVA then Tukey, n=6/group, mean ±SEM. Means with different letters are significantly different.

**Results:** Gut IR resulted in a significant increase in intestinal permeability, inflammation, and injury which were further increased in syndecan-KO mice. Glutamine, however, decreased intestinal permeability, inflammation, and injury in WT mice but these protective effects were abolished in syndecan-1 KO mice (table and figure).

**Conclusions:** Syndecan-1 plays a novel and critical role in the protective effects of enteral glutamine in the postischemic gut and supports the use of glutamine as a gut specific pharmacocounter. Further investigation into the precise mechanism by which glutamine modulates syndecan-1 warrants further investigation.

**Table:**

| Groups         | Permeability (ng/cm²/min) | MPO (mU/mg) | Fluorescence Intensity |
|---------------|---------------------------|-------------|------------------------|
| WT sham       | 28.3 ± 1.6 a              | 0.24 ± 0.01 a | 1.9x10⁸ ± 2.2x10⁷ a     |
| KO sham       | 33.8 ± 3.4 a              | 0.27 ± 0.01 a | 1.7x10⁸ ± 3.0x10⁷ a     |
| WT IR         | 87.8 ± 2.5 b              | 1.25 ± 0.12 b | 3.2x10⁸ ± 1.4x10⁷ b     |
| KO IR         | 119.8 ± 9.0 c             | 1.83 ± 0.13 c | 4.8x10⁸ ± 1.8x10⁷ c     |
| WT IR glut    | 59.2 ± 9.03 d             | 0.77 ± 0.06 d | 2.6x10⁸ ± 1.8x10⁷ d     |
| KO IR glut    | 116.8 ± 9.92 c            | 1.54 ± 0.13 c | 4.6x10⁸ ± 2.1x10⁷ c     |

**Figure:**

IVIS imaging of excised intestinal sacs 6 hours after reperfusion. Higher intensity (indicated by yellow) indicates greater injury.

HYPERTONIC SALINE RESUSCITATION WITHOUT DEXTRAN INHIBITS NEUTROPHIL ACTIVATION

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**Background:** Hypertonic saline (HS) resuscitation improves outcome in animal models at least in part by reducing neutrophil activation. However, in a recent clinical trial with prehospital bolus infusion of 250 ml HS (7.5% NaCl) or HS+6% dextran-70 (HSD) we have not been able to reproduce the beneficial effects observed in animal studies.

**Objectives:** To determine possible reasons for such disparate results, we studied how HS and HSD influence neutrophil activation in trauma patients using blood samples from a subset of patients enrolled in Toronto and Seattle as part of the HS Resuscitation Outcomes Consortium (ROC) trial.

**Design:** Shock patients (SBP<70 mmHg) were randomly assigned to receive HS (n=9), HSD (n=8), or normal saline (NS; n=17). Neutrophil activation (oxidative burst, myeloperoxidase, metalloproteinase 9) was assessed at admission to the emergency department (ED<3h) and 12 and 24 h after admission and compared to values of age-matched healthy controls (n=20).

**Results:** Trauma caused neutrophil priming and activation that was significantly higher in the NS group compared to healthy controls. This upregulation was inhibited in the HS group, but not in the HSD group.

**Conclusions:** These findings suggest that hypertonic resuscitation with HS but not HSD can reduce post-traumatic inflammation.
in shock patients. However, these results cannot explain why hypoten-
tonic resuscitation failed to improve clinical outcome in the parent
ROC trial.

Funding: Defence R&D Canada and NIH R01GM076101.

27

ALTERED GUT FLORA IN PATIENTS WITH SEPSIS: SURVIVORS VS. NON-SURVIVORS
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Background: The gut is considered an important target organ of injury after severe insult such as sepsis, trauma, and shock. The role of gut flora has been focused on in perspective of immunity such as regulatory T cells. Gut flora in patients with sepsis has not been thoroughly clarified. In the present study, we quantitatively evaluated changes in the gut microflora including Clostridium species in patients with sepsis by reverse transcription quantitative polymerase chain reaction (RT-qPCR) technique.

Methods: Sixty-two patients with sepsis were included in our study (mean age 62.1±20.6 years, APACHE II score 16.7±9.1). A fecal sample was used for quantitative evaluation of 13 kinds of microflora by reverse transcription quantitative polymerase chain reaction (RT-qPCR) technique. Data obtained from patients were compared between survivors (n=46) and non-survivors (n=16).

Results: Analysis of fecal flora confirmed that septic non-survivors had significantly lower obligate anaerobes (especially Clostridium cocoides group (survivors 7.8±1.8 vs. non-survivors 6.1±1.7)), C. leptum subgroup (7.7±1.8 vs.6.1±1.8), Bacteroides fragilis group (8.3±1.6 vs. 6.7±2.2), Bifidobacterium (7.3±1.8 vs. 6.2±1.8), Atopobium cluster (7.3±1.8 vs.5.9±1.3), and facultative anaerobes (Lactobacillus(6.6±1.8 vs. 5.5±1.6), Enterobacteriaceae (6.8±1.5 vs. 5.8±1.4) and Enterococcus (7.5±1.8 vs. 6.0±2.0)) than those in survivors. The number of pathogenic bacteria (Staphylococcus (5.1±1.7 vs. 5.2±1.5), Pseudomonas (3.7±1.7 vs. 3.7±1.3) had no significant difference between the two groups. (Log10 counts/g feces)

Conclusions: The gut flora is significantly altered in patients with sepsis especially in non-survivors. Altered gut flora may disrupt immunity and affect systemic inflammatory response in sepsis.

28

IMMUNE-ENHANCING PROPERTIES OF STATIN AND COENZYME Q10 TREATMENT IN SEPSIS
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We recently reported that treating septic mice with the farnesylation inhibitor drug, FTI-277, could improve mouse survival. Statins and FTI-277 modulate the mevalonate pathway and accordingly, we found that treating mice with Atorvastatin was nearly as effective as using FTI-277. One complication of statin treatment is the marked reduction of coenzyme Q10 (CoQ10) levels in cells leading to loss of mitochondrial function and integrity. In this study, we tested whether combined CoQ10 and Atorvastatin treatment might enhance the effectiveness of Atorvastatin treatment alone. Groups of mice underwent cecal ligation and puncture (CLP) sepsis or sham CLP and were treated with 20 mg/kg Atorvastatin and 40 mg/kg CoQ10 at 1 day after CLP. At 48 hours, we measured the effects of treatment on systemic cytokine levels and immune cell subset changes. To test for potential immune-enhancing activity, sham or CLP mice were immunized with the T cell dependent antigen, TNP-haptenated ovalbumin, and treated with Atorvastatin, CoQ10, or a combination. Antigen-specific antibody isotype formation was measured at 7 days after CLP to judge helper T cell responses in vivo. Combined Atorvastatin and CoQ10 treatment prevented sepsis-induced immune cell subsets losses. Plasma cytokine profiling showed that Atorvastatin/CoQ10 treatment significantly boosted systemic IFNγ, IL-10, IL12, and IL13 levels in septic mice. Immunized CLP mice showed marked suppression of antigen-specific IgG formation, which was partially restored by combined treatment, but not single drug therapies. Collectively, these findings demonstrate that combined Atorvastatin/CoQ10 treatment has immune system enhancing effects in a mouse sepsis model and suggests a potential for using statin with CoQ10 as treatment to improve immune function in patients that develop sepsis.

29

THE EFFECT ON GLYCEMIC CONTROL OF A LOW-CARBOHYDRATE, HIGH-FAT ENTERAL FORMULA IN CRITICALLY ILL PATIENTS ADMITTED TO A TRAUMA AND CRITICAL CARE CENTER
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Hyperglycemia is considered to be associated with the mortality and morbidity of critically ill individuals. We employed a high-fat low-carbohydrate enteral formula, reported to be useful for controlling blood glucose in diabetes, for patients admitted to our trauma and critical care center from April 2010 instead of the standard enteral formula. The aim of this study was to clarify the effect on glycemic control of this high-fat low-carbohydrate enteral formula in comparison with the standard enteral formula. A total of 147 mechanically ventilated patients hospitalized between April 2009 and March 2011 were enrolled in this study. Among them, 74 patients hospitalized between April 2009 and March 2010 received the standard enteral formula (Group S), while 73 patients hospitalized between April 2010 and March 2011 received the high-fat low-carbohydrate enteral formula (Group G). Enteral feeding was started within 7 days after admission. Each formula was given continuously starting at a rate of 20 mL per hour, which was increased to provide an appropriate calorie intake within a few days of starting nutritional support. We measured the maximum blood glucose level and need for insulin therapy after starting enteral nutrition in both groups. There were no significant differences between the two groups with regard to age, sex, underlying diseases, and blood glucose at the start of feeding. The maximum blood glucose level of group S (163±32.0 mg/dL) was significantly higher than that of group G (151±28.4 mg/dL) (p=0.022). The rate of starting insulin therapy was significantly higher in group S (12.2%) than in group G (1.37%) (p<0.018). In conclusion, a high-fat low-carbohydrate enteral formula is more effective for glycemic control in critically ill patients compared with a standard enteral formula.
PROTECTIVE ROLE OF THE GUT MICROBIOME IN THE HOST DEFENSE AGAINST STREPTOCOCCUS PNEUMONIAE INDUCED PNEUMONIA T. Schuit*, i.p. EGF or an mice to 1-/- L.S. Jaffree, Z. Liang, E.R. Breed, and EGF worsened 7-day survival in septic Rag-1 mice. This was accompanied by a decrease in in-Rag-1 were significantly decreased in mice receiving EGF. G mice were subjected to 2 2 Lymphocytes aid in mediating gut epithelial apoptosis. To determine if EGF interacts with lymphocytes to improve survival, which was analyzed using the Log rank test.

METHODS: Rag-1+/− mice were subjected to 2×25 cecal ligation and puncture and randomized to receive 150 μg kg⁻¹ i.p. EGF or an equivalent volume of saline. Groups were either compared at 24 hrs for villus length, permeability, apoptosis, and serum and peritoneal fluid inflammatory cytokines (n = 10–12) or followed for survival (n=18). Mann Whitney analysis was used for all comparisons except survival, which was analyzed using the Log rank test.

RESULTS: EGF worsened 7-day survival in septic Rag-1+/− mice to 16% while mice receiving saline had 61% survival (p=0.005). This is contrary to what has previously been shown in septic mice with intact immune systems where EGF improved survival by 50%. EGF did, however, decrease gut apoptosis in both H&E and active caspase 3 analyses in Rag-1+/− mice. This was accompanied by a decrease in intestinal Bax expression to undetectable levels. EGF treatment groups had no difference in villus length or intestinal permeability when compared to saline controls. Serum IL-1 and TNFα and peritoneal fluid TNFα were significantly decreased in mice receiving EGF.

CONCLUSION: EGF drastically diminishes survival in sepsis when lymphocytes are not present, however, retains its ability to improve gut apoptosis. These findings suggest that EGF’s ability to decrease apoptosis may not be responsible for its role in survival in mice with intact immune systems and demonstrate the critical role the adaptive immune system plays in the protective action of EGF in sepsis.

THE PROTECTIVE EFFECT OF EPIDERMAL GROWTH FACTOR DURING SEPSIS DEPENDS ON THE ADAPTIVE IMMUNE SYSTEM L.S. Jaffree, Z. Liang, E.R. Breed, and C.M. Cooper smith*. Emory University SOM, Atlanta, GA

Background: Lymphocytes aid in mediating gut epithelial apoptosis during sepsis. Epidermal growth factor (EGF) is a cytoprotective polypeptide that restores gut integrity and improves survival by 50% in septic mouse models.

Objective: To determine if EGF interacts with lymphocytes to improve survival and protect gut integrity in sepsis.

Methods: Rag-1+/− mice were subjected to 2×25 cecal ligation and puncture and randomized to receive 150 μg kg⁻¹ i.p. EGF or an equivalent volume of saline. Groups were either compared at 24 hrs for villus length, permeability, apoptosis, and serum and peritoneal fluid inflammatory cytokines (n = 10–12) or followed for survival (n=18). Mann Whitney analysis was used for all comparisons except survival, which was analyzed using the Log rank test.

Results: EGF worsened 7-day survival in septic Rag-1+/− mice to 16% while mice receiving saline had 61% survival (p=0.005). This is contrary to what has previously been shown in septic mice with intact immune systems where EGF improved survival by 50%. EGF did, however, decrease gut apoptosis in both H&E and active caspase 3 analyses in Rag-1+/− mice. This was accompanied by a decrease in intestinal Bax expression to undetectable levels. EGF treatment groups had no difference in villus length or intestinal permeability when compared to saline controls. Serum IL-1 and TNFα and peritoneal fluid TNFα were significantly decreased in mice receiving EGF.

Conclusion: EGF drastically diminishes survival in sepsis when lymphocytes are not present, however, retains its ability to improve gut apoptosis. These findings suggest that EGF’s ability to decrease apoptosis may not be responsible for its role in survival in mice with intact immune systems and demonstrate the critical role the adaptive immune system plays in the protective action of EGF in sepsis.
24 Abstracts

33

DISCOVERY OF A NOVEL SMALL MOLECULE INHIBITOR OF TOLL-LIKE RECEPTOR 4 (TLR4) WITH ANTI-INFLAMMATORY EFFECTS IN MURINE AND HUMAN TISSUE M.D. Neal1, S. Kim1, C.P. Sodhi1, P. Wipfa, J. Brodsky1, T.R. Billiar2, and D.J. Hackam3. 1University of Pittsburgh School of Medicine, Department of Surgery, Pittsburgh, PA, 2University of Pittsburgh Department of Chemistry, Pittsburgh, PA, 3University of Pittsburgh Department of Biological Sciences, Pittsburgh, PA

Introduction: TLR4 signaling plays a critical role in the pathogenesis of many inflammatory and infectious disorders. We sought to identify novel TLR4 inhibitors with potential anti-inflammatory properties and to test them directly in models of TLR4-mediated inflammation using an in silico to in vivo approach.

Methods: An in silico screen of 26 million compounds was performed to identify potential TLR4 antagonists based on homology to the TLR4-LPS binding domain. Selected compounds were screened for efficacy in vivo. Secondary screens for TLR4 inhibition were performed using in vivo imaging (IVIS, Xenogen) in an NFκB luciferase reporter mouse. Tertiary screens included murine models of TLR4-mediated inflammation: hemorrhagic shock and necrotizing enterocolitis (NEC).

Results: Sixty-five (65) compounds were identified as potential TLR4 antagonists and 21 compounds reduced the severity of endotoxia in vivo. Reduction of NFκB expression as a marker of TLR4 signaling occurred in 8 compounds, but one compound (C34) demonstrated robust TLR4 inhibition. C34 dramatically reduced disease severity and the expression of mucosal iNOS (p<0.01) in murine NEC. Additionally, pretreatment with C34 resulted in a significant reduction of liver injury as measured by AST/ALT expression (p<0.05) in mice subjected to hemorrhagic shock. To test the physiologic efficacy of C34, freshly isolated human intestinal mucosa from an infant with NEC was exposed to C34 ± LPS ex vivo. Strikingly, the production of mucosal iNOS was significantly inhibited by pretreatment with C34 (p<0.01).

Conclusions: We have discovered a novel inhibitor of TLR4 with potent anti-inflammatory effects using a combination of in silico and in vivo screening. These data suggest that C34 may provide a novel therapy in diseases of TLR4 mediated inflammation.

34

INHIBITION OF LIPOGENESIS REDUCES INFLAMMATION AND ORGAN INJURY INDUCED BY SEPSIS J.P. Idrovo1, 2, W. Yang1, 2, J. Nicastro1, G.E. Coppa1, and P. Wang2, 3. 1Hofstra North Shore-LIJ School of Medicine, Manhasset, NY, 2The Feinstein Institute for Medical Research, Manhasset, NY

Introduction: Sepsis is a life-threatening acute inflammatory disease and associated with metabolic complications. Accumulation of free fatty acids (FAA) induces inflammation and causes lipotoxic effects in the liver. Fatty acid synthase is a rate-limiting enzyme of generating FAA or lipogenesis and its activity can be inhibited by C75, a synthetic compound. We hypothesized that administration of C75 could alleviate the injury caused by sepsis.

Methods: Male mice were subjected to sepsis by cecal ligation and puncture (CLP). At 4 h after CLP, different doses of C75 (1 or 5 mg/kg BW) or vehicle (20% DMSO in saline) was injected intraperitoneally. Blood and liver tissues were collected at 24 h after CLP for various measurements. Murine macrophage RAW 246.7 cells were used to analyze the effect of C75 on TNF-α release induced by LPS (10 ng/ml).

Results: The levels of FAA in the liver of C75-5 mg group were lower than those in the vehicle group (29.1 ± 3.5 vs. 53.5 ± 3.8 pmol/g tissue, p < .05). Administration of C75 reduced the levels of serum proinflammatory cytokines and organ injury indexes in a dose-dependent manner. The measurements among the sham, vehicle, and C75 treated groups 24 h after CLP are summarized in the table below. Moreover, the levels of TNF-α release in RAW 246.7 cells after 4 h-exposure to LPS were reduced by 32% and 74% in the presence of 10 and 50 μM C75, respectively (p < .05). These doses of C75 did not affect the viability of RAW 246.7 cells.

Conclusions: C75 effectively lowered FAA accumulation in the liver, which was associated with inhibition of inflammation and reduction of organ injury after CLP. In addition, C75 had a direct activity on inhibiting inflammatory responses to LPS in macrophages. Thus, C75 has a potential to be developed as a novel therapeutic agent for treating sepsis.

Table 1. Effect of C75 on Inflammation and Organ Injury Induced by Sepsis

| Group   | Serum TNF-α (pg/ml) | Serum IL-6 (pg/ml) | ALT (UI/L) | AST (UI/L) | LDH (UI/L) |
|---------|---------------------|-------------------|------------|------------|------------|
| Sham    | 2.3 ± 0.2           | 1509.4 ± 304.2    | 28.6 ± 2.8 | 170.1 ± 7.6 | 501.0 ± 92.1 |
| Vehicle | 143.9 ± 39.8        | 2186.7 ± 206.6    | 28.6 ± 2.8 | 84.8 ± 12.0 | 284.3 ± 40.8 |
| C75-1 mg| 82.1 ± 32.1         | 197.3 ± 33.0      | 18.1 ± 4.1 | 64.3 ± 9.4  | 100.1 ± 5.1 |
| C75-5 mg| 12.4 ± 2.6*         | 284.3 ± 40.8      | 9.7 ± 2.0  | 9.6 ± 2.0   | 100.1 ± 5.1 |

Mean = SEM, n=5/group; one-way ANOVA, *p < .05 vs. Sham; p < .05 vs. Vehicle.

35

RESVERATROL IMPROVES COGNITIVE PERFORMANCE IN CONCUSED ATHLETES J. Gatson1, 2, J.R. Billiar1, 3, M. Cullum1, J. Barillas1, K. Abdel fattah1, S. Wolf2, J. Simpkins2, M. Cullum1, J. Wigginton3, and J. Minei4. 1UT Southwestern Medical Center, Dallas, TX, 2University of North Texas Health Science Center, Fort Worth, TX

Background: Each year, approximately three million sports-related concussions occur in athletes. Only about 5% of these concussions are treated in the hospital setting. To date, there are no effective interventions used at decreasing the levels of oxidative injury and inflammation within these athletes. In previous studies, following stroke and TBI, resveratrol decreased the levels of oxidative stress and lesion volume in the brain. These studies suggest that resveratrol may afford protection from secondary injury in individuals suffering from brain injuries such as sports concussions.

Methods: Boxers (n=12) were consented to participate in the Resveratrol and Sports Concussion Study (REPAIR) and prior to competition (within 2 weeks), a baseline Immediate Post-Concussion Assessment and Cognitive Testing (ImPACT) test was administered to the athletes. After injury (within 2 hours), the boxers that experienced a concussion were enrolled in this study. The boxers were randomized to either placebo or resveratrol treatment. At day 2, 7 and 30 after injury, the ImPACT test was administered.

Results: In the resveratrol group, the boxers had improved reaction time, speed, and accuracy. With respect to reaction time, in the placebo group, there was an approximate 4-fold increase in reaction
time. The boxers that received resveratrol treatment had post-injury reaction times similar to their baseline levels. In addition, the boxers that received resveratrol performed better (~2-fold increase) on the symbol match test (speed and accuracy).

**Conclusion:** In this safety and feasibility pilot clinical trial, we found that there was a modest improvement with reaction time, speed, and accuracy. These results suggest that resveratrol treatment after mild TBI (concussion) may afford athletes protection from secondary brain injury.

## 36

**CANNABINOID 2 RECEPTOR ACTIVATION IS BENEFICIAL DURING A MURINE MODEL OF SEPSIS**

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Our previous studies have demonstrated that endogenous cannabinoids, signaling through the cannabinoid receptor 2 (CB2R), are critical regulators of the immune response during experimental sepsis. We hypothesized that during sepsis, CB2R gain-of-function would improve the murine response to sepsis. To induce sepsis, mice were subjected to a cecal ligation and puncture (CLP). Consistent with our previous data, CB2R-agonist treatment in wild-type mice increased the mean survival time in response to CLP, while decreasing serum IL-6 levels, bacteremia, and damage to the lungs (Figure 1) compared to vehicle-treated mice. We observed that macrophages isolated from GP1a-treated septic mice demonstrated decreased MHC II expression and increased phagocytic ability. Previous biochemical studies showed that CB2R is a Gi-coupled receptor known to negatively regulate adenyl cyclase. The adenyl cyclase/CAMP circuit is a critical regulator of NF-kB and p38, molecules that play essential roles in inflammation and immune responses. Indeed, we found that CB2R agonist treatment decreased neutrophil recruitment into the peritoneal cavity, increased p38 activity, and increased phagocytic function. Altogether, we show that the CB2R plays a critical role in myeloid cell recruitment and function, thereby acting upon a major regulatory pathway of mortality in sepsis.

![Figure 1: Systemic treatment with the CB2R agonist decreases pulmonary injury. H&E-stained lung sections of A) wild-type, or B) mice treated with GPs 24 hours after CLP. C) Protein amount in lung BAL. The sample size equals 6 per group. Values represent the mean ± SEM. **p < 0.01, compared to wild-type.](image)

### 37

**A CARDIOVASCULAR HERBAL MEDICINE (TANSHINONE IIA) FACILITATES CELLULAR HMGB1 UPTAKE**

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The discovery of HMGB1 as a late mediator of lethal systemic inflammation has initiated a new field of investigation for the development of experimental therapeutics. We previously reported that a major ingredient of Danshen (Tanshinone IIA sodium sulfonate, TSN-SS) rescued mice from lethal sepsis by specifically inhibiting endotoxin-induced HMGB1 release.

**Objectives:** To investigate the underlying mechanisms by which TSN-SS effectively inhibits HMGB1 release.

**Methods:** We determined whether TSN-SS stimulated HMGB1 uptake in macrophage cultures; and whether genetic depletion of HMGB1 receptors (e.g., TLR2, TLR4, or RAGE) or pharmacological inhibition of clathrin-mediated endocytosis impaired TSN-SS-facilitated HMGB1 uptake.

**Results:** TSN-SS facilitated the uptake of recombinant HMGB1 protein into macrophage LC3-positive cytoplasmic vesicles (likely autophagosomes) in a time- and dose-dependent fashion. Simultaneously, it dramatically enhanced HMGB1-induced production of an autophagy marker, LC3-II, even in the presence of an autophagy inhibitor, bafilomycin A1, possibly facilitating autophagic degradation of cytoplasmic HMGB1 in macrophages. Genetic depletion of TLR2, TLR4, and/or RAGE did not affect TSN-SS-mediated enhancement of HMGB1 uptake, eliminating the potential involvement of these HMGB1 receptors in its cellular uptake. In contrast, a specific clathrin inhibitor, chlorpromazine, effectively abolished TSN IIA-SS-mediated HMGB1 uptake.

**Conclusions:** It is possible to employ herbal medicine to pharmacologically “recycle” potentially injurious proinflammatory mediators. (Supported by the National Institutes of Health Grants R01GM063075 and R01AT05076).

### 38

**MUNG BEAN (VIGNA RADIATA) EXTRACT IS PROTECTIVE AGAINST LETHAL SEPSIS IN MICE**

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The mung bean is commonly used as a food source worldwide, but has also been prescribed as a traditional Chinese herbal medicine in the treatment of a number of inflammatory ailments since 1050’s. Recent evidence has suggested a pathogenic role for HMGB1 as a “late” mediator of lethal systemic inflammation with a relative wider therapeutic window for pharmacological interventions.

**Objectives:** To explore the HMGB1-inhibiting capacity and therapeutic potential of Mung bean hull extract (MBH) in vitro and in vivo.

**Methods:** Human U937 or murine RAW 264.7 macrophage-like cell cultures were stimulated with bacterial endotoxin (LPS) in the absence or presence of MBH, and HMGB1 release was assessed 16 hours later. Male Balb/C mice (20-25 g, 6-7 weeks old) were subjected to experimental sepsis by cecal ligation and puncture (CLP), and MBH extract was orally administered at 24, 48, and 72 h post CLP.

**Results:** MBH extract dose-dependently abrogated LPS-induced HMGB1 release in both murine and human macrophage cultures. Meanwhile, it stimulated HMGB1 protein aggregation, and elevated LPS-induced production of an autophagy marker, LC3-II, thereby facilitating autophagic HMGB1 degradation in human and murine macrophage cultures. Oral administration of MBH extract significantly increased animal survival rates from 29.4% (in control saline group, N=17 mice) to 70% (in experimental Mung bean extract group, P < 0.05).

**Conclusion:** These data suggest that Mung bean hull extract is protective against lethal sepsis partly by attenuating endotoxin-induced HMGB1 release via stimulating its autophagic degradation.
RESVERATROL REDUCES ENDOPLASMIC RETICULUM STRESS FOLLOWING TRAUMA-HEMORRHAGE
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Resveratrol (RSV), a plant polyphenol, is an antioxidant and specific activator of Sirt1. Our functional mitogenomics experiments demonstrated a down regulation of Sirt1 following trauma-hemorrhage (T-H). Our subsequent experiments showed that RSV prevents T-H-induced Sirt1 downregulation, and has salutary effect on left ventricular contractility and mitochondrial (mt) function following T-H. However, the specific influence of RSV on metabolic perturbation following T-H remains unknown. Accordingly, we sought to determine the effect of RSV on endoplasmic reticulum (ER) stress following T-H. Previous studies from our laboratories have demonstrated the initiation of ER stress following T-H. In this study, we specifically tested the level of expression of ER stress proteins in the left ventricular tissue of Sprague Dawley rats subjected to T-H, followed by RSV (8 mg/Kg body weight); sirtinol (a specific inhibitor of Sirt1) or vehicle administration. RSV or vehicle was administered I.V. at 10 minutes after the onset of resuscitation. mt complex I activity and total ATP content were determined. Total proteins were tested for Bip (immunoglobulin heavy chain binding protein), IRE-1a (inositol-requiring kinase 1) and PDI (protein disulphide isomerase); and the pro-apoptotic protein, CHOP (C/EBP homologous protein, also known as GADD153) (n=3-4/group) by Western blot. RSV treatment improved total ATP content (p<0.05) and complex I activity (p<0.05). RSV treatment also abrogated the elevated expression of Bip, IRE-1a, PDI and CHOP following T-H. Sirtinol abolished the salutary effect of RSV. The results demonstrate that RSV reduces ER stress, when administered as an adjunct to resuscitation fluid. We conclude that RSV may be a useful adjunct to resuscitation fluid.

WATER SOLUBLE ESTROGENS PROLONG PERMISSIVE HYPTENSION FOLLOWING MAJOR BLOOD LOSS EVEN WITHOUT FLUID RESUSCITATION
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A DARPA research program termed SBL (surviving blood loss) targeted a practical means to enable warfighters to survive on the battlefield. Success was defined initially as 3-hour and subsequently as 6-hour survival from 60% blood loss in rats without resuscitation. We examined use of small volume estrogen (0.4 ml/kg) to facilitate life support, adding an additional “real world” injury of soft tissue trauma (midline laparotomy). Preliminary testing with this model revealed that efficacy hinged on delivering the hormone rapidly in a supraphysiologic dose in a water-soluble form. We explored 6 routes of delivery: intravenous (IV), intraosseous (IO), sublingual, intranasal, subcutaneous and intramuscular; we found IV and IO routes to be superior in attaining a rapid, high blood level. We selected IV administration and tested 3 soluble forms of estrogen (E2-cyclodextrin, E2-sulfate and ethynyl estradiol sulfate [custom synthesis, EE-SO4]). EE-SO4 was selected as the ultimate test article owing to its greater half-life and biological activity. Tests from a panel of 4 doses (0.1, 0.3, 1.0 and 3.0 mg/kg) ascertained that the 1 mg/kg dose was superior, based on 6-hour survival percentages of 16, 50, 87 and 50, respectively. The greater biological activity of EE-SO4 was confirmed through the use of isolated rat aortic rings in a vasorelaxation test system. Titrating the EE-SO4 in a logarithmic series from 1x10^-4 to 1x10^-1 M, we found that EE-SO4 was most effective at the lowest doses as compared to E2-SO4; it additionally demonstrated a steeper response curve. Our examination of the mechanism for the relaxation response revealed that it was endothelium-dependent and mediated by NO. Thus, this synthetic estrogen holds high potential for SBL therapy for both military and civilian use (DARPA W911NF-06-1-0219, W911NF-10-1-0130).

TLR4 ANTAGONISM RESTORED CARDIAC FUNCTION AFTER TRAUMA-HEMORRHAGE
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Trauma-hemorrhage (TH) induced cell and tissue injuries can release endogenous damage-associated molecular patterns (DAMPs) that are recognized by Toll-like receptors (TLRs) to initiate immune and inflammatory responses. Uncontrolled immune and inflammatory responses can lead to tissue damage, multi organ failure or death and cardiovascular collapse is one of the major factors contributing to the demise of TH patients. Our recent studies indicate that TLR4 deficient mice show attenuation of cardiac dysfunction due to TH. Eritoran tetrasodium (E5664), a TLR4 antagonist, has been evaluated in phase III trial as a treatment for severe sepsis. The primary objective of this study was to evaluate therapeutic effect of E5664 on cardiac dysfunction in our mouse model of TH. Trauma by soft tissue injuries and hemorrhage by blood withdrawal with mean arterial pressure maintained at 35±5 mmHg were established. Arterial and left ventricular (LV) pressures, as well as LV volumes were determined. Our results show that E5564 treatment significantly improved hemodynamic functions compared to placebo group after 60 min of TH in a dose dependent fashion (n=10/group). Using the optimal dose of E5564 (3 mg/kg), a single intravenous administration of the drug without fluid resuscitation prevented hemodynamic collapse when given at 1, 2, 3, or 4 hours after TH (n=10/group). E5564 also significantly attenuated TH-decreased phospho-akt/Akt ratio and ameliorated TH-increased NF-κB binding activity in the myocardium. In summary, TLR4 antagonist E5564 significantly attenuated TH-induced cardiac dysfunction. TLR4 antagonism may be a novel treatment strategy for managing cardiac dysfunction and enhancing recovery in TH patients.
ADIPONECTIN (ADPN) IS A KEY COMPONENT IN FRESH FROZEN PLASMA (FFP) RESPONSIBLE FOR ITS ENDOTHELIAL PROTECTIVE EFFECTS AFTER HEMORRHAGIC SHOCK (HS). X. Deng, T. In vitro, D.R. Lexcen. The only FFP fraction (#6) that contained the highest level of SEM. FFP was fractionated using fast performance liquid chromatography (FPLC) and tested for Adpn level and AMPK activation. Adpn was depleted from FFP by immunoprecipitation with an anti-Adpn antibody [FFP(Adpn-)] using an isotype IgG as control [FFP(IgG)]. Endothelial protective effects were evaluated using in vitro permeability assay and the mouse model of laparotomy/HS. Results: The only FFP fraction (#6) that contained the highest level of Adpn induced AMPKalpha phosphorylation in human pulmonary microvascular endothelial cells (HPMECs). Immunodepletion resulted in >90% removal of Adpn from FFP. In vitro, phosphorylation of AMPKalpha and inhibition of hypoxia-induced permeability were reduced by 70.8% and 73.8%, respectively, in HPMECs treated with FFP(Adpn-) compared with FFP(IgG). Laparotomy/HS mice resuscitated with FFP(Adpn-) had increased pulmonary vascular permeability as determined by Evans blue dye extravasation (0.28±0.59 vs 9.0±3.9 mmHg, P=0.042) compared with FFP(IgG).

Conclusions: Our study suggests that Adpn is a key component in FFP responsible for its endothelial protective effects after HS. Re-storing plasma Adpn level may be a novel strategy in the treatment of trauma/HS patients.

SERUM METABOLOMIC ANALYSIS OF THE EFFECTS OF GLUCOSE PRE-FEED ON METABOLISM IN A PORCINE MODEL OF POLYTRAUMA AND HEMORRHAGIC SHOCK. D.R. Lexcen1, E.R. Luszek1, N. Witowski1, J. Ashgar1, P. Iyegha1, K. Mulier1, and G. Beilman1. University of Minnesota, Minneapolis, MN

Hemorrhagic shock is a leading cause of trauma related deaths. Small animal studies demonstrated that pretreatment with glucose prior to hemorrhage results in a survival benefit. The mechanism of benefit is not completely understood. Thus, it was our goal to elucidate the metabolic response and effects on survival of a glucose pre-feed in a porcine polytrauma and hemorrhagic shock model.

Thirty-two male Yorkshire pigs underwent pulmonary contusion, liver crush injury, and controlled bleed. After polytrauma and shock, animals received 1 hour of limited resuscitation, followed by twenty hours of a standard resuscitation protocol. 1H-NMR spectroscopy was used to analyze serum samples from animals that were randomized to receive glucose prior to experiment (n=16) or fasted (n=16). Partial least squares discriminant analysis (PLS-DA) was used to identify differences in metabolic profiles.

Mortality was not significantly different between glucose pre-fed animals compared to fasted animals (50.0% vs. 31.3%, p=0.36). PLS-DA analysis revealed significant separation between the fasted and pre-fed groups. The most discriminant metabolites contributing to the model were glucose and metabolites related to alternate energy production pathways. PSL-DA also revealed distinct metabolic changes associated with mortality. Specific biomarkers of mortality were succinate, choline, hypoxanthine and uridine.

Contrary to previous studies, we observed no survival benefit with pretreatment of glucose in this study. Glucose prefeed was associated with a significantly altered metabolic profile in response to hemorrhagic shock. Serum biomarkers predictive of mortality in this large animal model include succinate, choline, hypoxanthine, and uridine. Future work will include confirmation of these biomarkers in human studies.
resuscitation time (100 min) followed by electron paramagnetic resonance spectroscopy.

Results: During reperfusion mean arterial pressure was higher in HG/NG compared to GG (p < .05). ROS were increased in GG versus both NG (plasma, 1.4-fold) and HG (ileum, 1.5-fold). Although thiobarbituric acid-reactive substances in ileum were higher in HG/NG compared to lab controls (p < .05), there was no difference in ileum, kidney and liver between groups. Lung myeloperoxidase activity was increased in all groups compared to lab controls (p < .05). Respiratory burst capacity increased at the end of experiment equally in all groups. Cellular/organ (liver, kidney) damage was higher in GG compared to other groups (p < .05).

Conclusions: Gradual reoxygenation after HTS and hypoxia is linked to increased ROS formation and cellular/organ damage. Hyperoxic and normoxic strategies may be preferred to a restricted reoxygenation.

45
DILUTING THE SURVIVAL BENEFIT OF HEMOSTATIC RESUSCITATION C. Guidry, E. Gleeson, P. Meade, N. McSwain, and J. Duchesne, Tulane University, New Orleans, LA

Background: High ratios of FFP:PRBC in Damage Control Resuscitation (DCR) are associated with increased survival. The impact of volume and type of resuscitative fluid used during this high ratio transfusion has not been analyzed. We hypothesize a direct mortality correlation between quantity and type of resuscitative fluids used in patients that received high ratios of FFP:PRBC.

Methods: This 4 year retrospective study included patients who received ≥ 10 units of PRBC with high ratio resuscitation, 1:2 of FFP:PRBC. Demographics and outcomes of the quantity and type of resuscitative fluids used were compared and analyzed. To further compare quantity of fluids given, Kaplan-Meier survival analysis was computed.

Results: There were 56 patients included (28 in crystalloid group, 28 in colloid group). Demographics were statistically similar. Mean units of PRBC: crystalloid vs colloid was 14.8 (SD 9.4) vs 17.3 (SD 8.0), p=0.246; mean units of FFP: 13.0 (SD 6.8) vs 14.0 (SD 5.9), p=0.33. Hospital and ICU LOS was similar. Odds Ratio for 10 day mortality in the crystalloid group was 8.41 [95% CI 1.65-42.76 (p= 0.01)]. Kaplan-Meier survival curve compared four groups: colloid (mean quantity = 1.4L), <3L crystalloid, 3-6L crystalloid, and >6L crystalloid. Lowest mortality was in the colloid group and higher mortality when increasing the amount of crystalloid (p=0.029).

Demographics and Outcomes

|                      | Crystallloid | Colloid  | p value |
|----------------------|-------------|---------|---------|
| Men (%)              | 24 (85.7)   | 27 (96.4) | 0.352   |
| Age, years (SD)      | 36 (13)     | 33 (13)  | 0.702   |
| Penetrating (%)      | 19 (67.9)   | 21 (11.5) | 0.768   |
| ISS (SD)             | 21.7 (9.6)  | 21.5 (11.5) | 0.154   |
| ER SBP, mmHg (SD)    | 112 (27)    | 96 (39)  | 0.495   |
| ER + OR PRBC, units (SD) | 14.8 (9.4)  | 17.3 (8.0) | 0.246   |
| ER + OR FFP, units (SD) | 13.0 (6.8)  | 14.0 (5.9) | 0.334   |
| ICU LOS, days (SD)   | 17.2 (13.3) | 8.5 (10.7) | 0.415   |
| Hospital LOS, days (SD) | 36.6 (31.3) | 22.0 (11.0) | 0.923   |

Conclusion: In high ratio DCR: higher volume of crystalloids decreased survival and low effective colloid use increased survival. With better understanding of limiting crystalloids, we postulate future guidelines will incorporate protocols targeting an effective low volume resuscitation with high ratio component resuscitation to improve outcomes without diluting the survival benefit of hemostatic resuscitation.

46
HMGB1 CYSTEINE 106 IS REQUIRED FOR BINDING TO MD-2 IN THE TLR4/M2 COMPLEX TO ELICIT INFLAMMATORY RESPONSES H. Yang*1, P. Lundback2, L. Ottosson2, Y. Al Abed*1, M. Ochani*1, J. Li2, B. Lu2, S. Chavan3, D.J. Antoine2, H. Harris3, U. Andersson3, and K.J. Tracey*1.

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High mobility group box 1 (HMGB1) is a cytokine mediator in the pathogenesis of inflammatory diseases (Yang et al, BBA, 2009). HMGB1 contains three conserved cysteine residues at position 23, 45 and 106. Previously, we showed that HMGB1 binds to TLR4/MD2 and that mutation of cysteine 106 (C106) prevents the binding interaction and subsequent stimulation of cytokine release from macrophages (Yang et al, PNAS, 2010). Recently we further demonstrated that both reduced C106 and a disulfide bond between C23 and C45 are required for HMGB1 cytokine activity (Yang et al, Mol Med, 2011). It is known that TLR4 activity and interaction with its ligands depends on the extracellular adaptor MD-2 (Visintin et al, 2006). Using biosensor-based surface plasmon resonance (BIAcore), we found that HMGB1 binds human MD-2 with high affinity (apparent Kd = 8nM), and it does not bind to TLR4 alone (data not shown). Inactive HMGB1, created by either exposure to mercury which reacts with C106 thiol or by mutation of C106 or C45 to alanine in full length and 106. Previously, we showed that HMGB1 binds to TLR4/MD2 and that mutation of cysteine 106 (C106) prevents the binding interaction and subsequent stimulation of cytokine release from macrophages (Yang et al, PNAS, 2010). Recently we further demonstrated that both reduced C106 and a disulfide bond between C23 and C45 are required for HMGB1 cytokine activity (Yang et al, Mol Med, 2011). It is known that TLR4 activity and interaction with its ligands depends on the extracellular adaptor MD-2 (Visintin et al, 2006). Using biosensor-based surface plasmon resonance (BIAcore), we found that HMGB1 binds human MD-2 with high affinity (apparent Kd = 8nM), and it does not bind to TLR4 alone (data not shown). Inactive HMGB1, created by either exposure to mercury which reacts with C106 thiol or by mutation of C106 or C45 to alanine in full length HMGB1, abolished the binding interaction with MD-2. This modification also prevents HMGB1-induced TNF release in cultured human macrophages (Yang et al, Mol Med, 2011). Thus, our data indicate that HMGB1 binds to MD-2 in the TLR4/M2 complex to elicit inflammatory responses and C106 is important for this binding interaction. Supported in part by grants from NIH, NIGMS (RO1GM62508 to KJT and RO1GM098446 to HY).
HISTONES ACTIVATE NLRP3 INFLAMMASOME THROUGH TOLL-LIKE RECEPTOR 9-DEPENDENT PATHWAY IN STEREILE INFLAMMATORY LIVER INJURY  H. Huang, H. Chen, W. Yan, G. Nace, L. Zhang, A. Tsung. University of Pittsburgh Medical Center, Pittsburgh, PA

Purpose: NLRP3 inflammasome activation induces pro-inflammatory and innate immune responses in stressful conditions. We recently showed that extracellular histones act as a new class of DAMPs and mediate inflammation and organ damage through TLR9 after liver ischemia/reperfusion (I/R). We sought to determine the mechanisms by which NLRP3 inflammasome activation results in organ damage and inflammatory response following liver I/R. 

Methods: NLRP3 KO, TLR9 KO, and WT mice were subjected to a segmental (70%) warm hepatic I/R. Anti-histone antibodies or exogenous histones were administered in selected I/R groups. Liver damage was assessed by ALT levels and histology. Protein, cytokines, mRNA and innate immune cell population was evaluated. 

Results: NLRP3 and TLR9 KO mice w/o histone treatment protected liver from I/R injury. Histone-treatment upregulated protein levels of NLRP3 inflammasomes (NLRP3, ASC and pro-caspase-1) and downstream activated caspase-1, IL-1β and IL-18 following liver I/R. Conversely, reduction of these proteins was observed in anti-histone-treated mice. Exogenous histones increased the production of reactive oxygen species (ROS) that promoted the association of thioredoxin-interacting protein (TXNIP) with NLRP3. This association was reduced by inhibiting TLR9, a receptor for histones. Furthermore, activation of NLRP3 inflammasome and its production of IL-1β and IL-18 in TLR9 KO mice were significantly less than in WT mice after I/R.

Conclusion: NLRP3 inflammasome is activated by endogenous histones during liver I/R, which contributes to organ damage through actvation of caspase-1 and increased proinflammatory IL-1β and IL-18 production. Activation of NLRP3 inflammasome by histones is through a TLR-9 dependent pathway that mediates ROS production and subsequently promotes the association of TXNIP to NLRP3. 

PKR IS REQUIRED FOR NLRP3 INFLAMMASOME ACTIVATION AND HMGBl RELEASE  B. Lu1,4, T. Nakamura1, K. Inouye2, S. I. Valdes-Ferrer1,4, P.S. Olofsson1, J. Li1, H. Wang1, U. Andersson1, H. Yang1, S.S. Chavan1, G.S. Hotamisligil1, and K. Tracey1,4 1The Feinstein Institute for Medical Research, Manhasset, NY, 2Harvard School of Public Health, Boston, NY, 3Karolinska Institute, Stockholm, Sweden, 4The Elmezzi Graduate School of Molecular Medicine, Manhasset, NY

The NLR family pyrin domain-containing 3 (NLRP3) inflammasome, an intracellular oligomeric protein complex, regulates release of caspase-1 activation-dependent cytokines, including IL-1β, IL-18, and high-mobility group box 1 (HMGBl). During the course of studying HMGBl release mechanisms from macrophages, we unexpectedly observed that double-stranded RNA dependent protein kinase (PKR) is required for NLRP3 inflammasome activation. PKR inactivation by genetic deletion or pharmacological inhibition blocked NLRP3 inflammasome activation in response to double-stranded RNA, ATP, monosodium urate, adjuvant aluminum, and live E.coli. Overexpression of PKR significantly enhanced the reconstituted NLRP3 inflammasome-induced caspase-1 activation and IL-1β maturation, whereas blocking PKR significantly inhibited the reconstituted NLRP3 inflammasome activation. PKR physically interacts with NLRP3, and that PKR autophosphorylation is required for efficient PKR and NLRP3 interaction. Importantly, PKR autophosphorylation in a cell free system with recombinant NLRP3, ASC and pro-caspase-1 reconstitutes inflammasome activity. Together, these results indicate that PKR is required for inflammasome activation, and that it should be possible to target this molecule to inhibit inflammasome activity during inflammation. Supported by grant from NIH (RO1GM62508 to KJT).

LOSS OF MYD88 OR TRIF DOES NOT IMPACT SURVIVAL TO NEONATAL POLYMICROBIAL SEPSIS  D.N. Joiner, A. Cuenca, J.L. Wynn, K. Kelly-Scumpia, P.O. Scumpia, D.C. Nacionales, P.A. Efron*, and L.L. Moldawer*. University of Florida, Gainesville, FL

Despite improvements in perinatal care, neonatal infection and sepsis are still global health concerns that claims 1 million lives a year. Neonates have distinct immunological responses relative to adults. In the adult, survival to polymicrobial sepsis has been demonstrated to be dependent on the downstream TLR signaling protein, MyD88; however, the role of MyD88 or the other TLR downstream signaling adaptor protein in the survival to neonatal polymicrobial sepsis is unknown. To address this, 5-7 day old wild-type (WT) B6, MyD88−/−, or TRIF−/− mice (on a B6 background) were inoculated intraperitoneally (ip) with a cecal slurry (CS), cecal contents from adult mice, and followed for survival. Surprisingly, WT, MyD88−/− and TRIF−/− neonates, had similar survival to a LD70 dose of CS (25% vs 22% vs 18% respectively, p=NS). Similar data were obtained using a LD30 dose. In addition, there were no differences in the percentage of macrophages and neutrophils recruited to the peritoneum 24 hours following CS challenge in WT vs MyD88−/− vs TRIF−/− neonates (70% vs 68% vs 68% respectively, p=NS). There were also no differences in the baseline respiratory burst and production of reactive oxygen species of neutrophils and macrophages in WT, TRIF−/− and MyD88−/− neonates in response to phorbol ester. These data suggest that neonates are able to compensate for the loss of one these critical downstream TLR signaling adaptor proteins in the face of polymicrobial sepsis. This
is contrast to adult data that has suggested that MyD88 is critical for survival to polymicrobial sepsis. Further studies that delete both of these proteins will elucidate which compensatory mechanisms elaborated by either TRIF and/or MyD88 are critical for the survival of neonates to polymicrobial sepsis.

50

THE ALARMIN S100A8/A9 AMPLIFIES VENTILATOR-INDUCED LUNG INJURY M.T. Kuipers1,2, T. Vogt1, H. Aslami1, G. Jongsm1, E. van den Berg1, A.P. Vlaar1, J.J. Roelofs1, N.P. Juffermans1, M.J. Schultz1, T. van der Poll1,2, J. Roth1, and C.W. Wieland1, 1Laboratory of Experimental Intensive Care and Anesthesiology Academic Medical Center, Amsterdam, Netherlands, 2Center of Experimental and Molecular Medicine, Academic Medical Center, Amsterdam, Netherlands, 3Department of Pathology, Academic Medical Center, Amsterdam, Netherlands, 4Institute of Immunology, University of Münster, Münster, Germany

Objective: Mechanical ventilation (MV) can induce lung injury in which innate immunity plays an important role. However, the exact triggers of the response remain unclear. S100A8/A9 (Mrp8/14) proteins are endogenous danger signals that activate innate immunity via TLR4. We hypothesized that S100A8/A9 proteins are released in acute lung injury (ALI) and mediate lung inflammation in a 2-hit model of LPS-induced lung injury combined with MV.

Methods: S100A8/A9 levels were measured in bronchoalveolar lavage fluid (BALF) of patients with and without ALI. Next, wild-type (WT) and S100A9 knock-out mice, naïve and with induced ALI (intranasal LPS), were randomized to 5 h of spontaneously breathing or MV. S100A8/A9 levels were measured, other endpoints were: lung wet/dry ratio, total protein level, neutrophil influx, cytokine and chemokine levels, and histology. In addition, healthy spontaneously breathing and ventilated WT mice received S100A8/A9, S100A8 or zvechle intratracheally. Neutrophils, total protein, cytokine and chemokine levels were measured in BALF.

Results: S100A8/A9 proteins were increased in BALF of ALI patients and in mice with LPS- or MV-induced lung injury. S100A8/ A9 levels further increased upon LPS/MV double hit. Targeted deletion of S100A9 attenuated lung inflammation in the 2-hit model. Spontaneously breathing mice receiving S100A8/A9 or S100A8 had higher KC levels and more neutrophil influx. In mice undergoing MV, S100A8/A9 or S100A8 instillation amplified lung inflammation; neutrophil influx, IL-6, KC, IL-1β, and MIP-2 levels were increased compared to ventilated vehicle-treated mice.

Conclusion: S100A8/A9 proteins are released in ALI and their presence enhances inflammation in a LPS/MV model. S100A8/A9 administration during MV amplifies the pulmonary inflammatory response towards ventilation.

51

ROLE OF CELL-FREE DNA IN SEPSIS PATHOPHYSIOLOGY: NOVEL STUDIES IN MURINE MODELS OF SEPSIS S. Mai1,2, A.L. Patrick1,2, A. Fox-Robichaud1,2, and P. Liaw1,3, 1McMaster University, Hamilton, ON, Canada, 2Hamilton Health Science, Hamilton, ON, Canada, 3David Braley Cardiac, Vascular & Stroke Research Institute, Hamilton, ON, Canada

Introduction: Sepsis is characterized by an exaggerated host response to microbial infection resulting in the systemic activation of coagulation and inflammation. We have recently observed that high levels of cell-free DNA (cfDNA) released in the circulation is a powerful prognostic marker in severe sepsis patients. Our objective was to investigate the role of cfDNA in inflammation during sepsis.

Methods: Healthy C57BL/6 male mice were subjected to cecal ligation and puncture (CLP), involving a ligation distal to the ileocecal valve and two punctures of the ligated cecum (mortality rate of 100% at 24 hrs). Mice were exsanguinated and organs harvested at times between 0 to 10 hours post-surgery and plasma levels of cfDNA, IL-6, TNF, and protein C were quantified. This was repeated in a lipopolysaccharide (LPS, 0.04 ug/mL) model of inflammation.

Results: Baseline levels of cfDNA in healthy mice were 1.1±0.2 ug/mL. At 6 hours post-CLP, cfDNA levels increased more than twofold compared to sham mice (5.5±0.4 ug/mL vs. 2.4±0.2 ug/mL; p<0.01) and remained elevated for 10 hours post-CLP. IL-6 and TNF levels increased and protein C decreased in mice subjected to CLP while these levels did not change significantly in sham-operated mice over 10 hours. Similarly, LPS-challenged mice had significant elevations in cfDNA versus healthy controls over 8 hours (6.2±0.3 µg/mL vs. 2.1±0.2 µg/mL respectively; p<0.01).

Conclusion: These studies are the first to show rapid elevations of cfDNA associated with an early proinflammatory response in experimental sepsis, supporting the importance of early intervention (ie. administration of resuscitation fluids and antibiotics within 1 hour of diagnosis). These findings suggest that early therapeutic interventions which modulate cfDNA levels may improve clinical outcome of septic patients.