1

PULMONARY CONTUSION IN A RODENT MODEL
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Pulmonary contusion (PC) is a common injury and associated with organ dysfunction and mortality. Mounting evidence suggests PC is also inflammatory in nature, but lacking are studies that characterize how PC modulates the inflammatory response to injury. In this study, a model of PC is described in the rat, and longitudinal Positron Emission Tomography (PET) is utilized to detect inflammation and resolution of the injury. Sprague-Dawley male rats underwent thoracotomy. A weight was dropped to strike the exposed lung (3,215 J/M^2). Sham animals underwent thoracotomy alone. Blood, bronchoalveolar lavage (BAL), and lung tissue were collected at 0, 3, and 24hrs. Systemic levels of IL-1β and IL-1ra were determined. Cell count/differential was obtained from BAL. Lung tissue was used for histology (hematoxylin and eosin) and immunohistochemistry (ICH). microPET scanning using [18F]2-fluoro-2-deoxy-D-glucose (FDG) uptake was performed at 1, 7, and 28d after PC and expressed as a percent of initial signal on day 1. Statistical analysis was performed using student's t-test ($p \leq 0.05$ significant). Data reported as pg/ml + SEM. Each group was composed of 6 animals. Grossly, a PC was noted on the lung, and had a neutrophil infiltrate/intraalveolar hemorrhage/septal edema. BAL demonstrated a neutrophil influx. IHC revealed enhanced local production of TNF-α and IL-1β. Systemic levels of cytokines are shown (Table). MicroPET revealed increased uptake of FDG, correlating with neutrophil influx. Signal intensity decreased at 7 (50%) and 28 (<10%) days. This model produces PC based on morphologic and histologic criteria and a local and systemic inflammatory response(IL-1ra). Also, microPET can be utilized to document the extent and resolution of the injury.

	IL-1β	IL-1ra
0 hrs	67±2	1668±568
3 hrs		
Sham	73.6±10	1085±267
Contusion	103±51*	8570±262*#
24hrs		
Sham	75.4±12	944±396
Contusion	80.1±3*	1233±245

* p<0.05 vs control #p<0.05 vs sham

2

HEME OXYGENASE-DERIVED CARBON MONOXIDE DECREASES CARDIAC CONTRACTILITY AND PROMOTES POST HEMORRHAGIC CIRCULATORY COLLAPSE.
R. Johnson, S. Appleton*, F. Johnson. Tulane Health Sciences Center, New Orleans, LA 70112
Heme oxygenase (HO)-1, catalyzes the metabolism of heme to generate carbon monoxide (CO), and is induced by surgical stress or other hemorrhagic losses. It is also known that environmental CO can decrease cardiac contractility. Objective: Determine if HO-derived CO decreases cardiac contractility in isolated hearts and promotes hemorrhage-induced circulatory collapse and in awake male Sprague-Dawley rats. Methods: *In vitro* studies were conducted on paced Langendorff isolated hearts, that were fitted with left ventricular balloons to measure changes in left ventricular pressures. Hearts were perfused with buffer and an HO substrate, heme-L-lysinate (HLL, 5μmole/L), an iron free substrate which promotes CO formation, delta-aminolevulinic acid (dALA, 80μmol/L), or exogenous CO (100μmol/L). *In vivo* studies examined awake rats (300-325g) with chronic indwelling arterial catheters. Exhaled CO rates were measured via gas-solid chromatography in subsets of awake animals pretreated 24 hours with HLL (45μmol/Kg q 24hr, IP) or an HO inhibitor, zinc deuteroporphyrin bis glycol (ZnDPBG, 45μmol/Kg q 12hr, IP). Animals were then exposed to a 9mL blood loss. Results: *In vitro* studies in perfused hearts revealed that HLL acutely decreases the slope of the dP/dt_{max} to flow ratio (-44±6%,n=6). Similarly, dALA decreased dP/dt_{max} 33±4% (n=6). In addition the HO product, CO, acutely decreased dP/dt_{max} by 35±7% (n=4). *In vivo* studies: With respect to untreated controls, ZnDPBG treated rats displayed a 45% decrease in expired CO; both groups displayed 100% survival 24 hours post hemorrhage. HLL pretreatment increased expired CO 78%, and led to 100% fatalities in less than six hours post hemorrhage. Conclusions: Endogenously-formed CO can decrease cardiac contractility and increase the risk of post hemorrhagic circulatory collapse. (support: R01HL76187)

3

ADRENOMEDULLIN (AM) AND ITS BINDING PROTEIN (AMBP-1) PREVENT METABOLIC ACIDOSIS AFTER UNCONTROLLED HEMORRHAGE VIA DOWNREGULATION OF ENDOTHELIN-1.
R. Wu, W. Dong*, M. Zhou, H.H. Simms, P. Wang. North Shore-Long Island Jewish Medical Center, Manhasset, NY 11030.
Management of trauma victims with uncontrolled hemorrhage remains a major problem in combat casualty care at the far-forward battlefield setting. Shock after uncontrolled hemorrhage is associated with metabolic acidosis, in which the upregulated endothelin-1 (ET-1) plays an important role. We have recently shown that vascular responsiveness to AM, a recently-discovered vasodilator peptide, is depressed after hemorrhage and resuscitation. Downregulation of AMBP-1 appears to be responsible for this hyporesponsiveness. We, therefore, hypothesize that administration of AM/AMBP-1 prevents metabolic acidosis after uncontrolled hemorrhage via downregulation of ET-1. A rat model of uncontrolled hemorrhage with an extremely low volume of fluid resuscitation was used to mimic the combat situation. Briefly, both lumbar veins of male adult rats were isolated and severed at the junction to the vena cava. The abdomen was kept open but covered with a saline wet gauze for 45 min and was closed in layers thereafter. The bleedout volumes in the vehicle group and the AM/AMBP-1 treatment group are 6.6±0.2 and 6.7±0.3 ml/rat, respectively. The animals then received 1 ml of normal saline with or without AM (12 μg/kg BW) and AMBP-1 (40 μg/kg BW) over 30 min. Various parameters were measured at 4 h after resuscitation. The results (means±SE, n=5-7/group) are as follows:

	Sham Control	Hemorrhage Vehicle	Hemorrhage AM/AMBP-1
Arterial blood pH	7.36±0.01	7.26±0.02*	7.37±0.01#
Actual bicarbonate (mmol/L)	24±0.3	19±1.2*	22±0.4#
Standard base excess (mmol/L)	-0.5±0.5	-6.0±1.3*	-2.3±0.4#
Lactate (mg/dl)	10±1	28±6*	16±1#
ALT (IU/L)	17.2±0.8	44.5±8.2*	28.9±1.8#
Creatinine (μmol/L)	88±10	157±14*	122±5*#
O_2 Content (mL/dL)	18.7±0.4	14.8±0.9*	17.2±0.8#
ET-1 (pg/mL)	0.34±0.02	1.11±0.15*	0.77±0.06*#

(One-way ANOVA: *P<.05 vs. Sham; # P<.05 vs. Vehicle)
The above results indicate that AM/AMBP-1 administration

prevented metabolic acidosis, mitigated organ injury and decreased plasma ET-1 levels after hemorrhage. Thus, AM/AMBP-1 may provide a novel approach for the treatment of uncontrolled hemorrhage. The beneficial effect of AM/AMBP-1 appears to be mediated by downregulation of ET-1. (NIH R01 HL076179)

4

BURN INJURY PROMOTES DEVELOPMENT OF PERSISTENT ENTEROCOCCAL BACTEREMIA IN RATS AND CAUSES HIGH MORTALITY. M.Goto*, V.Samonte*, T.Ravindranath*, R.L.Reed*, M.M.Sayeed, and R.L.Gamelli. Dept.Surg. Loyola Univ.Med.Ctr. Maywood IL 60163

Antibiotics-resistant Gram-positive infection is a significant complication in severe burn injury, because it results in high mortality and morbidity. We have developed a rat model of persistent *Enterococcal* bacteremia.

Materials and Methods Male Sprague-Dawley rats received an intragastric gavage of 14 mg/kg Ciprofloxacin daily throughout the experiment. On the 4th day, the rats were subjected to injuries. Group B: burn (30% TBSA); Group CLP: cecal-ligation and puncture (one puncture with a 22G needle); Group BCLP: CLP immediately after burn; and Group S: sham-burn and sham-CLP. Blood bacteria and mortality were monitored for 7 days after the injury, hemodynamic response on day 1 and 3 post-injury.

Results Burn did not induce bacteremia or alter hemodynamics. CLP induced transient Gram-positive bacteremia (Fig 1) and a hyperdynamic state on day 1 and 3 post-injury (Fig 3). B+CLP induced Gram-positive bacteremia first, then *Enterococcal* bacteremia (Fig2), and a hyperdynamic state on day 1 post-injury and a hypodynamic state on day 3 (Fig 3).

Conclusion In Ciprofloxacin-gavaged rats, burn injury made them susceptible to development of persistent Gram-positive bacteremia, and increased their mortality. With continued Ciprofloxacin administration, the population of bacteria changed from mixed to *Enterococci* alone by the 7th day post- injury.

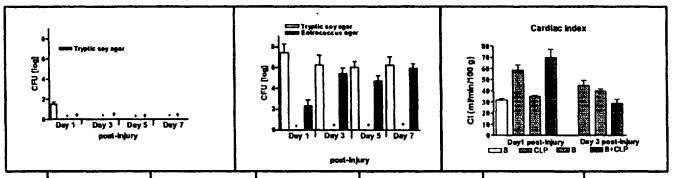

Fig 1 Fig 2 Fig 3

5

LOSS OF CD11c EXPRESSING DENDRITIC CELLS INCREASES MORTALITY IN MURINE POLYMICROBIAL SEPSIS P. Scumpia*, P. Efron*, P. McAuliffe*, T.Uchida*, R. Ungaro*, C. Tannahill*, M. Clare-Salzler*, L.L. Moldawer, Dep of Surgery, Univ of Florida, Gainesville, FL 32610

Introduction: Dendritic cells (DCs) regulate innate and adaptive immunity, and are lost from lymph nodes during polymicrobial sepsis. We hypothesized that DCs play an essential role in the successful host response to sepsis. **Methods:** 8-12 week old B6.FVB-Tg57Lan/J mice (DCKO; expressing diptheria toxin (DT) receptor on the CD11c promotor) were given 4 ng/g DT, and splenocytes were collected 24 hrs later for flow cytometry. DCKO and wild type littermates (WT) underwent cecal ligation and puncture (CLP) (n=11 per group) or received 5 mg/kg LPS (n=10) 24 hrs after DT or saline injection. 5-day survival and serum cytokines at 1.5 or 6 hours were measured. **Results:** Following DT treatment, DCKO, but not WT mice, showed a 90% reduction in $CD11c^{high}$ DCs by 24 hr * cting all subtypes of DCs, including $CD8\alpha^+$ lymphoid DCs (~95%), $CD11b^+$ myeloid DCs (85-90%) and $PDCA\text{-}1^+B220^+$ plasmacytoid DCs (75%). Increased mortality after CLP was seen in DCKO+DT compared to DCKO+saline or

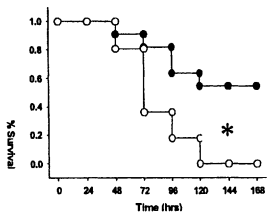

Fig 1. Survival to CLP

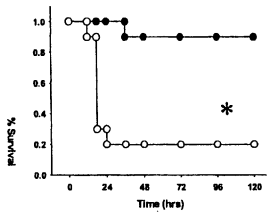

Fig 2. Survival to LPS Admin.

(open circle=DCKO mice; closed circle=WT mice) * $p<0.05$

WT+DT mice (100% vs 54% and 100% vs 54%, respectively; $p<0.05$). Mortality was also increased in DCKO+DT vs WT+DT mice following LPS administration (80% vs 10%; $p<0.05$), although there was no difference in plasma IL-12p70 or IL-6 at 1.5 and 6 hours ($p>0.05$). **Conclusions:** DCs are required for survival in both polymicrobial sepsis and endotoxemia. DCs may be an appropriate therapeutic target in sepsis, and preventing DC loss may improve outcome in patients.

6

A NOVEL CHOLINERGIC AGONIST IMPROVES SEPSIS: THE ROLE OF THE ENDOTHELIUM

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Background and Objectives: Previous studies show the anti-inflammatory effects of nicotine on experimental sepsis, endothelial cell activation, and leukocyte recruitment. One significant limitation of nicotine as a therapeutic agent is its toxicity. Therefore, a panel of cholinergic agonists was developed and tested for anti-inflammatory activity *in vitro*. CAP55 emerged as a lead compound. The purpose of this study was to characterize the effect of a novel cholinergic agonist, CAP55, in sepsis, as well as endothelial cell activation and leukocyte recruitment, critical components of sepsis pathogenesis.

Methods: CAP55 was chosen from a panel of cholinergic agonists based on its ability to inhibit TNFα release by cultured macrophages. The anti-inflammatory activity of CAP55 was tested in two experimental models of sepsis (endotoxemia and CLP). Further studies investigated the effect of CAP55 on endothelial cell activation and leukocyte recruitment *in vitro* and *in vivo*.

Results: CAP55, a novel cholinergic agonist, functions as an anti-inflammatory agent *in vitro*. CAP55, when tested in experimental models of sepsis, significantly improved survival and was less toxic when compared to nicotine. Further studies revealed that CAP55 modulates endothelial cell activation *in vitro*, as measured by adhesion molecule expression and cytokine/chemokine production. Finally, we observed that CAP55 significantly reduced leukocyte recruitment during inflammation *in vivo*. **Conclusions:** CAP55, a novel cholinergic agonist, improved sepsis outcome and suppressed endothelial cell activation and leukocyte recruitment *in vitro* and *in vivo*. CAP55 may improve sepsis by modulating endothelial cell activation and leukocyte recruitment.

7

MATHEMATICAL SIMULATION OF INFLAMMTION IN PORCINE SEPTIC SHOCK AND ARDS. G. Nieman*, J. Bartels, J. Wei, A. Baratt, S. Chang*, J. Puyana, D. Carney, G. Clermont, J. DiRocco and Y. Vodovotz*. Upstate Medical University, Syracuse, NY, 13210 and University Pittsburgh. Pittsburgh, PA, 15219.

Background: The progression of septic shock to acute respiratory distress syndrome (ARDS) is the result of complex interactions between inflammatory and physiologic elements. Mathematical modeling of complex systems is an approach for understanding the intricate interplay among these elements. Our existing model is based on ordinary differential equations and encompasses the dynamics of cells and cytokines of innate and adaptive immunity that participate in the acute inflammatory response. This mathematical model was previously calibrated in C57Bl/6 mice exposed to acute trauma, hemorrhage and/or endotoxemia. ***Methods:*** In the current study, we adapted our murine mathematical model of inflammatory dynamics to a porcine model that is well suited to pre-clinical studies in the settings of sepsis and ARDS (*Shock* 2005; 23:129-137). We incorporated matrix metalloproteinase (MMP) data not previously evaluated in the murine model. Data from our "two-hit" porcine model (peritoneal contamination + intestinal ischemia-reperfusion) was integrated with the data obtained from an extensive literature search. We constructed comprehensive mechanism diagrams for cytokines and each molecule deemed to play a significant role in MMP-2 and MMP-9 dynamics. These included the MMP precursors (pro-MMP-2, pro-MMP-9), endothelial cells, fibroblasts and plasmin. The integrated data was used to calibrate the current model. ***Results:*** The mathematical model was capable of describing the dynamics of TNF and IL-6 in swine and predicted qualitative dynamics of MMP-2 and MMP-9. In addition, an influence diagram of each molecule was built based on its biological process mechanisms. ***Conclusions:*** A mathematical model of inflammation in porcine sepsis-induced ARDS has been defined that will aid us in elucidating the pathogenesis of ARDS and may aid in identifying windows for novel therapeutics interventions.

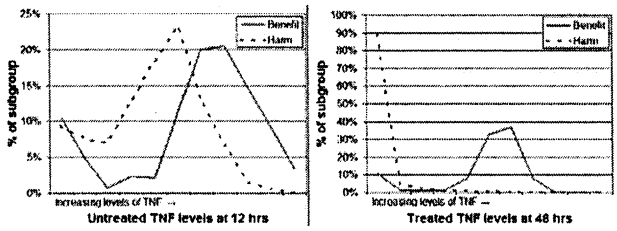
8

INTEGRATING ENVIRONMENTAL FACTORS INTO A MATHEMATICAL MODEL TO PREDICT MORTALITY OF SEPTIC PATIENTS S. Chang, F. Busche, Y. Vodovotz, G. Clermont, M. Fink, Immunetrics, Inc. (+ Pitt & IBM affiliations)

Objective: To determine the effects of aging and geographic distribution on the outcome of simulated clinical trials of sepsis. **Methods:** We simulated an interventional trial of a neutralizing antibody against tumor necrosis factor (anti-TNF) for treatment of sepsis. The simulation was based on a mechanistic mathematical model that includes bacterial infection, the host response, and a therapeutic intervention. Simulated cases differed by bacterial load and virulence as well as individual propensity to mount an inflammatory response modulated by age. Age distributions from three de-identified hospital populations were used. Aging effects were modeled by altering the effectiveness of macrophages and neutrophils with respect to killing capacity, production of superoxide, TNF, IL-6 and their cell half-lives. Cohorts were constructed with 10,000 patients to represent each hospital.

Results: Survival for control and treated groups is summarized in the Table. Compared to patients, who benefited from treatment, patients, who were harmed by the treatment, had lower levels of TNF during treatment despite similar levels at enrollment.

		Mortality		
Hospital	Mean Age	Placebo	Treated	Delta
A	61.5	40.2%	36.5%	3.6%
B	58.9	40.2%	34.0%	6.1%
C	56.9	39.1%	33.7%	5.4%



Conclusions: Environmental factors have a significant impact on the outcome of a drug trial. The model demonstrated that harmed patients exhibit evidence of immunoparalysis.

9

PULMONARY INSTILLATION OF FAS- BUT NOT CASPASE-8 SMALL INTERFERING RNA (siRNA) INTO LUNG EPITHELIAL CELLS AMELIORATES ACUTE LUNG INJURY.

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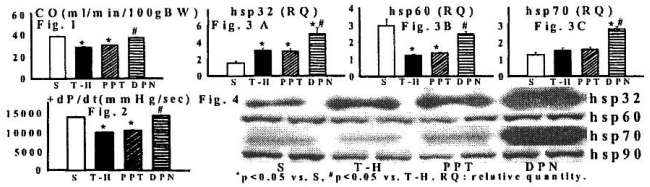
Apoptosis and inflammation in the lung are suggested to be major pathogenetic factors in acute lung injury. Thus, we tested the hypothesis that in vivo gene silencing of Fas or Caspase-8 by intratracheal, post-hemorrhage administration of siRNA would ameliorate acute lung injury in mice. To identify the target cells

male C3H/HeN mice (n=3/ group) received 100μg of Cy5-labeled Fas-siRNA intratracheally. 24 hrs later, frozen lung sections were stained for epithelial cells (Cytokeratin-18) and macrophages (CD115). Cy-5 labeled Fas-siRNA localized primarily in pulmonary epithelial cells and not alveolar macrophages. Subsequently, 100μg polyinosinic-polycytidylic acid sodium salt (P(I:C)) or siRNA or PBS were administered into mice lungs (n=4/ group). 18 hrs thereafter pulmonary levels of IFN-α, TNF-α and IL-6 displayed a marked increase in P(I:C) treated animals. Instillation of siRNA or PBS did not lead to an increase in IFN-α, TNF-α and IL-6 lung tissue concentrations. Finally, mice (n=12/ group) were subjected to hemorrhagic shock, were treated with 75μg of Fas-, Caspase-8 or control-siRNA intratracheally 4 hrs later and polymicrobial sepsis was induced 20 hrs thereafter. Inflammatory lung cytokines were determined 24 hrs after sepsis by cytometric bead array. Lungs were also assessed histologically and neutrophil influx was quantitated. Intratracheal delivery of Fas- or Caspase-8 siRNA significantly reduced lung tissue Fas- or Caspase-8 mRNA, respectively. However, only Fas- but not Caspase-8-siRNA treatment markedly diminished lung tissue TNF-α, IL-6, IL-10, IFN-γ and IL-12 levels. Fas-siRNA treatment also preserved alveolar architecture and reduced lung congestion as well as neutrophil infiltration. The ability of Fas- but not Caspase-8-siRNA to ameliorate acute lung injury suggests the relevance of an independent, Fas driven inflammatory pathway in the lung. Regulation of specific proteins in the lung using siRNAs should be further investigated for its therapeutic potential. (NIH-HL73525)

10

CARDIOPROTECTION BY A SELECTIVE ESTROGEN RECEPTOR (ER)-β AGONIST FOLLOWING TRAUMA-HEMORRHAGIC SHOCK: UPREGULATION OF CARDIAC HEAT SHOCK PROTEINS (hsp). HP Yu*, T Shimizu*, MA Choudhry, T Suzuki*, KI Bland, IH Chaudry. University of Alabama at Birmingham, Birmingham, AL 35294.

Although 17β-estradiol (E2) administration following trauma-hemorrhage (T-H) improves cardiac function in males, it is not known whether: 1) the salutary effects are mediated via estrogen receptor (ER)-α or ER-β and 2) cardiac heat shock proteins (Hsp) are affected by E2 administration. Male Sprague-Dawley rats (~250g BW) underwent T-H (mean BP 40mmHg for 90min, then resuscitation). ER-α agonist propyl pyrazole triol (PPT) (5μg/Kg), ER-β agonist diarylpropiolnitrile (DPN) (5μg/Kg), or vehicle (10% DMSO) was injected subcutaneously during resuscitation. At 24 h after T-H or sham operation, cardiac output (CO), stroke volume (SV), heart rate, mean arterial pressure (MAP), and ± dp/dt were measured (n = 6 rats/group). Cardiac Hsp32, 60, 70, and 90 mRNA/protein expressions were determined. One-way ANOVA and Tukey's test were used for statistical analysis. Cardiac output, SV and ± dP/dt decreased significantly after T-H, however, administration of ER-β agonist DPN after T-H significantly improved all parameters (Figs. 1-2). Moreover, DPN treatment prevented T-H-mediated decrease in hsp60 and hsp90 mRNA/protein expressions in the heart. Hsp32 and hsp70 mRNA/protein expression in the hearts were further increased in DPN treated T-H rats (Figs. 3-4). In contrast, no significant change in above parameters was observed in T-H rats treated with ER-α agonist PPT. The novel findings of our study are: 1) the salutary effects of E2 on cardiac function are mediated via ER-β, and 2) ER-β-induced upregulation of hsp likely plays a significant role in the E2-mediated cardioprotection after T-H. (Supported by NIH Grant R37 GM 36519)



11

DENDRITIC CELL (DC)-DERIVED EXOSOMES EXPRESSING MILK FAT GLOBULE EPIDERMAL GROWTH FACTOR VIII (MFG-E8) ATTENUATE PROINFLAMMATORY RESPONSES IN SEPSIS BY PROMOTING APOPTOTIC CELL CLEARANCE. M. Miksa*, R. Wu, W. Dong*, P. Das*, D. Yang*, P. Wang. North Shore-Long Island Jewish Medical Center, Manhasset, NY 11030.

Apoptotic cells can harm the host in sepsis if not cleared by phagocytes. Phagocytosis of apoptotic cells depends on "eat me" signals expressed on dying cells, such as phosphatidylserine (PS). PS can be recognized by phagocytes via its receptors. For complete engulfment of apoptotic cells, binding of PS to integrin $\alpha_v\beta_3$, mediated by the bridging protein MFG-E8, is needed. We hypothesize that phagocytosis of apoptotic cells is impaired in sepsis due to the decreased MFG-E8 and that adaptive transfer of bone marrow DC (BMDC)-derived exosomes rich in MFG-E8 is beneficial. To study this, sepsis was induced in rats by cecal ligation and puncture (CLP). MFG-E8 was assessed by Western blot in CLP rats and in RAW 264.7 macrophages (RMϕ) stimulated with LPS at 20 h. Exosomes (1000 μg), collected from supernatant of cultured BMDCs, were injected IV to CLP rats. TNF-α and IL-6 (pg/mL) was determined by ELISA and thymocyte apoptosis (TC-A_o) by Annexin V. Peritoneal Mϕ were cultured with exosomes (24 h) and their ability to engulf A_o-TC was determined *in vitro* (phagocytosis index, PI: A_o-TC/Mϕ). Our results indicate that MFG-E8 in spleen and liver decreased by 48% and 70% respectively at 20 h after sepsis (right table). MFG-E8 expression also decreased after *in vitro* stimulation of RMϕ with LPS (Fig). Injection of exosomes to CLP rats led to reduced detection of A_o-TC and plasma cytokines as shown below. Moreover, peritoneal Mϕ from exosome-treated rats displayed a 3.6-fold increased ability to phagocytose A_o-TC (PI: 0.84 ± 0.13 vs. 0.23 ± 0.04 in Vehicle, P<.05). We conclude that BMDC-derived exosomes attenuate systemic inflammation in sepsis, by enhancing clearance of apoptotic cells. The increased availability of MFG-E8, a factor indispensable for phagocytosis of apoptotic cells, is the underlying mechanism (NIH R01 GM057468).

Protein	Sham (n=5)	CLP (n=5)
Spleen	1.08 ± 0.19	0.56 ± 0.06*
Liver	0.67 ± 0.15	0.20 ± 0.04*

(MFG-E8/β-Actin; t-test: *P<0.05)

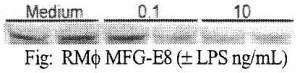


Fig: RMϕ MFG-E8 (± LPS ng/mL)

N=6	Sham	CLP Vehicle	CLP Exosomes
TC-A_o	4.7 ± 0.7%	12.2 ± 3.1%*	8.2 ± 0.3%#
TNF-α	4.9 ± 1.2	30.4 ± 8.6*	15.7 ± 2.1#
IL-6	90.9 ± 15.1	545.7 ± 119.5*	319.0 ± 28.5*

(Mean±SEM; ANOVA: P<.05:* vs. Sham; # vs. Vehicle).

12

GLUTAMINE ATTENUATES PULMONARY NF-κB ACTIVATION, INFLAMMATORY CYTOKINE RELEASE, AND PREVENTS THE ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS) FOLLOWING SEPSIS.

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Glutamine (GLN) has been shown to attenuate cytokine release from LPS-stimulated human peripheral blood mononuclear cells, however the in vivo anti-inflammatory effect of GLN in polymicrobial sepsis and ARDS is unknown. The aim of this study was to evaluate the effect of GLN on inflammatory cytokine release and the pathways that may mediate the anti-inflammatory effects of GLN in the lung. To examine this, 0.75 g/kg of GLN vs. a saline placebo (SP) was administered to male adult rats 1 h following initiation of sepsis via cecal ligation and puncture (CLP). NF-κB activation and IκBα degradation were evaluated in lung tissue 6 h post-CLP. Lung iNOS and eNOS expression were also measured. TNF-α, IL-6, IL-18, and CINC-1 levels were assayed. Finally, lung histopathology for the occurrence of ARDS and survival were examined. GLN administration 1 h post initiation of sepsis led to marked inhibition of lung tissue NF-κB activation ($p < 0.001$ vs. SP) and attenuated degradation of IκBα following sepsis. GLN led to significant attenuation of TNF-α, IL-6, and CINC-1 ($p<0.01$ vs. SP) 6 h post-CLP induced sepsis. IL-18 was attenuated by GLN at multiple time points post-CLP ($p<0.05$ versus SP). Further, GLN led to abrogation of increases in lung iNOS expression and enhanced lung eNOS expression following sepsis. Finally, GLN prevented the histopathological appearance of ARDS following sepsis and significantly improved survival ($p < 0.03$ versus SP). This data reveals that GLN exerts an anti-inflammatory effect in sepsis that may be mediated via attenuation of NF-κB activation and inhibition of increases in iNOS expression. This anti-inflammatory effect was associated with prevention of the occurrence of ARDS and mortality.

13

EFFECT OF RH-APC ON OVINE ACUTE LUNG INJURY

M.O. Maybauer, D.M. Maybauer, J. Fraser, L.D. Traber, K. Murakami, P. Enkhbaatar, N. Morita, A. Mizutani, D.L. Traber
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Objective: Recombinant human activated protein C (rhAPC) improves outcome in severe sepsis. This study investigates the effects of rhAPC on acute lung injury (ALI)(1). **Methods:** Eighteen sheep (33-38 kg) were operatively prepared for chronic study. After 7 days of recovery, sheep were randomly allocated to either the sham (group 1), control (group 2), or rh-APC group (group 3, n=6 each). After a tracheostomy, ALI was produced in the 2nd and 3rd group by in-sufflation of 48 breaths of cotton smoke. Then 30 mLs of live Pseudomonas aeruginosa solution ($2\text{-}5x10^{11}$cfu) were instilled into the lungs according to our protocol (1). Group 1 received only vehicle. All sheep were studied for 24h in awake state and were ventilated with 100% oxygen. In group 3, rhAPC (24μg/kg/h) was administered, beginning 1 h post injury. The animals were resuscitated with Ringer's Lactate Solution to maintain filling pressures and hematocrit. Peak airway pressures (PAWpeak = mmHg) and blood gases were determined every 3 h. Statistics: Two-way ANOVA and Student-Newman-Keuls post hoc test. Data: mean ± SEM. Significance $P<0.05$ *within group, † between groups. **Results:** PaO_2/FiO_2 ratio, PAWpeak, and pulmonary shunt fraction (Qs/Qt) remained stable in group 1. Group 2 showed a significant decrease in PaO_2/FiO_2 ratio, (0h: 521±22, 12h:72±5*, 24h:74±7*), as well as significant increases in PAWpeak (0h:20±1, 12h:31±2*, 24h:36±4*) and Qs/Qt (0h:0.14±0.02, 12h:0.58±0.07*, 24h:0.65±0.08*).Group 3 also showed a fall in PaO_2/FiO_2 ratio (0 h :541±12, 12h: 151±29 †*, 24h:118±20*), which was significantly improved after 12h compared with group 2. The PAWpeak (0 h:21±2, 12h: 27±3*, 24h:28±2*†) and Qs/Qt (0 h:0.24±0.04, 12h:0.53±0.08*, 24h: 0.45±0.02†*) increased significantly vs. baseline, and were significantly improved compared with group 2 after 24h. **Conclusion:** RhAPC significantly abrogates the deleterious response to combined severe smoke inhalation and pneumonia and should be assessed further in studies of ALI. **Reference:** (1) Murakami et al. Crit Care Med 2002,30(9):2083-90

14

EARLY FORMATION OF NITRIC OXIDE ADDUCTS IN HEMORRHAGIC HYPOTENSION

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Although it has been suggested that nitric oxide (NO) production is increased in early hemorrhagic shock (HS), this has not been measured and brisk production is generally thought to only occur after the appearance of newly formed inducible NO synthase (iNOS). This study is the first attempt to assess NO-derived intermediates (NO-IM), i.e., NO adducts with hemoglobin iron (HbNO) and protein thiols (RSNO), nitrites (NO2-), and nitrates (NO3-), which appear promptly in blood following hemorrhagic shock (HS). In an isobaric model of HS, a mean arterial blood pressure of 40 mmHg was maintained in anesthetized rats. The dynamics of HbNO adducts were assessed using electron paramagnetic resonance techniques during the first 90 minutes of HS conducted in the presence and absence of 1400W, a specific iNOS inhibitor. The total amounts of NO-IM in Red Blood Cells (RBC) and blood plasma were estimated using chemiluminescence techniques. The formation of RSNO adducts in RBC was examined with fluorescence imaging, 2-D gel and mass spectroscopy analysis of RSNO-derivatized products. We demonstrated that in comparison to sham-treatment, HS was accompanied by the accumulation of HbNO adducts in RBC (up to ~6 μM) in a time-dependent manner, independent of 1400W. RBC and plasma NO-IM and RSNO formation was substantial and was significantly higher in HS-treated animals than in sham-treated animals. To our knowledge this is the first demonstration of the production of NO-derived

adducts in RBCs as a prompt response to HS. These results provide strong evidence of brisk iNOS-independent NO production in early HS and raise the possibility that RBC serve as important transporters of bioreactive NO species beginning early in HS.

15

IMPORTANCE OF NORADRENERGIC REGULATION OF HEMODYNAMIC & INFLAMMATORY COUNTEREGULATORY RESPONSE TO TRAUMA-HEMORRHAGE. D. Niño, K. Williams, J. Varela, C. Vande Stouwe*, P. Molina. LSUHSC New Orleans, LA 70112.

The integrity of neuroendocrine control of host defense mechanisms is critical to ensure adequate immediate pro-inflammatory responses to injury and subsequent surgical (Qx) or infectious challenges. Previously we have demonstrated that chemical sympathectomy (SNx) with 6-OHDA accentuates the immediate pro-inflammatory response to trauma/hemorrhage (Tx/hem). We hypothesized that disruption of the sympathetic-mediated control of tissue cytokine expression during the early post-injury phase can lead to impaired host defense mechanisms resulting in increased morbidity and mortality from a second challenge. To test this hypothesis SNx (6-OHDA IP 50 mg/kg/d X 3 d) animals were subjected to Tx/hem followed by fluid resuscitation (FR) with Ringer's lactate (3 X total blood volume withdrawn). The impact of SNx on outcome from a Qx & infectious challenge was examined during recovery from Tx/hem + FR. Animals were subjected to Qx (laparatomomy) and infectious (ascending colon stent placement) challenge 48 h after completion of Tx/hem + FR. SNx impaired FR restoration of MABP resulting in greater mortality during the FR period despite similar blood loss and hypotension during Tx/hem as that of control animals. SNx increased mortality during the initial 48 h post Qx infection process. Peritoneal lavage TNF and IL-10 levels were significantly (50%) lower in SNx-Tx/hem than in vehicle-Tx/hem animals 24 h post Qx. These findings underscore the importance of noradrenergic modulation of early responses to Tx/hem. Furthermore, they demonstrate the relevance of noradrenergic tone on subsequent host defense mechanisms affecting morbidity and mortality from an infectious challenge during the recovery phase from Tx/hem. Supported by ONR 14-97-1-0248.

16

KUPFFER CELLS AND THEIR MEDIATORS: THE CULPRITS IN PRODUCING DISTANT ORGAN DAMAGE FOLLOWING TRAUMA-HEMORRHAGE (T-H). F Hildebrand*, WJ Hubbard*, KI Bland*, IH. Chaudry. CSR and Dept. of Surgery, University of Alabama at Birmingham, Birmingham, AL 35294.

Although Kupffer cells (KC) play a key role in the systemic inflammatory response and the release of cytokines following T-H, little is known about the function of KC-derived chemokines, and their role in distant organ damage. We hypothesized that a selective inhibition of KC will reduce chemokine release and associated organ injury after T-H. Female C57BL/J6 mice (not proestrus) were given iv injection of gadolinium chloride ($GdCl_3$, 10mg/kg BW) 48 hrs before the experiments to decrease the KC numbers. The KC reduced and normal mice (saline iv) then underwent T-H (laparotomy, BP 35±5 mmHg for 90 min, then resuscitated) or sham operation (laparotomy only). At 4 hrs, 1 day or 2 days thereafter, blood and tissue samples were collected and plasma cytokines (TNF-α, IL-6, IL-10)/chemokine (MCP-1) concentrations determined by FACS analysis (n=6/group). Lung and liver tissues were analysed for the wet/dry ratio. Neutrophil accumulation in the lungs was assessed by myeloperoxidase activity, and livers were subjected to histology analysis. One-way ANOVA and two-sided *t*-test were used and differences were considered significant at a $p<0.05$. Trauma-hemorrhage resulted in significantly higher plasma MCP-1, IL-6 and IL-10 levels at 4 hrs as well as one day after T-H ($p<0.05$). However, plasma MCP-1, IL-6 and IL-10 concentrations at 4 hrs and 1 day after T-H were markedly lower in mice receiving $GdCl_3$ ($p<0.05$), whereas TNF-α plasma levels were slightly increased at 4 hrs. Moreover, edema formation in liver and lung as well as MPO activity in lungs were markedly lower at all time points in mice receiving $GdCl_3$ compared to vehicle ($p<0.05$). Hepatic architecture was preserved in mice receiving $GdCl_3$, whereas infiltration was evident after T-H in vehicle-treated mice. We conclude that Kupffer cells are likely the most significant source of the chemokine MCP-1 following T-H, which appears to be affecting neutrophil infiltration into distal and proximate organs (lungs and liver, respectively).

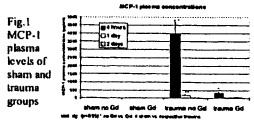

Fig.1 MCP-1 plasma levels of sham and trauma groups

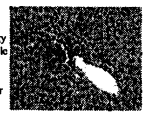

Fig.2 Liver histology in vecicle treated mice (4h after T-H)

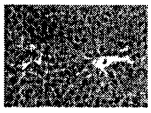

Fig 3. Liver histology in $GdCl_3$ treated mice (4h after T-H)

17

***IN SILICO* MODELS PREDICT AND *IN VIVO* STUDIES VERIFY THAT GUT-DERIVED LPS PLAYS A MINIMAL ROLE IN ACTIVATING THE INNATE IMMUNE RESPONSE AFTER HEMORRHAGE**

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Objective: The mechanisms underlying hemorrhagic shock (HS)-induced inflammation have not yet been fully elucidated, and this holds especially true for the role of Gram-negative bacterial lipopolysaccharide (LPS). We have previously developed a mathematical model incorporating major elements of the acute inflammatory response in C57Bl/6 mice, using input from experimental data. We found that a single model with different initiators including the autonomic system could describe the response to both HS and LPS *in vivo*. This mathematical model of acute inflammation could account for HS-induced inflammation without invoking endogenous release of LPS, a hypothesis that required *in vivo* validation. Recognition of LPS and subsequent cellular activation occur through the interaction of LPS with LPS binding protein (LBP), followed by binding of this complex to CD14. LPS-resistant CD14-null ($CD14^{-/-}$) mice do not respond to LPS.

Results: C57Bl/6 mice subjected to HS expressed elevated levels of hepatic CD14 protein. However, $CD14^{-/-}$ mice did not differ from their wild type controls with regard to circulating levels of TNF, IL-6, IL-10, and NO_2^-/NO_3^- following HS. With the exception of IL-6, elevations in liver mRNA expression of these cytokines and iNOS were not observed. Furthermore, no difference was observed between wild type and $CD14^{-/-}$ mice with regard to survival, hemodynamic parameters, the degree of shed blood, circulating AST and ALT, or hepatic TNF-α and ICAM mRNA levels. C57Bl/6 mice depleted of intestinal flora by antibiotic treatment and then subjected to HS also did not differ with regard to markers of inflammation and organ damage. Conclusion: Taken together, these data suggest that LPS release is not responsible for the pro-inflammatory changes observed in HS, and demonstrate the utility of mathematical modeling for the study of acute inflammation in shock states.

18

EFFECT OF FUSOGENIC ATP LIPID VESICLES ON HEMORRHAGIC SHOCK RATS

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This study was to determine the protective effect of intracellular ATP delivery on hemorrhagic shock rats. Methods: Intracellular delivery of ATP was achieved by encapsulating Mg-ATP in fusogenic lipid vesicles (F-ATP-LV). Adult Sprague-Dawley rats were anesthetized. Hemorrhagic shock was induced by draining 1/3 of total blood volume from jugular vein. Ten ml of F-ATP-LV or Lactated Ringer's solution (LRS) were infused intraperitoneally after shock initiated (6 rats in each group). Rats were euthanized at the end of 4 hours after shock or when blood pressure dropped below 5 mmHg. In another 2 groups, the shock duration was limited to one hour, blood and tissue high energy phosphates were measured. Results: The survival time of the F-ATP-LV group was significantly longer than that of LRS group 218±18* vs. 84±25 minutes. Blood pH was 7.20±0.12*, lactic acid level was 6.15±1.87*mmol/L in the F-ATP-LV group vs. 6.87±0.04, and 13.47±2.59 mmol/L in the LRS group. Blood ATP and total energy phosphates (TEP) were significantly higher at 60 minutes after shock in the F-ATP-LV group (Table 1), but, there were no significant differences in muscle, heart, lung, liver, kidney, and brain, between two groups. Discussion: The higher pH value, higher energy phosphate content, and lower lactic acid level may imply that F-ATP-LV supply energy to cells compensates for hemorrhagic shock showing a protective effect with longer survival time.

	F-ATP-LV		LRS	
	mean	sem	mean	sem
0' ATP	238.59	38.91	222.62	22.13
60' ATP	256.25*	30.27	133.22	31.37
0' TEP	450.26	54.09	466.59	22.36
60' TEP	513.73*	39.01	336.76	50.72

Table 1 * $p<0.05$ as compared to LRS group

19

ANTI-APOPTOTIC GENES MODULATED WITHIN THE HEARTS OF RATS FOLLOWING SUCCESSFUL IL-6 RESUSCITATION FROM HEMORRHAGIC SHOCK; NOT THE USUAL SUSPECTS.

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Half of adult trauma deaths are due to hypovolemic circulatory collapse (HCC). We developed a irreversible hemorrhagic shock (IHS) model in rats that leads to acute HCC secondary to cardiac apoptosis and dysfunction, which is completely reversed by administration of interleukin (IL)-6 at the start of resuscitation in IHS. The benefit of IL-6 in IHS was blocked by G-quartet-oligonucleotides that target Stat3, which is activated by IL-6 and has been shown to upregulate anti-apoptotic genes in the Bcl2 and IAP families. This supports our hypothesis that the protective effect of IL-6 in IHS results from Stat3-mediated modulation of anti-apoptotic gene transcription. To pursue this hypothesis, we performed microarray analysis. Rats were subjected to sham (n=4) or IHS protocol (mean arterial pressure of 35 mm Hg for 3 hr; n=8); IHS rats were blindly randomized to receive IL-6 (10 μg/kg, n=4) or placebo (P; n=4) at the start of resuscitation. Total RNA was extracted from hearts and hybridized to individual Affymetrix REA 230A GeneChips containing 15,923 genes, 150 which were apoptosis-related. 29 of these 150 apoptosis genes had significantly altered expression within the experimental groups (ANOVA $p<0.005$) falling into three sets: 1) 24 apoptosis genes were upregulated 1.3-8 fold in IHS/P vs sham ($p<0.05$); 2 of which were further upregulated 1.4 fold in IHS/IL-6 vs IHS/P ($p\leq0.03$); 2) 3 genes were decreased 1.2-2.7 fold in IHS/P vs sham ($p\leq0.01$); all three were upregulated 1.2-1.6 fold in IHS/IL-6 ($p\leq0.008$); 3) 2 genes demonstrated no change in expression in IHS/P vs sham; both were upregulated 1.2-1.5 fold in IHS/IL-6 vs IHS/P ($p\leq0.02$). Thus, IHS in the rat modulated mRNA levels of 29 apoptotic genes within the heart; IL-6 administration upregulated 7 of these 29 including 4 (Tob1, Jun, SHP2, Dnajc7) not previously identified as anti-apoptotic gene targets of Stat3. Increased activity of these 4 genes and perhaps others not currently classified in the apoptosis pathway may provide protection to the heart in IHS and prevent HCC.

20

PRE-HOSPITAL HEMOGLOBIN-BASED OXYGEN CARRIER (HBOC) RESUSCITATION REDUCES POST-SHOCK OXYGEN DEBT AND PREVENTS ACUTE LUNG INJURY IN A TWO-EVENT MODEL

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Pre-hospital resuscitation using HBOCs is currently being tested in Phase III clinical trials, but no pre-clinical studies have established its efficacy. The purpose of this study is to simulate an existing multi-center prehospital trial of HBOC versus crystalloid to determine the effects in a controlled two-event model. ***Methods***: Rats underwent hemorrhagic shock (30mmHg x 45min) and clinically relevant 2-hr resuscitation: 2x volume of shed blood (SB) using normal saline (NS) in the first 30 min; 50% vol. SB in the next 30 min; another 2x SB vol. with NS

over the remaining 60 min. HBOC (PolyHeme, Northfield Lab) was given as the initial resuscitation fluid over 30 min at vol. equal to the hemoglobin (Hb) content as 50% SB; then 4x SB vol. given using NS during the remaining 90 min. LPS (100 μg/kg) was given i.v. post- resuscitation as a second event. Global physiologic response via tissue oxygenation (StO_2) and base deficit (BD) and pulmonary response via lung neutrophil (PMN) accumulation and vascular permeability (Evans blue leak) were assessed. ***Results:*** Hb (g/dl) at the end of resuscitation were equivalent in the SB vs. HBOC groups (10.5±0.2 vs. 10.3±0.3). Compared to standard resuscitation with NS and SB, Pre-hospital HBOC restored StO_2 and BD more efficiently (table), decreased lung PMN accumulation both post-resuscitation (8.6±0.7 vs. 4.9±0.6) and post-second event (10.8±1.0 vs. 5.1±0.4), and attenuated subsequent acute lung injury (5.6±0.5% vs. 0.8±0.4%). ***Conclusion***: Pre-hospital HBOC resuscitation improves the recovery from post-shock oxygen debt and reduces acute lung injury in a clinically relevant two-event model.

*p<0.05		Shock	30min	60min	120min
StO_2	Std Resus.	36.5±1.5	73.5±0.5	77.0±2.0	80.5±2.5
	HBOC	39.3±0.7	83.3±0.7*	86.3±1.2*	87.3±1.2*
BD	Std Resus.	17.0±0.6	13.7±0.9	7.7±0.9	7.7±0.7
	HBOC	16.3±0.8	6.0±1.5*	3.7±0.3*	2.7±0.3*

21

ADENOSINE A2A RECEPTOR ACTIVATION REDUCES ORGAN FAILURE IN TRAUMA/HEMORRHAGIC SHOCK. J. Jabush*, D. Xu, Q. Lu*, Z. Nemeth*, S. Zaets, T. Berezina, G. Machiedo, E. Deitch, G. Hasko. UMDNJ-New Jersey Medical School, Newark, NJ 07103

Objective: Hemorrhagic shock and resuscitation trigger a global ischemia/reperfusion phenomenon, in which the activation of various inflammatory processes is considered a major contributor to the ensuing tissue damage. The adenosine A2a receptor is a member of the family of guanine nucleotide binding proteins and has become a focus of major interest primarily because of its ability to broadly inactivate the inflammatory cascade. The current study was designed to evaluate the effect of A2a receptor activation on organ injury and inflammation in the setting of global ischemia/reperfusion elicited by trauma/hemorrhagic shock (T/HS) and resuscitation. **Methods:** Male Sprague-Dawley rats were subjected to a laparotomy (trauma) and 90 minutes of hemorrhagic shock or trauma/sham shock (T/SS). The selective A2a receptor agonist CGS-21680 (0.5 mg/kg) or its vehicle was injected 30 min before shock or immediately after resuscitation. At 3 hours following resuscitation, animals were killed and samples of gut, lung, and blood were collected for analysis. Lung permeability and pulmonary myeloperoxidase levels were used to quantitate lung injury. Intestinal injury was determined by histologic analysis of terminal ileum. To assess red blood cell (RBC) injury, RBC deformability was measured using a LORCA device, as the RBC elongation index (RBC-EI). **Results:** Pretreatment with CGS-21680 protected the lung and gut against shock-induced injury and prevented the shock-induced decrease in RBC deformability. Post-treatment with CGS-21680 ameliorated shock-induced lung injury, as well. **Conclusion:** A2a receptor agonists may represent a novel therapeutic approach to prevent organ injury following T/HS.

22

JAK/STAT AND TLR PATHWAYS DIFFERENTIALLY REGULATE SOCS3 PROTEIN EXPRESSION DURING SEPSIS. C.S. Chung, Y. Chen*, L. Doughty, A. Ayala. Brown Univ./RI Hospital, Providence, RI 02903

The suppressor of cytokine signaling (SOCS) proteins have been identified as an important feed back regulatory mechanism in immune system. Studies indicate that SOCS proteins can be induced via traditional JAK/STAT (by cytokines/hormones) and TLR (by microbes) pathways. Our previous studies showed that induction of SOCS3 protein varies in cells/tissues and appears to be tightly regulated after polymicrobial sepsis induced by cecal ligation and puncture (CLP). However, the mechanism of such regulation is unclear. Therefore, the aim of this study was to determine the contribution of the JAK/STAT vs. TLR pathways to SOCS3 induction in sepsis. To study this, mice deficient in TLR4, MyD88 (TLR pathway), STAT1, IFNα/βR, IL-6, IL-10 (JAK pathway activators) and their background mice were subjected to CLP or sham-CLP procedure. 24h later, SOCS3 expression was determined by western blot in peritoneal leukocytes (Pleu), spleen, lung and liver harvested from these mice. Our data show that interruption of TLR signaling results in SOCS3 downregulated in Pleu but no change in the spleen and lung compared to background mice after CLP. However, the regulation of SOCS3 expression appears more complicated in JAK pathway depending on cytokines and cell types/tissue sites. While STAT1 (IFNγ) and IL-6 deficiency had no effect on SOCS3 in Pleu and spleen, they diminished SOCS3 in the lung and liver. To the contrary, SOCS3 was markedly decreased in Pleu, spleen and lung but no change in the liver in septic IFNα/βR-/- mice. Furthermore, IL-10 deficiency significantly reduced SOCS3 in Pleu, lung and liver. These results suggest that stimulation of SOCS3 is tissue-dependent and cytokine-specific in septic mice. (NIH GM46354 & Lifespan Award)

SOCS3 protein expression after CLP [Sham animals do not (or low) express SOCS3]							
Tissue/Strain	C3H/HeN[1]	TLR4-/-	MyD88-/-	STAT1-/-	IL-6-/-	IFNα/βR-/-	IL-10-/-
P. Leucocyte	+++	++*	+*	++	+++	+/-*	+/-*
Spleen	++	++	++	+	NT	-*	NT
Lung	+	+	+	-*	+/-*	+/-*	+/-*
Liver	+++	NT	NT	-*	-*	+++	+/-*
1. All background mice express similar levels of SOCS3, C3H/HeN as a representative strain. *P<0.05 vs. background CLP mice, ANOVA, n=4-6.							

23

THE SH2-CONTAINING TYROSINE PHOSPHATASE-1(SHP-1) PROMOTES NEUTROPHIL (PMN) APOPTOSIS THROUGH DEPHOSPHORYLATION OF CASPASE-8 S. Jia*, A Kapus*, O. Rotstein, J. Marshall University of Toronto.

PMN survival is prolonged during inflammation. We have previously found that LPS tyrosine phosphorylates caspase-8, inhibiting PMN apoptosis. We hypothesized that caspase-8 dephosphorylation by SHP-1 can permit apoptosis to proceed.

Methods: PMN from healthy controls or septic patients were cultured with or without 1 μg/ml LPS. HL-60 cells were differentiated to PMN with retinoic acid. Apoptosis was quantified by flow cytometry as propidium iodide uptake at 21 hours in permeabilized PMN. SHP-1 activity was inhibited by PTP Inhibitor I (10 μM) and measured using the malachite green assay; caspase-8 activity was measured as cleavage of IETD-FMK with a fluorometric assay. Co-immunoprecipitations and immunofluorescence microscopy (IF) used antibodies to SHP-1 and caspase-8; SHP-1 message was quantified by real time PCR.

Results: LPS exposure and sepsis induced sustained tyrosine phosphorylation of caspase-8. SHP-1 co-immunoprecipitated, and co-localized by IF, with caspase-8; both interactions were blocked by exposure to LPS. SHP-1 inhibitor delayed apoptosis and reduced caspase-8 and SHP-1 activity; LPS and sepsis reduced SHP-1 activity (± SD; N=4-6 *p<0.05 vs controls):

	Control	LPS	PTP Inh	Sepsis
Apoptosis (%)	45.8±3.2	19.4±2.9*	3.1±3.7*	8.9±3.2*
SHP-1 activity	186±47	84±43*	54±28*	50±29*
Caspase-8 Act.	0.6±0.2	0.2±0.1*	0.2±0.1*	0.1±0.1*

SHP-1 mRNA levels in resting PMN increased by 10 hours to 2.3±0.4 X baseline levels, but were reduced by LPS to 0.4±0.1, and in septic PMN to 0.3±0.1. SHP-1 message increased during the differentiation of HL-60 cells to 8.2±1.7 on day 5, coincident with an increase in spontaneous apoptosis to 57.6±2.2%.

Conclusions: The tyrosine phosphatase, SHP-1, binds to and dephosphorylates caspase-8 in resting PMN, promoting its cleavage and the progression of apoptosis. This interaction is blocked by LPS and in PMN from septic patients, resulting in prolonged PMN survival and the persistence of inflammation.

24

NEUTROPHILS MEDIATE TLR4 SIGNALING-INDUCED TLR2 UPREGULATION IN ALVEOLAR MACROPHAGES. J.Fan*, Y.Li*, Y.Vodovotz, T.Billiar and M.Wilson. Department of Surgery, University of Pittsburgh, PA 15213

Hemorrhage and resuscitation (H/R) renders patients susceptible to the development of ARDS in response to a second insult. The mechanisms underlying shock-primed lung injury remain unclear. We reported that neutrophils (PMN) mediated TLR4 upregulation of TLR2 in endothelia and thus sensitize endothelia to TLR2 ligands. In the present study, we hypothesized that PMN, which are primarily sequestered in the alveolar space following H/R, might mediate the cross talk between TLR4 and TLR2 in alveolar macrophages (AM), and thus exaggerate local inflammation. C57BL/6 (WT) mice were bled to a BP of 40 mmHg for 1 h and resuscitated with shed blood plus an equal volume of Ringer's lactate. LPS (30μg/kg) was given i.t. in 1 h. AM were recovered by BAL at 0 to 8 h after LPS, and TLR2 mRNA in AM was detected by RT-PCR. In some studies, PMN depletion was induced with i.p. injection of anti-mouse PMN Ab 16 h prior to the H/R. *In vitro* PMN-AM coculture experiments were performed using blood PMN isolated from H/R mice and AM recovered from WT or C3H/HeJ mouse BAL fluid. The co-cultures were challenged with LPS or sequential LPS-peptidoglycan (PGN). AM TLR2 mRNA expression markedly increased in the shock/LPS animals vs. the sham/LPS group, but failed to increase in PMN depleted shock/LPS mice. *In vitro* results showed that PMN derived from H/R mice, and not from sham mice, mediated an enhanced LPS-induced TLR2 upregulation in WT AM, but not in C3H/HeJ AM, and therefore resulted in an augmented expression of MIP-2 and MIF in the WT AM in response to PGN. The functional relevance of the TLR2 upregulation in AM was evident by significant increase in the AM-induced PMN migration in a dorsal air pouch. TLR2 and TLR4 recognize not only pathogen-derived products but also endogenous danger molecules, thus TLR2 activation in AM, signaled by TLR4 and regulated by H/R-stimulated PMN, is an important positive feedback mechanism that contributes to shock-primed PMN transmigration to alveolar space following primary PMN sequestration.?

25

TNF-α Induced Caspase Activation Mediates Endotoxin Related Cardiac Dysfunction D. Carlson, D.J. White, and J.W. Horton. UTSW, Dallas, TX 75390-9063

Objective: Sepsis-induced cardiac dysfunction is a serious clinical syndrome characterized by hypotension, decreased systemic vascular resistance, and elevated cardiac index. While cytokines such as TNF-α mount an early response, the downstream effects of TNF-α signaling on cardiac function, particularly its relationship to caspase (cas) activation and apoptosis, have not been elucidated. **Methods and Results**: To further delineate the role of LPS induced cas activation and apoptosis on cardiac dysfunction; hearts were collected from wildtype (WT) mice, and at 2, 4, 8, and 24h post LPS injection (4mg/kg, i.p.). Cas-3 was apparent by 2h, showing an 8-fold increase over WT; a 12-fold increase in cas-8 activity was also observed. Low levels of cleaved cas-3 (9.25 ± 0.96), were also evident by 2h after LPS. Cleaved cas-3 peaked at 8h after LPS (20.5 ± 4.7). TUNEL positive myocytes were apparent 24h post LPS. To determine whether cas activity was associated with differential expression of pro- (Bax) and anti-apoptotic (Bcl-2) genes in the heart, levels of Bax, and Bcl-2 were examined. 2h after LPS, protein concentrations of Bax had risen 6-fold over WT, while levels of Bcl-2 dropped 3-fold. To delineate the role of TNF-α, we challenged mice deficient for TNFR1 and TNFR2 with LPS. In these animals, LPS failed to induce cas activation, cas cleavage, and DNA fragmentation. No changes in Bax or Bcl-2 were observed. To determine whether cas activation influenced the development of cardiac dysfunction following LPS, we inhibited cas with the cas inhibitor zVAD (3.3mg/kg, i.v.). Administration of zVAD significantly inhibited myocardial cas activity, cleavage, and significantly preserved cardiac physiologic function (Langendorff preparation) following LPS infusion compared to WT. **Conclusion**: These data demonstrate that TNF-α dependent cas activation occurs in the heart *in vivo* after LPS in WT (but not TNFR1, R2 deficient mice) and this cas activation parallels the cardiac dysfunction observed. The LPS induced cardiac apoptosis and contractile dysfunction were ablated by cas inhibition, suggesting that TNF-α mediated cas activation is involved in cardiac dysfunction during sepsis.

26

INHIBITION OF MMP-9 ON HEPATIC TGF-β1 AND CASPASE-3 ACTIVATION IN SEPSIS

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We have previously demonstrated that hepatic matrix metalloproteinase-9 (MMP-9) and gelatinase activity increased significantly after sepsis, and pretreatment with chemically modified tetracycline (CMT-3) inhibited these expressions and improved survivability. Activation of MMP-9 may be associated with TGF-β1 and Caspase-3 signaling pathways. We have been interested in investigating the role of post treatment with CMT-3 on hepatic MMP-9, TGF-β1 and Caspase-3 activity following sepsis. In this study, sepsis was induced in rats by cecal ligation and puncture (CLP) and 2 h later received either CMT-3 (25 mg/kg) or vehicle by gavage. Twenty four and 48 h after sepsis induction, blood and liver samples were collected. Plasma glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) levels were determined by enzymatic method and the activation states of hepatic MMP-9, MMP-2, tissue inhibitor of metalloproteinase-1 (TIMP-1), TGF-β1 and Caspase-3 were determined by Western immunoblotting. Plasma GOT, GPT and hepatic MMP-9 activity increased 2.5 fold, and TGF-β1 and Caspase-3 activity increased 1.5 to 2 fold at 24 h and 48 h post CLP; CMT-3 treatment blocked these increases. Furthermore, CMT-3 treatment also led to increased TIMP-1 level, an *in vivo* inhibitor of MMP-9. MMP-2 level was unaffected by CLP. The 24 h and 48 h mortality for CLP rats were 29% and 50%, where as post-treatment with CMT-3 resulted in 0% mortality. These results indicate the beneficial effects of CMT-3 post treatment in preventing an increase in GOT, GPT, MMP-9, TGF-β1, and Caspase-3 activity with the potential for improvement of hepatic injury due to sepsis.

27

PENTOXIFYLLINE DECREASES NEUTROPHIL OXIDATIVE BURST INDEPENDENT OF PKA ACTIVATION: EFFECTS ON MAPK P38.

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We have previously shown that phosphodiesterase inhibition by pentoxifylline (PTX) attenuates LPS-induced neutrophil oxidative burst by intercepting signals apart or downstream from PKC. Classically, PTX acts by increasing cAMP and by activating PKA. However, it is not clear whether PKA activation by PTX plays an important role in the attenuation of neutrophil oxidative burst or if PTX affects other intracellular signaling pathways independent of PKA. We hypothesized that PTX decreases neutrophil oxidative burst by MAPK p38- and PKA-dependent mechanisms.

Whole blood was incubated with HBSS (control), LPS (100 μg/mL), fMLP (1 μM), LPS+PTX (2 mM) and fMLP+PTX for different time intervals at 37C. Oxidative burst was measured by flow cytometry. In additional experiments, whole blood was preincubated either with the MAPK p38 inhibitor SB203580 (10 μl) or with the PKA inhibitor H89 (10 μl) for 1 hour prior to stimulation with LPS or fMLP and concomitant PTX treatment. PTX significantly decreased fMLP- and LPS-induced neutrophil oxidative burst. SB203580 markedly decreased fMLP-and LPS-induced neutrophil oxidative burst to levels similar to PTX. The addition of PTX following pre-incubation with SB 203580 showed an additive and downregulatory effect on neutrophil oxidative burst than each drug alone in both fMLP- and LPS-stimulated whole blood. The inhibition of PKA using H89 did not affected PTX downregulation of neutrophil oxidative burst. Our results indicate that PTX may exert its effects by acting on the MAPK p38 pathway, although its effects on steps that control the NADPH oxidase assembly cannot be ruled out. PKA activation by PTX seems not to participate in the regulation of neutrophil oxidative burst.

In conclusion, PTX downregulates fMLP- and LPS-induced neutrophil oxidative burst at least in part, by a MAPK p38-dependent and PKA-independent mechanism.

28

NRF2- A NOVEL REGULATOR OF SEPSIS.

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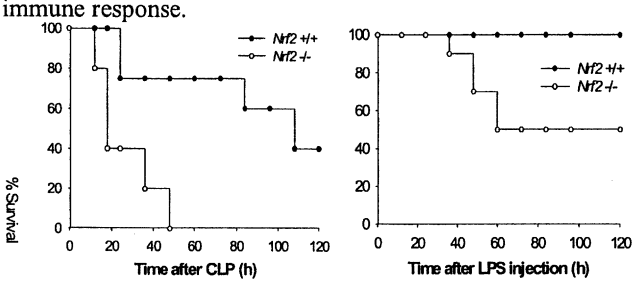
Objective: We have recently reported that nuclear factor-erythroid 2 p45-related factor 2 (Nrf2), a bZIP redox sensitive transcription factor protects against pulmonary emphysema by inhibiting oxidative stress and inflammation. The present study was designed to investigate the role of Nrf2 in sepsis.

Methods: Nrf2 -/- and +/+ ICR (CD-1) mice [n= 10 /group] were subjected to (a) cecal ligation puncture (b) endotoxin shock (intraperitoneal injection of LPS) and effect on mortality was determined. Bacteremia in the mice after CLP was determined by blood bacterial count. Serum TNF-α level was measured by ELISA.

Results: Disruption of *nrf2* gene enhanced the sensitivity of mice to polymicrobial as well as LPS induced septic shock resulting in early death while its wild-type littermates survived. The Nrf2 -/- mice had greater bacteremia and higher serum TNF-α levels relative to the wild type as a result of CLP. LPS instillation also resulted in markedly higher degree of lung inflammation in *nrf2*-deficient mice while a moderate inflammatory response was observed in the wild type mice. Direct instillation of LPS in the lungs resulted in greater influx of inflammatory cells predominantly neutrophils as well as edema in *nrf2*–deficient mice relative to the wildtype mice indicating exaggerated immune response.



Conclusion: Nrf2 plays a critical role in sepsis by effecting a controlled immune response to infection.

29

ABROGATING DAP12 SIGNALING IMPROVES CLP SURVIVAL BY DECREASING INFLAMMATION WITHOUT COMPOMISING BACTERIAL CONTROL.

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Background and Hypothesis: DAP12 is an adaptor protein that transduces activating signals for a family of immune receptors, amplifying the response to microbial products. We hypothesized that DAP12 contributes to inflammation and bacterial control during sepsis.

Methods: WT and DAP12-deficient mice were subjected to cecal ligation and puncture (CLP) and observed for survival or tissues were harvested. Peritoneal cells were stimulated ex-vivo with LPS; supernatants were reserved for cytokine determination and cells were lysed for western blot. Cytokine were measured by cytometric bead array. ERK phosphorylation in cell lysates was measured by immunoblot. Bacterial load was measured by culturing peritoneal lavage fluid on sheep's blood agar.

Results: 100% of WT mice died while only 40% of DAP12-deficient mice died (n=19-20, $p<0.05$). There was no difference in the number or type of cells recruited to the peritoneum or in the bacterial load in the peritoneal lavage. 24 hours after injury, DAP12-deficient mice had decreased plasma levels of pro- and anti-inflammatory cytokines (IL-6, MCP-1, TNF-α, and IL-10). Peritoneal cells isolated from septic DAP12-deficient mice produced significantly less TNF-α, IL-10, and MCP-1 after ex-vivo stimulation than did cells from WT septic mice. Increased cytokine production was correlated with amplified ERK phosphorylation in cells from WT mice.

Conclusions: DAP12-deficient mice have improved sepsis survival due to a decreased inflammatory response but a normal anti-microbial response. These results suggest that DAP12 signaling potentiates inflammation by amplifying the cellular response to microbial products through ERK activation, but that this signal is not required for bacterial control during sepsis.

30

THE EFFECT OF INTERMITTENT HYPOXIA ON NITRIC OXIDE PRODUCTION

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INTRODUCTION: Hypoxia influences nitric oxide (NO) production at several levels: gene expression, enzyme activity and substrate limitation. Many disease states result in dynamic fluctuations in tissue pO_2, e.g. cyclical recruitment in acute respiratory distress syndrome (ARDS) and nocturnal desaturations in obstructive sleep apnea. The influence of intermittent hypoxia on NO synthesis has been limited to *in vivo* models, which have multiple confounding factors influencing NO production. Until recently, *in vitro* studies have been unable to reproduce moderate to high frequency intermittent hypoxia. **METHODS:** RAW 264.7 macrophages were cultured in a specially designed forced convection culture system that allowed for 1) rapid switching between 2 precisely controlled culture pO_2, 2) collection and freezing of cells without exposure to ambient O_2 and 3) on-line measurement of NO production. Cultures were randomly assigned to sustained hypoxia (24 Torr O_2) or intermittent hypoxia (cycles of 40 Torr O_2 for 90 sec and 8 Torr for 30 sec) for 4 hours. RNA purification was followed by quantitative RT-PCR. On line NO measurements were obtained after 4 hours of hypoxia or intermittent hypoxia using an NO electrode. **RESULTS:** Both sustained hypoxia and intermittent hypoxia resulted in a 2 fold induction of the gene for inducible nitric oxide synthase compared to normoxia. NO production was higher in cells exposed to intermittent hypoxia than in cells exposed to hypoxia. **CONCLUSIONS:** Brief exposure of murine macrophages to intermittent hypoxia and hypoxia leads to increased iNOS gene expression, however intermittent hypoxia results in greater NO production than hypoxia. The dynamic nature of oxygen regulated NO production is potentially important in the pathophysiology of several diseases including ARDS and obstructive sleep apnea.

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31

ACUTE TESTOSTERONE INFUSION DECREASES POST-ISCHEMIC MYOCARDIAL RECOVERY AND INCREASES ACTIVATION OF MYOCARDIAL MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) FAMILY.

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Objectives: There are gender differences in post-ischemic myocardial function, and sex hormones may be partly responsible for these disparities. On the basis of the results of several recent papers in the trauma literature, we hypothesized that acute testosterone infusion (ATI) prior to ischemia worsens myocardial functional recovery through the activation of MAP kinases and caspase-3, and increases myocardial production of TNF, IL-1, and IL-6. Methods: Isolated-perfused rat hearts (Langendorff) (n=5-9/group) from adult females and castrated males were subjected to 25 min ischemia and 40 min reperfusion with and without ATI (17b-hydroxy-4-androstenone, 24nM/min) prior to ischemia. Myocardial contractile function (LVDP, +dP/dt, -dP/dt) was continuously recorded. After I/R, hearts were assessed for expression of TNF, IL-1, and IL-6 (ELISA) and activation of p38 MAPK, JNK, ERK and caspase-3 (Western blot). Data were analyzed with two-way ANOVA or student's t-test, $p<0.05$ statistically significant. Results: All indices of post-ischemic functional recovery were decreased with ATI compared to the untreated groups. Recovery of LVDP in females (68.7±2.5%) and castrated males (63.4±6.3%) was significantly higher ($p<0.001$) than in their treated groups (26.8±7.9% and 7.1±1.3%, respectively). ATI worsened markedly ($p<0.001$) recovery of +dP/dt and –dP/dt in females (619±155 vs. 1969±98, -408±125 vs. -1056±65) and in castrated males (180±40 vs. 1899±239, -118±31 vs. -1130±126). ATI increased activation of p38 and JNK following I/R, but caspase-3 activation was increased in female ATI hearts only. There were no differences in the myocardial production of TNF, IL-1, IL-6 and ERK activation. Conclusions: These results suggest that ATI prior to acute ischemia: 1) worsens myocardial functional recovery; 2) increases activation of p38 and JNK; and 3) activates caspase-3.

32

GENETIC INHIBITION OF PARP-1 REDUCES COX-2 EXPRESSION AND ABROGATES ACTIVITY OF PPARγ DURING MYOCARDIAL ISCHEMIA AND REPERFUSION. B. Zingarelli, P.W. Hake*, T.J. Burroughs*, A. Denenberg*. Critical Care Medicine, Cincinnati Children's Hospital Medical Center and University of Cincinnati College of Medicine, Cincinnati, Ohio 45229, USA.

Poly (ADP-ribose) polymerase-1 (PARP-1) is a nuclear enzyme activated in response to DNA injury in the nucleus and leads to cell dysfunction and death. Recent studies have shown that PARP-1 participates in the signaling mechanisms of the NF-κB pathway during inflammation. It has been proposed that activation of cyclo-oxygenase 2 (COX-2), a NF-κB dependent mediator, leads to formation of the cyclopentenone prostaglandin 15d-PGJ_2, which is a ligand for peroxisome proliferator-activated receptor (PPAR)γ, a nuclear receptor with putative cytoprotective functions. Therefore, aim of our study was to evaluate the effect of inhibition of PARP-1 in the expression of COX-2 and the activation of the PPARγ pathway. PARP-1 deficient (PARP-$1^{-/-}$) and wild-type (PARP-$1^{+/+}$) mice were subjected to myocardial ischemia (30 min) and reperfusion (2 h). In PARP-$1^{+/+}$ mice, extensive myocardial injury was associated with induction of gene expression of COX-2 as evaluated by microarray analysis (7.02±0.16 fold increase) and elevation of plasma levels of 15d-PGJ_2 (24.9±7.8 ng/ml). This event was associated with nuclear translocation (as evaluated by Western blotting) and DNA binding of PPARγ in the heart (as evaluated by electrophoretic mobility shift assay). On the contrary, in PARP-$1^{-/-}$mice a significant cardioprotection was observed. Gene expression of COX-2 (4.25±0.95 fold increase) and endogenous production of 15d-PGJ_2 (8.4±7.8 ng/ml) were significantly reduced when compared with wild-type mice ($p<0.05$). Reduction of COX-2 induction was associated with abrogation of PPARγ nuclear translocation and DNA binding in the heart. These data suggest that PPARγ does not contribute to the cardioprotection afforded by PARP-1 inhibition. Furthermore, our data support the hypothesis that induction of COX-2 represents an important requisite of endogenous PPARγ activation. (Supported by NIH grant R01 HL-60730).

33

ROUTE OF C5A RECEPTOR ANTAGONIST ADMINISTRATION DIFFERENTIALLY INHIBITS ORGAN INJURY IN A MODEL OF RUPTURED ABDOMINAL AORTIC ANEURYSM. S. Gryn*, S. Nicholson*, S. Taylor*, B. Rubin*, T. Lindsay. Department of Surgery, University Health Network and University of Toronto, Toronto, ON.

The rupture and repair of an abdominal aortic aneurysm is a two-hit ischemia-reperfusion event that initiates an inflammatory response, leading to local and remote organ injury. The complement cascade plays an important role in this organ injury. This study compared oral and intravenous administration of a peptide C5a receptor antagonist (AcF-[OpdChaWR]) in a rat model of RAAA. Male Sprague-Dawley rats were subjected to 1 hour of hemorrhagic shock at a mean arterial pressure of 50 mm Hg, followed by 45 minutes of supramesenteric aortic clamping, and 2 hours of resuscitated reperfusion. The animals were divided into the following groups: sham, control shock and clamp, oral C5a receptor antagonist-treated shock and clamp (10 mg/kg prior to anesthesia), or intravenous C5a receptor antagonist-treated shock and clamp (2 mg/kg, 5 minutes prior to aortic clamping). Vascular permeability to ^{125}I-labelled albumin was measured in the intestine and lungs, and mRNA expression of TNF-α in the lung was measured using real time PCR. Required fluid volume was reduced from 87 ± 4 mL to 71 ± 7 mL with oral treatment (no significance, P=0.189), and to 63 ± 9 mL with intravenous treatment (no significance, P=0.06). During reperfusion, total intestinal albumin was reduced from 257 ± 49 to 90 ± 17 mg albumin/g dry intestine with oral C5a receptor antagonist ($P<0.01$), and to 73 ± 11 mg/g dry intestine with intravenous treatment ($P<0.01$). Lung permeability index was reduced from 0.14 ± 0.02 to 0.090 ± 0.016 with oral treatment (no significance, P=0.149), and to 0.046 ± 0.006 with intravenous treatment ($P<0.005$). Lung TNF-α/GAPDH was reduced from 1.37 ± 0.28 to 0.38 ± 0.12 with oral treatment ($P<0.05$), and to 0.062 ± 0.046 with intravenous treatment ($P<0.05$). Both routes of administration have significant effects on vascular permeability in the intestines and TNF-α mRNA expression in the lungs, but only intravenous treatment has a significant effect on vascular permeability in the lungs.

34

ROLE OF CHANGES IN SURFACE HYDROPHOBICITY IN THE MECHANISM OF ISCHEMIA/REPERFUSION-INDUCED INTESTINAL INJURY. E. Dial*, R. Landry*, M. Khalil*, L. Lichtenberger*, and F. Moore. The University of Texas Medical School, Houston TX 77030.

Our laboratory initially reported that the gastrointestinal mucosa is protected, in part, by a phospholipid-based hydrophobic lining (Amer J Physiol, 1984), and that lipopolysaccharide treatment of rats injures this GI mucosal hydrophobic surface (SHOCK, 2002). We have now examined the changes in ileal mucosal hydrophobicity after ischemia/reperfusion (I/R) induced by superior mesenteric artery occlusion (SMAO), and have also tested a potential therapeutic modality, exogenous intraluminal phosphatidyl-choline (PC). Methods: In Study 1, rats were subjected to 60 min SMAO or sham laparotomy, followed by 0.5, 2.5, or 5 h of reperfusion. Ileal tissue was collected for analysis of contact angle as a measure of surface hydrophobicity, and flushes of the ileum were analyzed for hemoglobin (Hb) as a measure of GI injury and bleeding. In study 2, rats were orally pretreated with 0, 100, or 300 mg/kg PC two hours prior to I/R (I=60min/R=2.5h). Ileal tissue was analyzed for myeloperoxidase (MPO) as a measure of inflammation. Results: In Study 1, ileal contact angle was unchanged after 30 min of R, but was significantly reduced at 2.5 (sham=42±6; I/R=27±5°) and 5 h of R (sham=39±7; I/R=21±7°). Similarly, ileal bleeding into the lumen was unchanged at 0.5 h of R, but was significantly elevated at 2.5 (sham=358±37; I/R=1760±47 μg Hb) and 5 h of R (sham=206±8; I/R=789±105 μg Hb). In study 2, I/R alone induced a significant increase in MPO (sham=20±2; I/R=122±36 mU MPO/g tissue). This elevation was significantly and dose-dependently reduced by PC (100 mg/kg=76±23; 300 mg/kg=31±5 mU MPO/g tissue). Conclusions: Intestinal I/R induces a time-dependent reduction in ileal surface

hydrophobicity that is associated with intestinal bleeding. Addition of PC into the intestinal lumen prevents I/R-associated inflammation, and may provide a novel means to block traumatic shock-induced GI injury. (Supported by NIGMS P50 GM38529).

35

INHALATION OF 100% OXYGEN REDUCES BOWEL DAMAGE, ACCELARATES ENTEROCYTE TURNOVER AND IMPROVES INTESTINAL RECOVERY FOLLOWING ISCHEMIA-REPERFUSION INJURY. I. Sukhotnik*, V. Brod, J.G. Mogilner*, M. Lurie*, E. Shiloni*, H. Bitterman. Bnai Zion and Carmel Medical Centers, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa 34362, Israel.

OBJECTIVE: Inhalation of oxygen improves the hemodynamic status and the inflammatory response after intestinal ischemia/reperfusiom (I/R). The aim of the present study was to examine the effect of hyperoxia on small bowel damage, enterocyte turnover and intestinal rehabilitation following ischemia-reperfusion (IR) injury in rats. METHODS: three experimental groups were studied: 1) sham rats underwent laparotomy and breathed air, 2) I/R rats underwent occlusion of the superior mesenteric artery and vein for 30 minutes + air, and 3) I/R–O_2 rats underwent IR and inhaled 100% for 6 hrs starting 10 min before reperfusion. Intestinal structural changes, enterocyte proliferation and enterocyte apoptosis were determined 24 hours following IR. RESULTS: IR resulted in a significant increase in Park's injury score ($P<0.05$), and significant decreases in bowel weight in the jejunum, mucosal weight, mucosal DNA, and mucosal protein in the jejunum and ileum ($P<0.03$ or less), as well as villus height and crypt depth in the jejunum and ileum ($P<0.01$). Breathing 100% oxygen resulted in a significant decrease in Park's injury score in the ileum (0.6 ± 0.01 vs. 1.7 ± 0.4, $P<0.05$). Rats treated with oxygen also demonstrated a significant increase in bowel weight in the jejunum ($P<0.005$), mucosal weight, mucosal DNA and protein content, and villus height in the jejunum and ileum ($P<0.05$ or less) and crypt depth in the ileum ($P<0.01$). Oxygen therapy also increased enterocyte proliferation (assessed by BrdU uptake) in the jejunum and ileum, and significantly diminished apoptosis index in the jejunum ($P<0.05$).

CONCLUSION: Hyperoxia reduces small bowel injury, accelerates enterocyte turnover, and improves intestinal rehabilitation after I/R.

36

HEPATIC SINUSOIDAL ENDOTHELIAL CELLS CONTRIBUTE TO THROMBOXANE A2 RELEASE FOLLOWING OXIDATIVE STRESS. A. Karaa*, H. Xu*, W. Kamoun*, J. Zhang and M. Clemens. Department of Biology, University of North Carolina at Charlotte, 9201 University City Blvd, NC 28223.

Shock, sepsis and ischemia sensitize the liver microcirculation to Endothelin-1 (ET-1) leading to severe vasoconstriction. Endotoxemia, ethanol intoxication, hypoxia and bile duct ligation can mediate the hyperresponsiveness to ET-1 by increasing liver Thromboxane A_2 (TXA_2) release primarily in kupffer cells. Free radical production is a common thread in these stresses. Therefore, we determined if sinusoidal endothelial cells (SEC) can contribute to the release of TXA_2, whether this release is affected in response to oxidative stress (OS) and whether OS-primed SEC can raise TXA_2 release in response to ET-1. Method: Primary liver SEC cultures were pretreated with H_2O_2 (25μM) acutely (1h) or subacutely (6h) prior to 10 min ET-1 (10 nM) stimulation. The culture media was sampled for the enzyme immunoassay for TXB_2 a stable metabolite of TXA_2. Results: 1h and 6h OS induced a significant increase in ET-1 mRNA expression (1.6 and 1.4 fold increase $p< 0.005$). TXA_2 derives from arachidonic acid via activation of cyclooxygenase (COX). The inducible COX-2 protein levels in SEC extracts were found to be significantly higher after 1h (1.4 fold $p<0.05$) and 6h (1.45 fold $p<0.05$) OS. We found that SEC can synthesize TXA_2 at baseline in the absence of any stress (~ 670 pg/ml). The upregulation of COX-2 by OS was associated with a further elevation of TXA_2 (1.7 and 1.3 fold increase compared to the control group $p<0.05$). ET-1 stimulation of OS-primed SEC did not affect TXA_2 release compared to control cells treated with OS alone. Conclusion: Our results indicate that normal SEC are capable of releasing basal TXA_2 levels and we propose that the OS-induced ET-1 expression in SEC may result in maximal production of TXA_2 through an upregulation of the COX 2 pathway mimicking the effects seen in kupffer cells. Thus SEC may constitute an additional source of vasomodulators that might play a role in liver microcirculation regulation concomitantly with kupffer cells. (Supported by DK38201)

37

PULMONARY SOCS EXPRESSION WITH INCREASING HEPATIC ISCHEMIA-REPERFUSION (IR) INJURY SEVERITY.

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Liver IR is known to elicit a secondary pulmonary injury. Suppressors of Cytokine Signaling (SOCS) proteins have been identified as negative feedback control mechanisms in acute inflammation. We have previously shown that SOCS-3 mRNA is expressed in both ischemic and perfused liver after IR but is independent of injury severity. By contrast, liver SOCS-1 mRNA expression increases with ischemia time and injury severity. We hypothesize that pulmonary injury secondary to liver IR is also associated with a SOCS-mediated negative inflammatory response, and reflects the severity of primary liver injury.

METHODS: Male C57Bl6 mice (6-8wks, n=5) underwent 20, 45, 60, or 90 min. of partial liver IR under isoflurane anesthesia. Lungs were harvested 1, 2, 4, 8, or 24 hr post liver reperfusion and analyzed for mRNA expression of SOCS-1, SOCS-3, and various cytokines (TNF, IL-1, IL-6, IFN) by RT-PCR. Changes in cytokine and SOCS mRNA expression were compared to their expression patterns from ischemic and perfused liver.

RESULTS: Lung expression of TNF, IL-6 and IFN paralleled liver IR injury severity. Lung IL-1, SOCS-1 and SOCS-3 were expressed across the spectrum of liver IR, induced

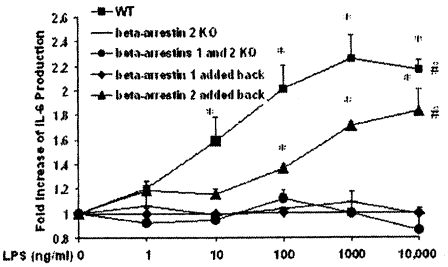
even in the face of a relatively minor liver injury (20min ischemia) that did not ultimately result in significant liver damage as evidenced by late histology. Pulmonary SOCS-1 expression increased with prolonging liver ischemia time, and paralleled the severity of hepatic injury. Lung SOCS-1 showed a delayed and sustained expression compared to that of post-IR liver, peaking 4-8 hr after liver reperfusion. **CONCLUSION:** SOCS-1 and SOCS-3 are expressed in lung after liver IR. This expression is associated with the induction of pulmonary cytokines following even minor remote organ ischemia-reperfusion and parallels the severity of the primary hepatic IR injury.

Supported by Veterans Administration and University of Washington Department of Surgery.

38

β-ARRESTIN 2 IS REQUIRED FOR LIPOPOLYSACCHARIDE (LPS) -INDUCED IL-6 EXPRESSION. H. Fan, L.M. Luttrell*, O.M. Peck, G.E. Tempel, P.V. Halushka and J.A. Cook. The Med. Univ. of S.C., Charleston, SC 29425

Previous studies have implicated a novel role of heterotrimeric G_i proteins in signaling leading to inflammatory mediator production induced by LPS. β-arrestins 1 and 2 are ubiquitously expressed proteins that alter signaling by G protein coupled receptors (GPCR). Recently it has been demonstrated that β-arrestins play a multifaceted role as signaling adaptors and scaffolds connecting GPCR to an ever growing list of signaling pathways. We hypothesized that β-arrestins 1 and/or 2 play a role in LPS-induced pro-inflammatory mediator gene expression. Since double KO of β-arrestins 1 and 2 in adult mice is lethal, we employed mouse embryonic fibroblasts (MEFs). MEFs from WT, β-arrestin 2 KO, double β-arrestins 1 and 2 KO mice and double KO MEFs into which β-arrestin 1 or 2 had been reintroduced were studied. The expression of β-arrestins 1 and 2 determined by Western blot confirmed the presence or absence of β-arrestin 1 or 2 in each group. LPS induced a concentration-dependent increase in IL-6 production in WT cells (2.3±0.2 fold; $p<0.05$). In contrast, double KO and β-arrestin 2 KO MEFs were refractory to LPS stimulation. Restoring β-arrestin 2 but not β-arrestins 1 expression to the double KO MEFs reestablished the WT response (1.8±0.2 fold, $p<0.05$). This is the first evidence that β-arrestin 2 is required for LPS-induced cytokine expression. Understanding the role of β-arrestins in regulation of LPS signaling pathways will provide novel insight into LPS regulation of inflammatory gene expression. (Supported by NIH GM 27673)



*, p<0.05 compared to unstimulated cells. #, p<0.05 compared to β-arrestin 2 KO, β-arrestins 1 and 2 KO, and β-arrestin 1 added back MEF groups.

39

INSULIN REGULATIONS MACROPHAGE ACTIVITY THROUGH SHIP PRODUCTION J. Cuschieri, E. Bulger, R. Grinsell*, I. Garcia, and R. Maier University of Washington, Seattle, WA 98104.

Strict control of serum glucose with insulin has been associated with a reduction in nosocomial infection rates and reduced mortality in critical illness. The tissue-fixed macrophage is critical to the eradication of Gram-negative bacteria, but unregulated activity, characterized by the overproduction of pro-inflammatory mediators, results in the development of Multiple Organ Dysfunction Syndrome. It is our hypothesis that insulin regulates endotoxin-mediated macrophage activation resulting in controlled pro-inflammatory mediator production.

Methods: Monocytic THP-1 cells were stimulated with endotoxin, with or without pretreatment with insulin for 24 hours. Cellular protein was extracted and analyzed by immunoblot for factors essential to TLR4 mediated signaling. Supernatants were analyzed by ELISA for TNF-α and TGF-β production. In addition, the effect of insulin on the regulatory factors, AKT and SHIP, were analyzed by immunoblot.

Results: Endotoxin exposure resulted in the activation of ERK 1/2, p38, JNK, and NF-kβ, and the production of TNF-α. Insulin pretreatment did not attenuate endotoxin mediated ERK 1/2, p38, JNK, or NF-kβ activation. However, endotoxin-induced TNF-α production was significantly reduced. Insulin pretreatment resulted in an increase in cytoplasmic SHIP and the activation of AKT. These changes were preceded by insulin-mediated TGF-β release.

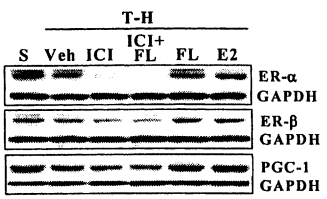
Conclusion: Insulin results in regulation of macrophage activity in response to endotoxin through the release of TGF-β and the subsequent production of SHIP. This increase in cytoplasmic SHIP results in enhanced activity of AKT, which attenuates TNF-α production though an alternative pathway to MAPK signaling.

40

FLUTAMIDE (ANDROGEN RECEPTOR ANTAGONIST) RESTORES CARDIAC FUNCTIONS FOLLOWING TRAUMA-HEMORRHAGE VIA ESTROGEN–DEPENDENT PATHWAY. YC. Hsieh*, S. Yang, MA. Choudhry, LW. Rue, KI. Bland* and IH. Chaudry. Department of Surgery, University of Alabama at Birmingham, Birmingham, AL 35294.

The mechanism by which flutamide (FL) improves cardiac functions following trauma-hemorrhage (T-H) remain unknown. We hypothesized that FL mediates its beneficial effects via estrogen (E2)-dependent upregulation of peroxisome proliferator-activated receptor coactivator-1 (PGC-1) pathway through estrogen receptor (ER-α and -β). PGC-1, a key regulator of mitochondrial function is known to play a role in cardiac functions. Male rats (~275g) underwent T-H (BP 40 mmHg for ~ 90 min) followed by resuscitation. At the onset of resuscitation,

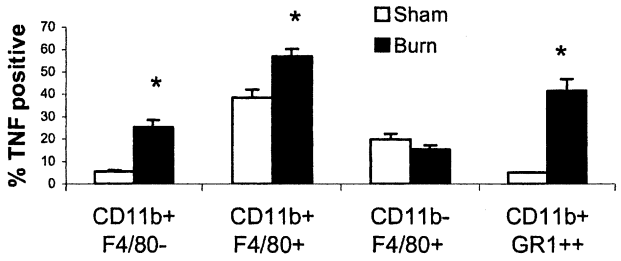
rats (n=6/group) received either vehicle, FL (25 mg/kg) or E2 (50 μg/kg), respectively. Another group of rats received the ER antagonist ICI 182,780 (3 mg/kg) with or without FL. At two hrs thereafter, cardiac functions were measured and blood and heart tissue collected. One-way ANOVA and Tukey's test were used and differences were considered significant at $p<0.05$. The depressed cardiac output, stroke volume and +/-dP/dt after T-H were restored by FL treatment, however, they remained depressed if ICI 182,780 was administered with FL. Plasma E2 and testosterone levels also increased after T-H by FL treatment. As shown in the figure, cardiac ER-α, ER-β, and PGC-1 protein levels were increased by FL treatment ($p<0.05$); however, FL-induced increase in ER-α, ER-β, and PGC-1 were abolished if ICI 182,780 was given with FL. The restoration of PGC-1 in FL-treated rats also normalized cardiac mitochondrial ATP, and downstream effectors: NRF-2, Tfam, COX IV, and β-ATP synthase expressions. These novel findings suggest that the salutary effects of FL on cardiac functions following T-H are mediated via E2-dependent pathway through ER-α, and ER-β upregulation of PGC-1 (NIH R37 GM 39519).



41

EFFECT OF THERMAL INJURY UPON THE INFLAMMATORY POTENTIAL OF SPLENIC MYELOID SUBPOPULATIONS G. Noel, D Byrum*, X. Guo*, C. Caldwell, A. Martignoni*, and C.K. Ogle. Shriners Hospital for Children, U. of Cincinnati, Cincinnati, OH. 45229

We and others have shown that thermal injury increases the TNFα production of splenic macrophages. In both humans and mice, histological examination of normal spleen suggests that the splenic macrophage population is heterogenous, with the CD11b and F4/80 antigens capable of discriminating white pulp and red pulp macrophages respectively. **Methods:** Male C57Bl/6J mice were given a full thickness 18% scald burn injury and sacrificed 8 days later. Intracellular TNFα was determined for phenotypes of macrophages and for neutrophils after incubating with LPS. **Results:** Burn injury increased the number of $CD11b^+$ $F4/80^-$ macrophages by 330% and decreased the number of $CD11b^-$ $F4/80^+$ macrophages by 60%. Burn injury induced a 476%

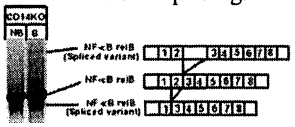


increase in the number of macrophages expressing F4/80 and CD11b antigens. Burn injury increased the number of neutrophils by 315%. The accompanying graph shows that $CD11b^+$ $F4/80^-$ and $CD11b^+$ $F4/80^+$ macrophages from burn mice had significantly more cells producing TNF than did sham mice. Only about 18-20% of $CD11b^-$ $F4/80^+$ macrophages from sham and burn mice produced TNF. The percentage of PMN making TNF was increased 8-fold in burn mice. **Conclusion**: Burn injury has a differential effect on the various splenic myeloid subpopulations, and only a portion of these cells exhibit an inflammatory phenotype after burn injury.

42

ACTIVATION OF ALTERNATIVELY SPLICED NF-κB AFTER BURN INJURY IS CD14 DEPENDENT. H. Phan*, K. Cho, K. Sainz*, S. Shin*, D. Greenhalgh. Shriners Hospitals for Children Northern CA and UCDavis, Sacramento, CA 95817

Nuclear factor kappa-B (NF-κB) transcription factor, an important regulator of the inflammatory response, is a key downstream player of the LPS signaling pathway. Recent studies from our laboratory have suggested that CD14, a component of the LPS receptor complex, may play a role in the regulation of alternative splicing of many genes, including Toll-like receptor 4 and endogenous retroviruses in response to burn injury. In this study, we examined the effect of CD14 on alternative splicing of different members of the NF-κB after burn injury. CD14 knockout and control mice were sacrificed 3 hours and 1 day after 18%TBSA burn. RT-PCR analysis of lung tissues using primer sets specific for the coding sequence of each member of the NF-κB family revealed varying sizes of PCR products. Subsequent cloning and sequencing of these PCR products revealed that splicing variants existed for p65, p100 and relB. Interestingly, all these splicing variants are up-regulated 1 day after injury in only CD14 knockout and not wild-type mice. These splicing variants result from either failure of splicing of a single intron or from exclusion of an exon, resulting in a frameshift and premature termination of translation. Because most of these splicing events occur near the 5' end, the resulting proteins contain only a small portion of the rel homology domain in the N-terminus, and are likely to be inactive. However, one splicing variant of p100 is missing an exon 20, which is closer to the 3' end, corresponding to the loss of the death domain in the C-terminus of the p100 protein. This protein retains most of its functional structure, and loss of the death domain may lead to its constitutive activation, according to some authors. These results suggest that the CD14 mediated regulation of the systemic inflammatory response after burn injury may occur through the activation of NF-κB alternative splicing.



43

GENDER DIFFERENCES IN THE POSTBURN HYPERMETABOLIC RESPONSE. MG Jeschke*, CT Pereira*, CC Finnerty*, R Przkora*, RE Barrow, RP Mlcak*, and DN Herndon Shriners Hospital for Children and Dept. of Surgery, Univ. of TX Medical Branch, Galveston, TX 77550

Objective: Recent evidence suggests that endogenous anabolic hormone levels are higher and hospital stays shorter in female

pediatric burn patients compared to males. To identify mechanisms responsible for these gender-dependent outcomes, muscle protein synthesis, hormone and cytokine levels, resting energy expenditures (REE), and liver sizes in female and male burned children. Methods: One hundred eighty-six patients (1-16 yrs old) with ? 40% TBSA were divided into females (n=74) and males (n=112). Muscle protein synthesis was determined by stable isotope techniques. REE was measured by indirect calorimetry, body composition by dual energy x-ray absorptiometry, and liver size by ultrasound. Serum hormones, proteins, and cytokines were also measured. Results: Demographics, nutritional intake, and concomitant injuries were not significantly different. During acute hospitalization, muscle protein net balance was 0±0% and -0.05±0.01% while change in lean body mass was 8±4% and -1±3% for females and males respectively, $p<0.05$. Percent of predicted REE was significantly lower in females compared to males, $p<0.05$. Females had higher levels of IGF-1, IGFBP-3, and GH compared to males, $p<0.05$. IL-6 and TNF-α levels were significantly lower in females compared to males, $p<0.05$. Female burn patients had a lower percent increase in liver size compared to males, $p<0.05$. Length of ICU stay was significantly less for females than males (29±3 vs. 38.3 days, $p<0.05$). Conclusion: Data suggest that in burned children, females have attenuated inflammatory and hypermetabolic responses compared to males. This decrease in hypermetabolism is reflected in improved muscle protein net balance and lean body mass, which relate to a shorter hospital stay.

44

AN IMMUNE MODULATING ROLE FOR PLATELETS DURING THE HOST RESPONSE TO INJURY. S. Fujimi*, A.A. Maung*, M.P. MacConmara*, A. Delisle*, J.A. Mannick, J.A. Lederer, and P.H. Lapchak* Dept. of Surgery, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, 02115

Although platelets are associated with hemostasis, coagulation, and inflammation, their role in immunity is undefined. To test whether platelets modulate the host response to injury, we examined injury-induced changes in platelets, as well as changes in the injury response after platelet depletion. **Methods:** At different times after burn injury, we measured leukocyte and platelet numbers, and platelet activation in murine whole blood samples. Platelets were identified by CD61 while activation was identified by CD62P. We then developed a novel model using mice made thrombocytopenic by treatment with anti-platelet Ig 3 days prior to injury. Mortality of platelet-depleted vs. control mice was measured. Whole blood samples were analyzed, and plasma cytokine levels were measured by cytokine bead arrays. **Results:** Circulating platelet numbers decreased by three hours after injury but returned to normal levels by 18 hours; a time where a marked increase in the number of platelets expressing CD62P. Mice depleted of platelets showed lower survival of 14.3% at 48 hours after injury compared to 100% survival in sham- and burn-injured control mice ($p<0.0001$). Hypovolemia and hemorrhage were ruled out as causes of death. Platelet-depleted injured mice had markedly lower numbers of blood leukocytes ($p<0.05$), lymphocytes ($p<0.01$), and neutrophils ($p<0.01$), whereas monocytes ($p<0.002$) were significantly increased when compared to controls. Plasma TNFα ($p<0.02$), IL-6 ($p<0.02$), and MCP-1 ($p<0.03$) levels were markedly increased in injured platelet-depleted mice when compared to injured control mice. **Conclusion:** Using a new mouse model to study the role of platelets in the host response to injury, we show that platelets play a significant role in controlling the injury response at both the cellular and protein levels. This model will further enable us to characterize the role of platelets in injury.

45

HORMONE METABOLISM IN SEVERELY BURNED CHILDREN RECEIVING LONG-TERM GROWTH HORMONE TREATMENT

R. Przkora*, M. G. Jeschke, W. F. Gold*, C.C. Finnerty*, D. L. Chinkes*, D.N. Herndon

Background: Major trauma and burns are leading to hormonal dysregulation, whole body catabolism and hypermetabolism. Anabolic agents such as growth hormone (rhGH) exert positive effects during the acute phase after burn injury. The aim of the present study was to determine the effect of rhGH administered at a dose of 0.05 mg/kg on hormone levels of severely burned children over a period of 2 years who received rhGH treatment for the first 12 months after burn. Methods: One hundred and fifty-two children with over 40% total body surface area burn (TBSA) were included and followed for 24 months after injury. Patients were treated with placebo or rhGH (0.05 mg/kg body weight sc) for the first 12 months. Treatment was stopped and patients received no treatment for the following year. Serum levels of human growth hormone (HGH), insulin like growth factor-I (IGF), IGF binding protein-3 (IGFBP-3), insulin, cortisol, osteocalcin, parathormone (PTH), total T4, T3 uptake and FTI were measured during hospital stay and at 6, 9, 12, 18 and 24 months after burn injury. Statistical analysis was performed using ANOVA followed by Tukey (significance at $p<0.05$). Results: HGH, IGF-I and IGFBP-3 serum levels were reduced after burn injury. RhGH treatment significantly increased hGH, IGF-I and IGFBP-3 compared to control patients, $p<0.05$. Cortisol concentrations were significantly decreased in the rhGH group compared to the controls patients, $p<0.05$. Osteocalcin levels were higher in the GH group at 18 months post burn, $p<0.05$. Insulin, FTI, total T4 and T3 uptake showed no significant differences. Conclusions: Long-term rhGH administration positively affects hormone metabolism. Some of the effects persevered even when the treatment was ended and may improve the rehabilitation potential of the children.

P1

GENTAMICIN FAILED TO IMPROVE OXYGENATION IN OVINE SEPSIS AFTER ACUTE LUNG INJURY

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University of TX Medical Branch, Galveston 77555-0833, USA

Objective: Our group developed an ovine model of Pseudomonas aeruginosa (Pa) sepsis associated with acute lung injury

(ALI). Aim of this study was to modify this model by administration of gentamicin (GM) to simulate a more clinically relevant situation. This could be the basis for future studies investigating new treatment strategies in sepsis. **Methods:** Eighteen sheep (35-40 kg) were operatively prepared for chronic study. After 7 days of recovery, sheep were randomly allocated either to the sham, control, or GM group (n=6 each). After a tracheostomy, ALI was produced in the control and GM group by insufflation of 48 breaths of cotton smoke under deep halothane anesthesia. Then, live Pa ($2\text{-}5x10^{11}$ cfu) were suspended in 30 mL saline solution and instilled into the lungs. The sham group received the vehicle. The sheep were studied for 24h in the awake state and were ventilated with 100% oxygen. GM (80mg) was intravenously administered at 6, 12, and 18h post injury. Statistical analysis: two-way ANOVA and Student-Newman-Keuls test. Data: mean ± SEM. Significance *P<0.05. **Results:** PaO_2/FiO_2 ratio, peak airway pressure (PAWpeak = mmHg), and pulmonary shunt fraction (Qs/Qt) remained stable in sham animals. The control group showed a significant decrease in PaO_2/FiO_2 ratio, (BL: 492±18.8 vs. 24h: 79.8±4.1*), as well as significant increases in PAWpeak (BL: 20.7±1.2 vs. 24h: 30.3 ±2.2*) and Qs/Qt (BL: 0.17±0.02 vs. 24h: 0.55 ±0.03*). The GM group showed similar changes in PaO_2/FiO_2 ratio (BL : 492.8 ±9.9 vs. 24h: 82.3±14.3*) PAWpeak (BL: 20.5±1.6 vs. 24h: 32.8±3.8*) and Qs/Qt (BL: 0.16±0.02 vs. 24h: 0.54±0.1*) as the control group. There were no significant differences between those two groups. **Conclusion:** GM failed to improve pulmonary function, when given 6h post injury. Lung tissue damage may have been manifested and a reduction of bacteria and toxins at this late time point has no effect on the course of ARDS. Future studies are warranted to determine a time point for effective antibiotic treatment in this model.

P2

Hyperbaric Oxygen Protects From Sepsis Mortality and Reduces Splenic Bacterial Load J. Buras, D. Holt, S. Pavlides, D. Orlow, B. Belikoff B, W. Reenstra. Department of Emergency Medicine, Beth Israel Deaconess Medical Center, Boston, MA 02215.

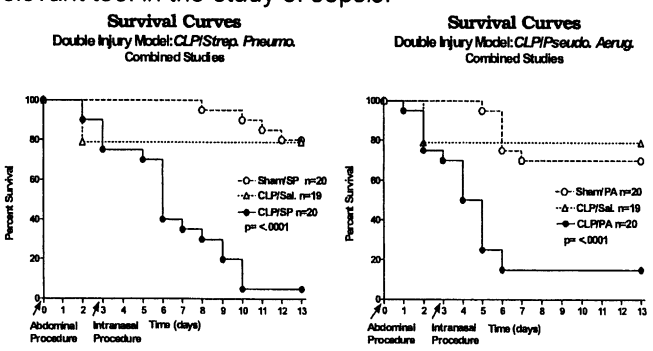
Objective: Hyperbaric oxygen (HBO) may be used adjunctively in treatment of severe soft tissue infections. Optimal treatment schedules and oxygen dosing have not been systematically defined. Our hypothesis is that HBO therapy in sepsis may be protective in sepsis if delivered with the correct dosing schedule.
Methods: Sepsis was induced in mice by cecal ligation and puncture (CLP) by an IACUC-approved protocol. Mice were randomized to receive HBO for 90 min immediately following the CLP surgery and then every 12 hrs at treatment pressures of 1, 2.5, or 3 atmospheres absolute (ATA). A control group contained mice subjected to CLP without HBO treatment. Animal survival was noted over 108 hr. In separate experiments, animals were harvested at 24 hr for determining splenic bacterial load. Statistical analyses employed the Log Rank test and ANOVA.
Results: A HBO treatment schedule of 90 min BID was effective in preventing mortality from CLP sepsis: CLP group (n=28) 79% mortality Vs CLP+HBO at 2.5 ATA (n=20) 65% mortality (P=0.003, Log-Rank). Mortality rates were not different between CLP (n=28) and HBO at 1.0 ATA (n=10) groups, 79% Vs 70%, respectively (P=0.84, Log-Rank). Treatment with HBO at 3.0 ATA significantly worsened outcome: mortality HBO at 3.0 ATA (n=20) Vs CLP group (n=28) 100% Vs 79%, respectively (P<0.001). Treatment with HBO at 2.5 ATA once daily did not improve survival (P=0.79). Analysis of bacterial load by culture of spleen homogenates showed a reduction in colony forming units in mice treated with HBO at 2.5 ATA BID (n=14) Vs control CLP (n=8), 160714+/-126822 Vs 357500+/-153320 respectively (P=0.002).
Conclusion: Our results demonstrate a therapeutic window for HBO treatment of peritonitis with a reduction in splenic bacterial load.

P3

DEVELOPMENT OF A CLINICALLY RELEVANT "2-HIT" MODEL OF SEPSIS

C. Davis*, D. Osborn*, K. Chang*, W. Dunne* and R. Hotchkiss Washington University School of Medicine Dept. of Anesthesiology St. Louis, Mo. 63110

Introduction: Septic patients are immunosuppressed and prone to develop secondary infections. Although cecal ligation and puncture (CLP) reproduces many of the signs of clinical sepsis, it isn't representative of patients who develop secondary, hospital-acquired infections, typically ventilator associated pneumonia (VAP). Our aim was to develop a "2-hit" model of sepsis that mimics the clinical scenario of primary infection followed by secondary, nosocomial pneumonia. **Hypothesis:** A primary, sub-lethal infection (CLP) will impair the immune system, enabling a secondary, otherwise sub-lethal pneumonia to induce a high mortality. **Methods:** Female ND-4 mice (~10 wks.) were subjected to a sub-lethal CLP. At 3 days post-CLP an intra-nasal infusion of either *Streptococcus pneumonia* (SP) or *Pseudomonas aeruginosa* (PA) was administered. Control animals received either a mock CLP (Sham) followed by intra-nasal infusion of SP/PA, or CLP followed by intra-nasal saline (Sal.). **Results:** See graphs below. **Conclusions:** A primary, sub-lethal infection impairs the immune system, rendering the host more susceptible to secondary infection and leading, as demonstrated, to a higher mortality rate. This model provides a clinically relevant tool in the study of sepsis.



P4

SUSPENDED ANIMATION ALLOWS NORMAL RECOVERY AFTER EXSANGUINATION CARDIAC ARREST IN RATS.

T. Drabek, J. Stezoski, L. Jenkins, M. Chadha, X. Wu, W. Stezoski, J. Henchir, S. Tisherman, P. Kochanek (Support: DAMD 17-0102-0038) Safar Center for Resuscitation Research, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260

Introduction: Suspended animation (SA) is a novel method for resuscitation of exsanguination cardiac arrest (ExCA) using aortic flush to achieve deep hypothermia circulatory arrest (DHCA) followed by delayed resuscitation. We have successfully studied SA in a dog model. A rat SA model would enable study of mechanisms and drug screening.

Hypothesis: Survival from 20 min SA at 15°C after ExCA is achievable.

Methods: ExCA was achieved by removal of 12.5 ml of blood over 5 min, followed by KCl-induced CA and 1 min of no-flow. Three groups were studied: (1) hypothermic SA (H-SA, 0°C flush with Plasma-Lyte A, n=5); (2) normothermic SA (N-SA, 38°C flush, n=6); (3) control group (n=6). After 20 min of H-SA or N-SA, resuscitation was attempted via miniaturized CPB over 60 min. Controls were subjected to 60 min CPB only. Surviving rats were weaned from mechanical ventilation and extubated 2 h later. Survival, Overall Performance Category (OPC), Neurologic Deficit Score (NDS) and weight were assessed at Day 7.

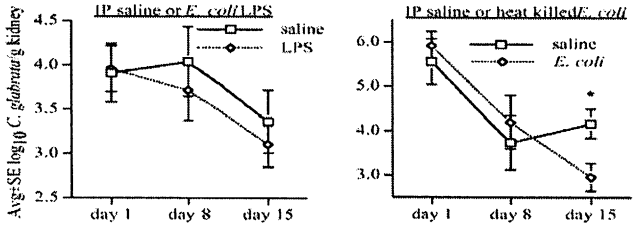
Results: All rats in H-SA and control groups achieved OPC 1. None of the rats in N-SA group had restored cardiac activity or survived. NDS was normal in H-SA and control rats. There were no differences in NDS (2±4.5 vs. 7.5±8.8; p=0.24) and weight (339±9 g vs. 339±29 g; p=0.96) between H-SA and control groups.

Conclusion: We have established a rat SA model that includes 20 min of SA. This new model should greatly facilitate study of both mechanisms of neuronal injury and therapies in DHCA.

P5

EFFECT OF LPS AND NONVIABLE *ESCHERICHIA COLI* ON MURINE *CANDIDA GLABRATA* CANDIDEMIA. R. Garni, M.A. Johnson*, C. Bendel*, M. Henry-Stanley and C. Wells. Univ. of MN, Minneapolis, 55455, USA.

Candida glabrata is now the 2nd or 3rd most frequent cause of candidemia (after *C. albicans*) in trauma patients. Although considered less pathogenic than *C. albicans*, *C. glabrata* candidemia has similarly high mortality (~30-40%). Relatively little is known about *C. glabrata* pathogenesis, but patients with a *C. glabrata* blood isolate often have concomitant or preceding isolation of a bacterium or another *Candida* sp. Because others have reported that *C. albicans* candidemia can be augmented by intact *E. coli* or by *E. coli* LPS, experiments were designed to clarify the effect of *E. coli,* and *E. coli* LPS, on *C. glabrata* candidemia. Mice were inoculated IV with 10^8 *C. glabrata* and sacrificed 1, 8 and 15 days later. To study the effect of LPS on the course of systemic *C. glabrata* infection, mice were also inoculated 16 hr before each sacrifice with IP saline or with IP 100 μg *E. coli* LPS (n=10-15/treatment/ day). Compared to saline controls, fewer *C. glabrata* were recovered from the kidneys (left figure) and livers (not shown) of LPS-treated mice, although these differences were not significant. In a follow-up experiment, $10^{8.7\text{-}9.7}$ heat-killed *E. coli* was substituted for LPS. In mice with long-standing (15-day) *C. glabrata* candidemia, IP *E. coli* was associated with a 14-fold (P<0.05) and 6-fold (P=0.1) decrease in the numbers of *C. glabrata* recovered from kidneys (right figure) and livers (not shown), respectively. Thus, *E. coli*



LPS and intact *E. coli* did not augment *C. glabrata* candidemia, and these bacterial agents actually appeared to ameliorate the course of systemic *C. glabrata* infection.

P6

VAGUS NERVE PREVENTS SYSTEMIC INFLAMMATION BY INHIBITING TNF TRANSCRIPTION IN THE SPLEEN. Luis Ulloa, Hong Liao*, Mahendar Ochani*, Kanta Ochani*, Jared M. Huston*, Christopher J. Czura, & Kevin J. Tracey . Center of Immunology and Inflammation, North Shore-LIJ Research Institute. North Shore University Hospital. 350 Community Drive, Manhasset, New York 11030. Lulloa@nshs.edu

Endotoxin stimulates macrophages to release pro-inflammatory cytokines including tumor necrosis factor (TNF), which can cause lethal shock and tissue injury. We have recently discovered that vagus nerve stimulation attenuates circulating TNF levels during endotoxemia. This mechanism was named "the cholinergic anti-inflammatory pathway" because acetylcholine, the principle neurotransmitter of the vagus nerve, inhibits TNF production in macrophages. The object of this study is to determine how vagus nerve regulates TNF production in different organs. Here we report that stimulation of the vagus nerve prevents lethal systemic inflammation by inhibiting TNF transcription in the spleen. Splenectomy attenuates circulating TNF levels during endotoxemia (sham = 181.3 +/- 31 pg TNF/ mL serum vs. splenectomy = 41.2 +/- 13.4 pg TNF/mL serum; p<0.05). Vagus nerve stimulation fails to further attenuate circulating TNF levels in splenectomized mice. Electrical stimulation of the vagus nerve activates a splenic neuronal network, and sectioning of the common celiac trunk of the vagus nerve, proximal to the spleen, ablates vagus nerve regulation of TNF in both

spleen and serum. This research was supported by the Faculty Awards Program of the North Shore Research Institute, NIGMS, the North Shore-LIJ GCRC, and DARPA.

P7

LOCAL CONTROL OF BLEEDING BY QUIKCLOT OR TOURNIQUET IN UNCONTROLLED HEMORRHAGIC SHOCK INDUCED BY RAT TAIL RESECTION. M.M. Krausz, L. Semenikhina*, and M. Hirsh. Dept of Surgery A, Rambam Medical Center and Technion, Haifa 31096, Israel.

Background: The mineral granular hemostatic agent QuikClot (QC) has been recently recommended for control of external cutaneous bleeding in military trauma. In the present investigation the efficiency of hemostasis by QC was studied in uncontrolled hemorrhage induced by rat-tail resection (RTR).
Methods: The animals were randomly divided into 5 groups: Group 1 (n = 8) - Sham operated. Group 2 (n = 8) - RTR untreated. Group 3 (n = 8) - RTR treated by a tourniquet. Group 4 (n = 8) - RTR treated by application of dry gauze on the terminal portion of the bleeding tail. Group 5 (n = 8) - RTR treated by application of QC on the terminal portion of the bleeding tail. Results: Rat tail resection in group 2 was followed by a drop in MAP from 105.3 ± 5.8 to 47.7 ± 7.8 mmHg ($p < 0.001$), and heart rate decreased from 388 ± 13 to 296 ± 16 beats/minute, in 15 minutes. A similar early change in MAP and pulse rate was also observed in groups 3, 4, and 5. After 120 minutes MAP in group 2 dropped to 19.6 ± 9.8 mmHg ($p < 0.001$) and 62.5% of the animals were dead. Mean blood loss (MBL) was 41.3 ± 5.2 mL and mean survival time (MST) was 87.3 ± 11.7 minutes. In group 3 MAP after 120 minutes was 64.1 ± 8.2 mmHg and no animal died. MBL in this group was 25.0 ± 1.8 ($p < 0.01$) and MST was 120 minutes. In group 4, MAP after 120 minutes was 30.8 ± 12.3 mmHg and 50.0% of the animals were dead. The MBL was 32.4 ± 4.1 mL and the MST 102.3 ± 7.7 minutes ($p < 0.05$). In group 5, MAP after 120 minutes was 70.4 ± 9.4 mmHg and no animal has died. The MBL was 25.2 ± 1.4 mL ($p < 0.01$) and MST was 120 minutes. Conclusions: Control of bleeding with QuikClot significantly reduced blood loss from the bleeding rat-tail and improved survival similar to tourniquet application.

P8

THE EFFECT OF HYPOTHERMIA ON THE GENE EXPRESSION OF iNOS IN HEMORRHAGIC SHOCK IN RATS. K. Kim*, W. Kim*, G. Suh*, Y. Youn. Seoul National University College of Medicine. Seoul, Korea.

Objective: To evaluate that hypothermia(HT) in hemorrhagic shock (HS) would decrease the induction of the iNOS, and that it would in turn, improve the hemodynamic and metabolic parameters.
Methods: Male Sprague-Dawley rats were randomly divided into the hypothermia(HT, n=8, 27-30°) and the normothermia group(NT, n=8, 36-37°). Orotracheal intubation was done and hemorrhagic shock was induced by withdrawing blood (2cc/100g of body weight) via the femoral artery over 30 minutes (induction phase of HS). After blood withdrawal, rats were observed for one hour with monitoring of hemodynamic parameters(maintenance phase), and then half the withdrawal blood was reinfused(resuscitation phase). Rats were sacrificed after the observation for 30 minutes(post-resuscitation phase). Malondialdehyde(MDA), the expression of iNOS mRNA in the lung, and plasma NO concentration were measured. Arterial blood gas analyses(ABGA) and lactate levels were also checked.
Results: In the induction, maintenance and post-resuscitation phases, the mean arterial pressures were significantly increased in the HT group compared with the NT group. In ABGA, PaO_2 was higher in the HT group than in the NT group(106.3±37.3 mmHg vs 46.0 ±38.6mmHg, p=0.015), while blood lactate level was lower in the HT group.(1.1±1.1 nmol/L vs 6.4±5.0 nmol/L, p=0.021). Lung MDA contents were significantly decreased in the HT group.(63.8 ± 6.2 nmol/g vs 44.6 ± 4.5 nmol/g, $p<0.001$). Plasma NO concentrations were reduced in HT group, but there was no significant changes between the two groups(11.71 ± 1.97 μmol/l vs 12.94 ± 3.23 μmol/l, $p = 0.383$). The band density of the expression of iNOS mRNA in the lung was significantly increased in the NT group compared to the HT group (9088.4 ± 3984.0 vs 1313.0 ± 924.4, $p < 0.001$).
Conclusion: In hemorrhagic shock, hypothermia may improve hemodynamic and metabolic parameters, and inhibit the lipid peroxidation and the gene expression of iNOS.

P9

COMPARISON OF HYPOTENSIVE RESUSCITATION WITH POLYHEME (HBOC) OR BLOOD IN A SWINE HEMORRHAGE MODEL. M.A. Dubick, J.L. Sondeen, M.D. Prince,* J.B. Holcomb*. US Army Institute of Surgical Research, San Antonio, TX 78234, USA.

Hemoglobin therapeutics are proposed as improved resuscitation fluids due to their oxygen-carrying capacity. The present study investigated whether hypotensive resuscitation with HBOC would improve hemodynamic and metabolic recovery from severe hemorrhage as effectively as fresh whole blood (FWB). Anesthetized, splenectomized and instrumented 40 kg swine (n=9-10/gp) were subjected to a controlled hemorrhage of 20 ml/kg over 5 min that duplicated the blood loss profile of an uncontrolled hemorrhage. After 30 min, fluid resuscitation was initiated with HBOC or shed blood along with a second hemorrhage of 8 ml/kg. Fluid infusion was controlled to return systolic blood pressure (SBP) to 80 mmHg, as necessary throughout the experiment. Hemodynamic and metabolic variables were monitored continuously and blood samples were drawn at baseline and at select times throughout the 3.5 hr experiment or until death. Survival rates were 8/10 and 9/9 in the Polyheme and blood groups, respectively. Hemorrhage reduced MAP to about 36 mmHg and lowered cardiac output (CO) to 36% of BL in all groups. Both HBOC and FWB improved MAP and CO similarly. Heart rate, systemic vascular resistance and oxygen delivery were also similar between groups. Although pulmonary artery pressures were higher in the HBOC than FWB group, values were still within clinical norms.

Plasma lactate was about 2-fold higher in the HBOC than FWB group after 60 min. Base excess showed a similar trend between the groups. Hypotensive fluid resuscitation with HBOC and FWB to SBP of 80 mmHg required similar volumes of fluid (24 ± 2 vs 21 ± 3 ml/kg, respectively). In summary, although PolyHeme matched the hemodynamic response of FWB, it may not correct some indices of tissue hypoxia associated with hemorrhage and hypotensive resuscitation. Additional studies would be required to evaluate whether stored blood would be equivalent to FWB in this model.

P10

TRAUMA-ASSOCIATED SEVERE HEMORRHAGE (TASH)-SCORE: A SCORING SYSTEM TO IDENTIFY MULTPLY INJURED PATIENTS WITH HIGH RISK FOR BLEEDING.
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The aim of this investigation was to develop a scoring system that allows an early and reliable estimation for the risk of bleeding in multiply injured patients. Materials and Methods: Based on data from the Deutsche Gesellschaft für Unfallchirurgie (DGU)-Trauma Registry (1993-2003; n=17.200) clinical and laboratory parameters were subjected to uni- / multivariate analysis related to the risk of relevant bleeding, i.e. administration of ≥ 10 PBC's (packed red blood cell concentrates) from hospital admission to ICU. Results: Six independent variables which are available within the first 15 minutes following hospital admission were identified to be significantly associated with an increased risk for bleeding in multiply injured patients ($p<0.0001$), i.e. systolic blood pressure (< 60mmHg = 10pts, <100mmHg = 7pts), haemoglobin (< 7g/dl = 24pts, < 9g/dl = 18pts, < 10g/dl = 14pts, and < 12g/dl = 10pts), free intraabdominal fluid by ultrasonography (10pts), complex bone fractures of the extremities (10pts), lactate (> 4mmol/l = 6pts), and base excess (< -10 mmol/l = 11, < -6 mmol/l = 5, and < -2mmol/l = 2). The TASH-score was calculated by the summation of the scores from each variable. The higher the TASH-Score the higher the risk for relevant and life-threatening bleeding: A TASH-score of 43 points corresponds to a 50% risk. Conclusion: The TASH-score allows an easy and reliable estimation for the risk of bleeding in patients with severe multiply trauma. Apart from surgical approaches early application of substances to stabilize coagulation can be more targeted thus substantially improving outcome.

P11

HBOC-201 RESUSCITATION IN A SEVERE SWINE HEMORRHAGIC SHOCK MODEL SIMULATING COMBAT CASUALTIES WITH DELAYED EVACUATION
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OBJECTIVES: To compare the effects of small-volume resuscitation with bovine polymerized hemoglobin (HBOC-201) and hetastarch (HEX) in a combat-relevant swine hemorrhagic shock (HS) model with delay to hospital care. **METHODS:** 24 pigs were anesthetized and invasively monitored. A soft tissue injury was created and pigs were hemorrhaged 55% estimated blood volume. At 20-min, pigs were resuscitated with HBOC-201, HEX, or nothing (NON) followed by additional infusions as needed to normalize HR and BP. Pigs were monitored for a 4-h "pre-hospital" period until simulated hospital arrival: surgical sites repaired, blood or saline administered, pigs recovered from anesthesia and monitored for 72-h. **RESULTS:** 100% (8/8) of HBOC-201-, 75% (6/8) of HEX-, and 38% (3/8) of NON-resuscitated pigs survived to 72-h. Mean arterial pressure and transcutaneous tissue oxygenation were greatest in HBOC-201 pigs ($P < 0.001$). Increased mean pulmonary arterial pressure and systemic vascular resistance were observed in HBOC-201 pigs ($P<0.001$). HBOC-201- and HEX-pigs had comparable heart rate, cardiac output, pre-hospital fluid requirements, base deficit, and urine output. At 4-h, 12.5% HBOC-201 pigs required blood transfusions compared to 100% of HEX-resuscitated pigs ($p < 0.01$). **CONCLUSIONS:** In a severe model of HS, in comparison to HEX, low-volume HBOC-201 resuscitation improved hemodynamics, tissue oxygenation, and transfusion avoidance. These beneficial results, similar to findings in a less severe model (40%), further substantiate HBOC-201 as a superior fluid compared to HEX for resuscitation from HS with controlled hemorrhage and delayed definitive care.

P12

RELATIVE EFFICACY OF GUT PROTEASE INHIBITION POST HEMORRHAGIC SHOCK – NAFAMOSTAT IS BEST
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One mechanism for the gut origin of multiple organ failure post hemorrhagic shock may be immune priming by intraluminal gut proteases. Prior work has shown that gut protease activity can be blocked by nafamostat mesylate (Futhan®), a serine protease inhibitor, with decreased post shock fluid resuscitation requirements and neutrophil activation. Another class of serine protease inhibitors is the chloromethyl ketones which can also block LPS and cytokine induced NFkB activation. The purpose of this study was to evaluate the relative efficacy of the chloromethyl ketone serine protease inhibitors tosyl lysyl chloromethyl ketone (TLCK) and thioester peptide chloromethyl ketone (TPCK) to Futhan in inhibiting gut protease activity. Yorkshire pigs (21 to 26 kg) underwent laparotomy with placement of enterostomy tubes in the duodenum, mid-jejunum and terminal ileum. Pigs were bled 30 ml/kg over 30 min and a MAP ? 30 torr maintained for 60 minutes. Resuscitation was then performed with shed blood to maintain MAP = 45 torr for 3 hours. Once resuscitated, GoLYTELY® was administered via duodenal catheter (1 L/hr) and samples of enteral content were collected at baseline, after resuscitation, and at 30 minute intervals thereafter. Enteral samples from the 3 gut sites were assessed for protease activity in vitro without inhibitor, with

Futhan alone (0.3mM), with TLCK (1mM) and TPCK (2mM), and with Futhan, TLCK and TPCK. TLCK and TPCK provided partial inhibition of gut protease activity in the duodenum, equivalent inhibition in the mid jejunum, and minimal inhibition in the terminal ileum. Futhan provided the best inhibition of enteral protease activity in all of the sites with no added benefit seen from the addition of TLCK and TPCK to Futhan. This demonstrates that Futhan is the protease inhibitor with the greatest therapeutic potential for successfully inhibiting enteral proteases post hemorrhagic shock to attenuate the systemic inflammatory response and secondary organ dysfunction.

P13

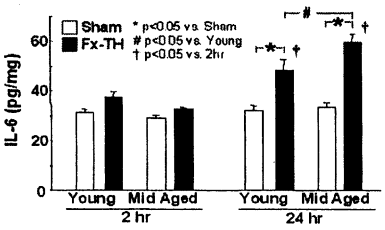
A PEPTIDE INHIBITOR OF c-JUN N-TERMINAL KINASE PROTECTS AGAINST HEMORRHAGE/RESUSCITATION INDUCED INJURY IN THE RAT. M. Lehnert*, C. Czerny*, V.S. Young*, D. Henrich*, H. Lind*, T. Borsello*, I. Marzi, J.W. Goethe University, 60560 Frankfurt, Germany

Activation of the c-Jun N-terminal kinase (JNK) pathway represents an early signalling event that is associated with the systemic stress response after hemorrhage/resuscitation (H/R). Here, using a cell-penetrating, protease resistant peptide that blocks the access of JNK to many of its targets, we tested the hypothesis that in vivo inhibition of JNK blunts organ damage and proinflammatory changes after H/R. **Methods**: Male Sprague Dawley rats received an intraperitoneal injection with d-JNKI-1 (11mg/kg, Alexis Corp., n=6) or vehicle (n=9). 30 min later, rats were subjected to hemorrhagic shock for 1 hour at 30-35mmHg. Rats were resuscitated over a 20min period with twice the shed blood volume as Ringers Lactate and 60% of the shed blood. Two hours after the end of resuscitation, serum was collected and superoxide generation from polymorphnuclear leukocytes (PMNs, oxidative burst) was quantified by flowcytometry by measuring the superoxide mediated conversion of DHR123 to the greenfluorescent rhodamin123. The mean fluorescence intensity (MFI) is proportional to the quantity of superoxide radicals. **Results:** Serum alanine aminotransferase (ALT) as a marker of liver injury increased to 2963 ± 670 (mean ± S.E.M.) IU/L. In rats treated with d-JNKI-1, ALT level was blunted to 955 ± 166 IU/L ($p<0.05$). Serum Creatine kinase (CK) levels were also attenuated in rats that received d-JNKI-1 treatment to 5839 IU/L (25% quartile 5343 IU/L, 75% quartile 10075 IU/L) compared to 14366 IU/L in the vehicle group ($p<0.05$) (25% quartile 11024 IU/L, 75% quartile 28133 IU/L). d-JNKI-1 treatment showed a tendency to reduce pancreatic injury (786 ± 267 IU/L vs. 172 ± 43 IU/L, $p=0.08$). Production of superoxide radicals by PMNs decreased by 33% after d-JNKI-1 treatment (45 ± 4.2 vs. 31 ± 3.6 MFI, $p<0.05$). **Conclusion:** H/R cause severe organ damage, which is mediated by JNK. JNK is also involved in activation of PMNs to produce reactive oxygen species. Therefore, JNK inhibition might represent a reasonable therapeutic target for H/R induced injury.

P14

AGE-RELATED DIFFERENCES IN PULMONARY INJURY FOLLOWING BONE FRACTURE AND SOFT-TISSUE TRAUMA AND HEMORRHAGE. T Matsutani, S Kang*, KI Bland*, IH Chaudry. Center for Surgical Res. and Department of Surgery, University of Alabama at Birmingham, AL 35294.

Trauma is an important risk factor for the development of the acute lung injury. Although it is known that the immune responses after bone fracture and soft-tissue trauma-hemorrhage (Fx-TH) are different in young (6-8 weeks) and middle aged (12 months) mice, it is not known whether any differences in the extent of pulmonary injury exits between young and middle-aged following the above mentioned injury. To examine this, young and middle-aged C3H/HeN male mice were subjected to right lower leg fracture, midline laparotomy) and hemorrhage (BP to 35 ± 5 mmHg for 90 min) followed by adequate crystalloid resuscitation. The mice were euthanized at 2 and 24 h thereafter and lung tissue was harvested. TNF-α levels in the lung significantly increased at 2 h following Fx-TH in both young and middle-aged mice, however, the TNF-α levels in middle-aged mice were significantly higher than the young mice at 24 h (n=6-8/group. One-way ANOVA and Tukey's test). IL-6 levels in the lung increased significantly at 24 h following Fx-TH in both groups, whereas IL-10 levels increased in the middle-aged mice but not in the young mice at 24 h after Fx-TH ($p<0.05$). Moreover, MCP-1 levels increased significantly in the middle-aged mice but not in the young at 2 h after Fx-TH. The protein and mRNA levels of cytokeratin, which is released from injured bronchial epithelium, were significantly higher in the lung from middle-aged compared to young mice after Fx-TH ($p<0.05$). These results collectively suggest that the different patterns of cytokine production in young vs. middle mice following Fx-TH may play a significant role in the development of acute lung injury under those conditions *(Supported by NIH Grant GM 37127).*



P15

17β-ESTRADIOL ATTENUATES THE ENDOTHELIN-1 VASOCONSTRICTION: ESTRADIOL RECEPTOR-β PLAYS AN IMPORTANT ROLE: Z.F. Ba*, T. Shimizu*, L. Rue, K. Bland*, I. Chaudry. Center for Surgical Research and Department of Surgery, University of Alabama at Birmingham, AL 35294

Although 17β-estradiol (E2) reduces the production of vasoconstrictor endothelin-1 (ET-1) following trauma-hemorrhage, it remains unknown whether estradiol attenuates the ET-1 vasoconstriction. To study this, the thoracic aorta was isolated from normal male Sprague-Dawley rats and cut into ~ 2.5 mm rings. Isolated aortic rings were placed in the organ bath that contained aerated Krebs-Ringers buffer (95%O_2 and 5%CO_2). The dose response curves for ET-1 were determined

with and without 17β-estradiol (E2) pretreatment. The vaso-relaxation capacity of E2, DPN (ER-β agonist) and PPT (ER-α agonist) on ET-1 vasoconstriction were also examined (n=6/group). Additionally, the effects of E2 on ET-1 vasoconstriction were determined in aortic rings with L-NNA (L-arginine antagonist), and indomethacin (cyclooxygenase inhibitor) pre-treatment, or denuded endothelium. One-way ANOVA and Tukey's test were used for group comparison, and differences were considered significant at $p<0.05$. The results indicate that ET-1 vasoconstriction in aortic rings were significantly attenuated by E2 pretreatment ($p<0.05$). DPN induced greater vasorelaxation on ET-1-induced vasoconstriction compared to E2 ($p<0.05$); however, PPT did not induce significantly relaxation until a concentration of 10^{-5} M. The results also indicate that the attenuated effects of E2 on ET-1 vasoconstriction were prevented by endothelium denudation or pretreatment with L-NNA. However, the attenuated effects of E2 on ET-1 vasoconstriction were still observed if indomethacin pre-treatment was carried out. These results therefore indicate that E2 significantly reduces ET-1 vasoconstriction and that ER-β plays an important role in this process. Moreover, the salutary effects of E2 on ET-1 vasoconstriction appear to be endothelium-dependent and NOS-mediated. (Supported by NIH Grant R37 GM 36519)

P16

DOWNREGULATION OF CARDIOMYOCYTE IL-6 IS ONE OF THE MECHANISMS RESPONSIBLE FOR THE PROTECTION OF CARDIAC FUNCTION IN PRO-ESTRUS (PE) FEMALES FOLLOWING TRAUMA-HEMORRHAGE (TH). S. Yang*, S. Hu, Y. Hsieh, M. Choudhry, L.W. Rue, K.I. Bland, and I.H. Chaudry. Center for Surgical Research and Dept. of Surgery, University of Alabama at Birmingham, Birmingham, AL 35294

Cardiac function is depressed and circulating IL-6 levels increase in both males and females following TH. However, such changes do not occur in PE females under those conditions although the mechanism responsible for the protection of PE females is not known. Moreover, a sustained elevation of IL-6 correlates with poor outcome. Although cardiomyocyte IL-6 levels and IL-6 gene expression increase after TH in males and females, it is not known if cardiac IL-6 levels and IL-6 gene expression are also unaffected in PE females following TH. Male and female adult S-D rats (225-275 g) were divided into 6 groups: male sham and TH, female sham, PE-TH, met-estrus (ME)-TH and ovariectomized (OVX)-TH (n=6/group). Sixty percent of the circulating blood was withdrawn from femoral artery over ~45 min and after 90 min of severe hypotension, rats were resuscitated (4 x the shed blood volume in the form of Ringer's lactate over 1 h). At 2 h thereafter, cardiac output (CO) was determined by ICG dilution technique, blood samples were collected and the hearts harvested to isolate cardiomyocytes. Cardiomyocyte intracellular IL-6 levels were measured by Flow Cytometry and IL-6 gene expression was determined by real-time PCR. In additional groups, fresh heart tissues were harvested to measure cardiac IL-6 protein levels using western blot. Plasma IL-6 and steroid hormones were measured by ELISA or EIA kits. Data are mean ± SE. ANOVA and Tukey's test was employed for comparison among groups of animals and differences were considered significant at $p\leq0.05$. The results indicate that following TH: 1) CO was depressed in male, ME and OVX females but not in PE females, 2) plasma estrogen levels increased in PE females, 3) plasma IL-6 levels increased in male, ME and OVX females, however, they were markedly decreased ($p<0.05$) in the PE group, 4) cardiomyocyte intracellular IL-6 levels increased in male, ME and OVX females, however, they were significantly decreased in PE group, and 5) cardiomyocyte IL-6 gene expression significantly increased in male, ME and OVX females, however, it was attenuated in PE females. Thus, downregulation of IL-6 protein and gene expression following TH appears to be one of the mechanisms responsible for the protection of cardiac functions in PE females under those conditions (NIH grant R37 GM 39519).

P17

TRAUMA-HEMORRHAGIC SHOCK (T/HS)-INDUCED RBC RIGIDIFICATION IS ASSOCIATED WITH ALTERATIONS IN THE RBC LIPID COMPOSITION.

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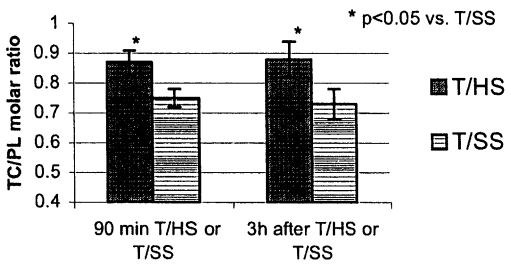
Objective: The lipid bilayer plays an important role in the maintaining of RBC deformability (RBCD). RBCD is decreased following T/HS. However, the effect of T/HS on the lipid composition of the RBC membrane remains unknown. We hypothesize that T/HS alters the distribution of RBC lipids and that this is related to decreased RBCD. **Methods**: Lipid composition in plasma and RBC was studied in rats subjected to T/HS or trauma-sham shock (T/SS). Total cholesterol (TC) and phospholipids (PL) were determined using commercial kits. RBCD index (DI) was determined by Reid filtration technique. **Results:** *Plasma TC* at the end of 90 min T/HS (before resuscitation) was lower than after T/SS (1.3±0.4 vs. 1.9±0.6 mmol/L, $p<0.05$) and remained lower 3h after T/HS. *Plasma PL* were also decreased in T/HS rats compared to T/SS rats. *RBC TC* was higher in T/HS rats (3.5±0.4 vs. 2.9±0.4 μmol/g, $p<0.05$). T/HS rats demonstrated higher *RBC TC/PL* molar ratio compared to T/SS animals (*Fig.1*). Concentration of *RBC PL* did not differ in T/HS and T/SS rats. RBC DI in T/HS rats was significantly lower at all points of investigation. There was an



inverse correlation between *RBC TC/PL* ratio and DI ($r=-0.67$, $p<0.01$). **Conclusion**: These data demonstrate that T/HS alters lipid distribution in RBC increasing their TC/PL ratio, which is associated with RBC rigidification.

P18

CROCETIN INHIBITS EXPRESSION OF INFLAMMATION-RELATED GENES IN HEMORRHAGIC SHOCK

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Introduction. Hemorrhagic shock (HS) following acute injury often leads to multiple organ failure and death. Crocetin, a saffron-derived carotenoid, has been shown to improve survival after hemorrhagic shock. The present study was designed to investigate its mechanism of action. Our hypothesis was that crocetin supresses post-shock over-expression of mRNA for tumor necrosis factor (TNF-α). interleukin 1 (IL-1β), and inducible nitric oxide synthase (INOS). **Methods**. HS was produced in male Spague-Dawley rats (350±30g) anesthetized with isoflurane by withdrawing blood through a femoral arterial cannula until the mean arterial pressure (MAP) was 25-30 mmHg, and maintaining that level for 30 minutes with further withdrawals. Resuscitation was done with 21 ml/kg Ringer's lactate (LR), with and without crocetin (2mg/kg), followed by return of shed blood. Controls were HS only (no resuscitation), sham (no HS) and normal (anesthesia only). Each group had 9 animals. The animals were sacrificed 30 minutes after completion of resuscitation. Total RNA was isolated from liver samples for RT-PCR (TNF-α, INOS, IL-1β and β-actin). **Results**. Increased expression of liver mRNA for TNF-α, INOS, IL-1β was seen following HS. Crocetin treatment significantly reduced mRNA expression for TNF-α, IL-1β, and INOS. All animals expressed β-actin mRNA. **Conclusion**. Crocetin modified the expression of genes controlling cytokines and INOS in the liver. Subsequent investigations will determine whetherthis action is primary or is secondary to increased oxygen transport. This agent continues to show potential for treatment of hemorrhagic shock. (Supported by the Office of Naval Research, ONR N00014-01-0151 Van Way 11/2001, and by a grant from the American Heart Association)

% of animals expressing mRNA			
	Shock	LR	Crocetin
TNFα	66.7	77.8	33.3
INOS	50.0	88.9	33.3
IL-1β	77.8	88.9	22.2

P19

TRAUMA-HEMORRHAGIC SHOCK LYMPH KILLS MULTIPLE CELL TYPES AND ITS TOXICITY IS NEUTRALIZED BY ALBUMIN

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Previously we have published that trauma-hemorrhagic shock mesenteric lymph (T/HS) but not trauma sham-shock (T/SS) lymph kills endothelial cells via an apoptotic mechanism and that albumin is protective. This study was performed to test the hypothesis that T/HS mesenteric lymph cytotoxicity is not limited to endothelial and that albumin would protect these other cell populations. **Methods:** The following cell lines were tested: A7r5: rat smooth muscle cells, L-929: mouse fibroblast cells, IEC-6: rat enterocytes; CaCo-2: human enterocytes; CCL-64: mink lung epithelial cells, CCL-95.1: human lung epithelial cells and HUVEC: human umbilical vein endothelial cells. The protective effects of both bovine and human albumins were tested at 0.5% or 1% concentrations. Each cell type was incubated with 5% rat T/HS lymph with or without albumin for 18 hours and then cell cytotoxicity was determined by measuring cell viability using the MTT assay. Cell viability was expressed as a percentage of the viability of cells incubated in medium. **Results:** After 18 hrs incubation with 5% T/HS lymph, the viability of each of these cell lines was reduced by 50% to 88%, depending on the cell line (i.e. viability 12 to 50%). T/SS lymph had no effect on cell viability. Both bovine and human albumin protected against T/HS lymph induced cytotoxicity in a dose-dependent fashion. The viability of the cell lines incubated with T/HS lymph plus 0.5% albumin ranged from 60% to 90% ($p<0.01$), while the viability of the cell lines incubated with 1% albumin ranged from 92% to 100% ($p<0.01$). **Conclusion:** Albumin has a general protective ability against T/HS lymph-induced cytotoxicity indicating it may have broad protective effects. Whether its beneficial effects are due to its anti-oxidant, scavenging or other properties will require more study.

P20

EFFECTS OF SUPPLEMENTAL INSPIRED OXYGEN DURING HEMORRHAGE IN THE RODENT.

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The following study was undertaken utilizing a fixed pressure hemorrhage model to evaluate if administration of oxygen (O2) during an ongoing hemorrhage would alter the volume of blood removed. **Methods**: Three O2 concentrations were initially tested. All rats (n=20; 443±10 gm) were anesthetized with 1.5 to 2.0% isoflurane and allowed to spontaneously breath. The initial inspired O2 content was the same for each group, air at 21% O2. After vascular cannulation and a 45 min stabilization period, they were bled over 40 min to a mean arterial pressure (MAP) of 40-45 mmHg and maintained at that pressure for 30 additional minutes by removing blood as required. Group 1 received only air (21% O2). Beginning at 40 min, Group 2 was given supplemental O2 at 40% and Group 3 was given 60% O2. At 30 min post hemorrhage Group 2 and 3 were changed to 100% O2 and observed. No resuscitation fluids were given. **Results:** Data are mean ± SEM. Initial MAP in Group 1 was 86 ± 4 mmHg and was similar between groups as was the MAP at 40 min and 70 min being about 43 mmHg. The total hemorrhage volume at 70 min was 21 ± 2 ml/kg in Group 1, 24 ± 2 in Group 2, and 20 ± 2 in Group 3. These differences were not significant. However, after inspired O2 was changed to 100% at 30 min post hemorrhage in Groups 2 and 3, MAP was significantly increased for up to 20 min compared to Group 1 (P?0.02). MAP was 62±6 mmHg in Group 1, 77±6 mmHg in Group 2, and 87±4 mmHg in Group 3. **Conclusion**: Increasing the percentage of inspired oxygen up to 60% during an ongoing fixed pressure hemorrhage does not appear to alter the volume of blood that can be

removed from a rat. However, in the post hemorrhage period, administering a very high concentration of inspired oxygen (100% FIO2) will help maintain MAP transiently even if resuscitation fluids are not administered.

P21

WHOLE BODY HYPERTHERMIA INDUCES HSP72 IN THE BRAIN AFTER RESUSCITATED HEMORRHAGE

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Head trauma produces cerebral cellular injury that contributes to morbidity following trauma/hemorrhage. Heat shock proteins (HSPs) generated in response to stress, attenuate protein denaturation and may preserve cellular function. This study was performed to determine the effect of whole body hyperthermia (WBHT) on cerebral HSP72 expression after resuscitated hemorrhage. Three groups were studied in rats: 1. HS; 20% bld vol. 2. HS-WBHT. HS followed by WBHT (39.5-40°C for 6hrs) Rats were resuscitated with shed blood and lactated ringers. 3.Control (no HS). The cerebrum was assayed for HSP72 18 hr post-treatment using Western analysis.

	HSP72 (densitometry)
HS	0.0288 +/- 0.0274
HS-WBHT	2.4289 +/- 1.0037*
Control	0.0097 +/- 0.0109

*p<0.05 vs. HS & Control via ANOVA Tukey Kramer HSD

These results demonstrate that HSP72 is induced in brain after hemorrhage and is upregulated when hemorrhage is followed by WBHT suggesting a role for WBHT in decreasing cerebral injury after trauma hemorrhage.

P22

RESUSCITATION OF COMBINED TRAUMATIC BRAIN INJURY (TBI) AND HEMORRHAGIC SHOCK (HS) WITH RINGER'S ETHYL PYRUVATE (REP) VS. LACTATED RINGER'S (LR).

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Combined TBI & HS is a common clinical scenario with high morbidity and mortality. LR is considered the crystalloid of choice for resuscitation of these patients. Pyruvate is an energy substrate with antioxidant and positive inotropic properties. REP (28mEq ethyl pyruvate/L of Ringer's) has been shown to be protective in models of sepsis and ischemia-reperfusion. Hence, REP may be an ideal resuscitative agent for combined TBI & HS. OBJECTIVE: To compare the effects of limited resuscitation with REP vs LR in a model of combined fluid percussion (FP)-TBI & HS. METHODS: 24 anesthetized swine(20kg) with sagittal sinus, left and right cerebral microdialysis catheters, and ICP monitors, underwent 3.0atm FP-TBI and were bled to a mean arterial pressure(MAP) of 35mmHg. Group I(N=13) was resuscitated with REP, Group II(N=11) with LR. For the first 60 min of resuscitation, solutions were infused to maintain MAP=60mmHg. At 60 min, animals were aggressively resuscitated to receive a total of 75mL/kg of study solution, followed by shed blood to normalize physiologic parameters. Animals were observed for 4 hours. RESULTS: 4-hr mortality was 23% and 9% for Groups I & II (Fisher exact; P=0.596; 95%CI diff: -0.145, 0.425). Transfusion requirements did not differ [REP (22±6mL/kg) vs LR (24±6mL/kg); P=0.464; t-test]. Serum pyruvate increased 2-fold to maximum levels of 114±12μM in REP, and 119±13μM in LR animals (P=0.760; rmANOVA). Base deficit reached levels of -14.7±8.4mmoL/L in REP, and -11.4±9.1 mmoL/L in LR animals (P=0.403). Arterial lactate:pyruvate ratio(L/P) increased 4-fold (P=0.854; rmANOVA), while cerebral L/P increased 6-fold in both groups (P=0.445; rmANOVA). Cerebral glutamate, cardiac output, and cerebral and total body O2 consumption also did not differ. CONCLUSION: In a clinically relevant model of combined TBI & HS, resuscitation with REP offered no advantage over LR. REP did not decrease transfusion requirements, or improve survival or measured physiologic parameters.

P23

EFFECT OF BLOOD EXCHANGE WITH LIPOSOME-ENCAPSULATED HEMOGLOBIN ON OXYGEN METABOLISM IN RABBITS

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The present study was performed to clarify the effect of blood exchange with liposome-encapsulated hemoglobin (LEH) on oxygen metabolism. Fifteen New Zealand White rabbits were anesthetized with pentobarbital sodium and randomly divided into three groups (n = 5, each) based on the materials of blood substitute: group 1 with LEH suspension ([Hb] = 6 g/dL in saline containing 3% alubumin), group 2 with shed blood diluted 1:1 with saline containing 3% albumin, and group 3 with saline containing 3% albumin without hemoglobin. After baseline measurements, 9 mL/kg of circulating blood was withdrawn in 150 sec and the identical amount of blood substitute was subsequently infused in 150 sec. This procedure was repeated eight times in 5-min cycles for 40 min to achieve blood exchange at approximately 90% of total circulating blood. Hemodynamics and oxygen metabolism parameters were measured at 60, 90, 120 min after the beginning of blood exchange. Rabbits were subjected to stepwise cardiac tamponade to reduce oxygen delivery (DO_2) thereafter. The oxygen consumption (VO_2)/DO_2 relation was analyzed by the dual line method. Mean values of [Hb] after blood exchange in groups 1, 2, and 3 were 5.7 g/dL, 6.0 g/dL, and 1.2 g/dL, respectively. Mean arterial pressure, heart rate, and cardiac output in groups 1 and 2 at 60, 90, and 120 min did not significantly differ from the corresponding baseline values. All animals in group 3 died by 120 min due to circulatory shock. After blood exchange, DO_2 values for groups 1 and 2 were reduced by approximately 50%, but VO_2 values remained consistent in comparison with the baseline values. In the VO_2/DO_2 relation, the slope of the supply-dependent line for group 1 ($y = 0.69x + 1.8$) was not significantly different from that for group 2 ($y = 0.68x + 2.3$). These results indicate that LEH is a potent blood substitute with respect to the ability of tissues to extract oxygen even in case of massive transfusion.

P24

eNOS DEFICIENCY UPREGULATES iNOS AFTER HEMORRHAGE/RESUSCITATION AND INCREASES ACTIVATION OF c-JUN. H. Lind[*,$], S. Siegmund[*,&], Z. Zhong[*,$], M. Lehnert[*,#], I. Marzi[#], D.A. Brenner[*,&] and J.J. Lemasters[*,$]. Dept. of [$]Cell & Developmental Biology, Univ. of North Carolina at Chapel Hill, [&]Department of Medicine, Columbia University, and [#]Dept. of Trauma Surgery, J.W. Goethe University, Frankfurt/Main.

Previous studies show that eNOS deficiency increases and iNOS deficiency decreases hemorrhage/resuscitation (H/R)-induced liver injury, but the importance of iNOS and proinflammatory stress kinases on H/R injury in eNOS deficient mice is not known. Accordingly, our **AIM** was to test the hypothesis that eNOS deficiency upregulates iNOS expression and activates proinflammatory c-Jun N-terminal kinase (JNK). **METHODS:** eNOS knockout ($eNOS^{-/-}$) and wild-type (WT) mice were hemorrhaged to a maintained mean arterial pressure of 40 mm Hg. After 3h, mice were resuscitated over 30 min with the shed blood plus half the shed volume of lactated Ringer's solution. Serum samples were collected at 1 h and 6 h after resuscitation, and the livers were then harvested for histology and measurement of phospho-c-jun (phosphorylated substrate of JNK) and iNOS by immuno blotting. **RESULTS:** Serum alanine aminotransferase (ALT) was 789 ± 259 and 557 ± 119 IU/L (mean ± SE, n=7) at 1 and 6 h after resuscitation, respectively, in WT mice, which increased more than 5-fold to 4254 ± 808 and 3248 ± 917 IU/L in $eNOS^{-/-}$ mice (n=6, $p<0.01$). Compared to sham operation, phospho-c-Jun increased substantially in wild-type mice after H/R. In $eNOS^{-/-}$ mice, phosphor c-jun increased even more ($p<0.05$). Compared to sham, iNOS expression decreased in wild-type mice after H/R but increased in $eNOS^{-/-}$ mice ($p<0.05$). **CONCLUSION**: eNOS deficiency causes iNOS upregulation after H/R, which may promote JNK activation and liver injury.

P25

GELDANAMYCIN TREATMENT PREVENTS HEMORRHAGE-INDUCED ATP LOSS IN MOUSE ORGANS. J.G. Kiang, P.D. Bowman*, X. Lu*, X.Z. Ding*, B Zhao*, J.L. Atkins, and G.C. Tsokos*. Walter Reed Army Institute of Research, Silver Spring, MD 20910 and US Army Institute of Surgical Research, San Antonio, TX 78234.

Objective: To limit tissue damage following shock induced by hemorrhage. Treatment with geldanamycin (GA, 1 μg/g body weight) increases inducible heat shock protein 70 kDa (HSP-70i) and decreases inducible nitric oxide expression in hemorrhaged mouse organs. This increased expression of HSP-70i is coupled to increased expression of iNOS and its transcriptional enhancer KLF6 (J App Physiol 97:564-569, 2004). The aim of this study was to determine if hemorrhage affected the levels of ATP and whether GA treatment reduced the hemorrhage-induced ATP changes. Methods: Male Swiss Webster mice were subjected to a 40% hemorrhage without resuscitation and allowed to respond to hemorrhage for 1, 3, 6, 12, 24, or 48 h. The cellular ATP levels of lysates prepared from jejunum, lung, heart, kidney, brain, and liver were measured using the ATP Bioluminescence Assay Kit HS II. Results: Hemorrhage decreased ATP levels in jejunum, lung, kidney, heart, and brain, but not liver. In jejunum, the ATP levels continued to decrease up to 48 h, whereas in lung, heart, kidney, and brain they decreased to the lowest point at 6 h and then started returning to the basal levels at 48 h. Pre-treatment with GA inhibited the hemorrhage-induced ATP loss in jejunum, lung, heart, kidney, and brain. The GA inhibition may be due to activation and increased expression of pyruvate dehydrogenase. In cultured intestinal epithelial T84 cells, HSP-70 gene transfection but not iNOS gene transfection inhibited hypoxia-induced ATP loss. Conclusion: These results suggest that GA inhibits the hemorrhage-induced ATP loss probably by inducing the expression of HSP-70i. GA may be of therapeutic use in reducing hemorrhage-induced ATP loss when used as a pre-surgical treatment or when added to resuscitation fluids. (Supported by DOD RADII STO R)

P26

INHIBITION OF THE Na^+/H^+ ANTIPORTER SUPPRESSES TRAUMA-HEMORRHAGIC SHOCK-INDUCED GUT INJURY AND LUNG INJURY

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Background: Trauma-hemorrhagic shock (T/HS) is a major contributor to development of acute respiratory distress syndrome (ARDS) and the multiple organ dysfunction syndrome (MODS) in patients sustaining major trauma. Recognition of the current limitations of standard crystalloid resuscitation regimens has led to an increased interest in alternative resuscitation regimens as well as the use of hypertonic saline (HTS) and other agent. The purposes of this study was to test the hypothesis that the amiloride-inhibitable Na^+/H^+ antiporter would decrease T/HS induced lung and gut injury, neutrophil activation and deterioration of red blood cell (RBC) deformability in vivo. **Methods:** Male rats were subjected to T/HS or trauma sham-shock (T/SS) and resuscitated with Ringers lactate (RL), HTS, amiloride, or the combination of them. The T/HS model consisted of a laparotomy plus 90 minutes of shock (MAP 30mmHg). Three hours after the end of the shock or sham-shock period, lung permeability, gut injury, pulmonary neutrophil sequestration, RBC deformability and neutrophil activation were assessed. **Results:** Both HTS and amiloride reduced T/HS-induced lung permeability and gut injury, the combination of both reduced more effectively, as compared to resuscitation with RL. Amiloride could not, however, reduce deterioration of RBC deformability and neutrophil activation without HTS. **Conclusion:** The combination of HTS and amiloride is more effective on T/HS-induced lung and gut injury than HTS alone.

P27

HEMORRHAGE-INDUCED SKELETAL MUSCLE INSULIN RESISTANCE. L. Holland*, Y. Ma*, H. Kim*, I. Chaudry and J. Messina. University of Alabama at Birmingham, Birmingham, AL, United States, 35294-0019

Insulin is an important regulator of cellular metabolism and exerts its effects by first binding to the insulin receptor.

Phosphorylation and activation of the insulin receptor leads to activation of two major signaling pathways, the phosphatidylinositol 3-kinase (PI3-kinase)/Akt pathway and the mitogen-activated protein kinase (MAPK)/extracellular regulated kinase (ERK) pathway. Severe injuries, such as thermal injury (burn), surgical trauma, hemorrhage and sepsis, lead to a hypermetabolic condition that is characterized by insulin resistance. The molecular defects that lead to the insulin resistant state following severe injury are not well characterized. A male rat (Sprague-Dawley) model of surgical trauma and hemorrhage is utilized in the current studies. In brief, rats are acutely hemorrhaged over a period of 10 minutes and maintained at a blood pressure of 35-40 mmHg for 60 minutes and then sacrificed. This time point is referred to as trauma-hemorrhage 60 minutes (TH60'). Serum insulin and glucose levels were determined and skeletal muscle tissue (triceps) were harvested for use in Western blot analysis. There was no change in serum insulin levels, but there was an elevation of fasting serum glucose concentrations (TH60'=190 mg/dL; sham hemorrhage=117 mg/dL). There was an almost complete reduction of insulin-induced Akt activation (phosphorylation) in tricep muscle following only 60 minutes hemorrhage, with little change after surgical trauma in the absence of hemorrhage. In contrast, there were no changes in insulin-induced MEK/ERK signaling following either trauma alone or following trauma and hemorrhage. These data suggest that muscle insulin resistance occurs rapidly following surgical trauma and hemorrhage and this resistance is selective for insulin-induced activation of the PI3-kinase/Akt pathway.

P28

TRENDING BASE DEFICIT DURING HEMORRHAGE WITH CHEST SKIN PCO_2 OR SUBLINGUAL PCO_2.

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Base deficit is an indicator for degree of metabolic insult/oxygen debt from hemorrhage and is mortality risk related in trauma patients. We investigated the use of less invasively available skin and sublingual PCO_2 as surrogates for base deficit. **Methods:** Anesthetized dogs were hemorrhaged (H, 90min MAP=35-40mmHg). Arterial base deficit was recorded every minute (Diametrics Neotrend® fiber-optic sensor, n=24) as were chest skin PCO_2 (SensorMedics 7650® Severinghaus type sensor, n=12) or sublingual PCO_2 (SensorMedics 7650®, n=12). **Results:** Average (±SD) of correlation coefficients (r) in each dog = 0.95 ± 0.03 for skin PCO_2 versus base deficit and 0.84 ± 0.10 for sublingual PCO_2 versus base deficit. When the chest skin or sublingual PCO_2 and arterial base deficit values from all dogs (n=12 and n=12) were grouped together before correlation, the coefficients with arterial base deficit dropped to 0.47 for skin PCO_2 and 0.49 for sublingual PCO_2, respectively. The reason for the difference in coefficients appears to be variability between dogs in the distance separating arterial base deficit from either chest skin PCO_2 or sublingual PCO_2. For example, the dog with the lowest starting and ending sublingual PCO_2 values had starting and ending arterial base deficits in the highest quartile. **Conclusions:** Either chest skin PCO_2 or sublingual PCO_2 can be used to trend arterial base deficit during hemorrhage (similar slopes). However, the amount of variation between individual animals in the difference between the chosen site PCO_2 and the arterial base deficit would preclude the use of either chest skin PCO_2 or sublingual PCO_2 for indicating actual arterial base deficit unless a time-matched pair were taken at some point in that particular individual. (Funding: IA Space Grant Consortium, Eagles)

P29

BLOOD FLOW AND PRESSURE EFFECTS OF ENALAPRILAT AIDED HYPOTENSIVE STABILIZATION BEFORE RESUSCITATION FROM HEMORRHAGE.

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We are investigating pharmacologic interruption of angiotensin II production (enalaprilat) combined with either 7.8% hypertonic saline dextran (HSD) or a hemoglobin based oxygen carrier (HBOC, Oxyglobin®) as methods to improve blood flows with minimal IV fluid during intentional, severe post-hemorrhage hypotension. **Methods:** 13 anesthetized dogs were hemorrhaged (H, 90min MAP=35-40mmHg), "stabilized" (S, 120min saline vehicle 1mL/kg/hr or enalaprilat [enal] 0.01mg/kg/hr ± HSD or HBOC for MAP =40-45mmHg), resuscitated (R, 60min lactated Ringer's [LRS] for MAP=75-80mmHg), then only monitored (M, 60 min no fluids), and euthanized. **Results:** Only dogs receiving enal with HSD or HBOC survived S (3 of 4 HSD, 1 of which died at R, and 2 of 3 HBOC). Cardiac output (CO) was more than restored with HSD but not HBOC (1.8±0.5 to 0.7±0.3 to 3.6±2.1 L/min HSD versus 1.6±0.4 to 0.8±0.5 to 0.9±0.1 L/min HBOC from start H to S to end S). CO increased during R (5.6±1.6 L/min HSD versus 7.6±4.1 L/min HBOC at mid R), but the LRS required to achieve 75-80mmHg MAP was substantial, especially in HSD dogs (120±57mL/kg versus 75±32mL/kg). The COs and MAPs continuously declined in all dogs after R ended (≤2.1 L/min and 32±26mmHg HSD and ≤1.1 L/min and 51±42mmHg HBOC by end M). **Conclusions:** HBOC with enal may not sufficiently restore blood flows for use as a hypotensive "stabilizing" combination. The resuscitation and monitoring findings are concerning. Considering the H and S durations with those findings and with the growing hemorrhage and vasopressin literature, we believe that vasopressin depletion may be playing an important role in our resuscitation and monitoring period results. (Funding: IA Space Grant, Eagles)

P30

BASE EXCESS AND Na^+, Cl^-, AND ALBUMIN CONTRIBUTIONS DURING HEMORRHAGE, HYPOTENSIVE STABILIZATION, AND RESUSCITATION.
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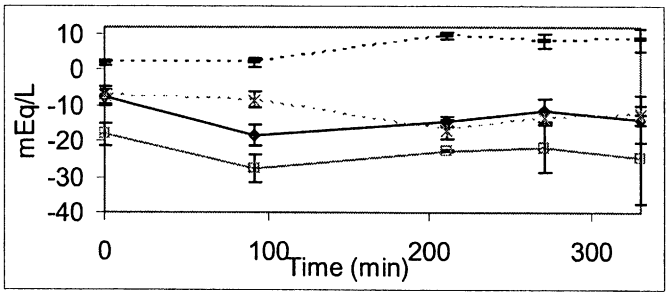
Alterations in Na^+, Cl^-, and albumin, if present and not accounted for, may lead one to false conclusions concerning perfusion status as indicated by base excess (BE_{total}). **Methods:** Anesthetized dogs were hemorrhaged (H, 90min MAP=35-40mmHg), "stabilized" (S, 120min saline vehicle 1mL/kg/hr [n=3] or enalaprilat [enal, n=3] 0.01mg/kg/hr ± 7.8% hypertonic saline dextran [HSD, n=3] for MAP=40-45mmHg), resuscitated (R, 60min lactated Ringer's for MAP=75-80mmHg), then only monitored (M, 60min no fluids), and euthanized. Equations used were: BE_{total}=BE reported as part of blood gas, BE_{Na}=0.3(Na-140), BE_{Cl}=102-(Cl x 140/Na), $BE_{albumin}$=0.34(45-albumin g/L), $BE_{unmeasured}$= BE_{total}-(BE_{Na}+ BE_{Cl}+ $BE_{albumin}$). **Results:** $BE_{albumin}$ remained 15.2 to 15.3±0.0mEq/L throughout. Only 2 HSD dogs survived S, requiring 12.4 and 5.5 mL/kg of HSD as well as 160 and 78 mL/kg of LRS, respectively.



Conclusions: The BE_{total} versus $BE_{unmeasured}$ relationship remained relatively constant in this short term model, but the Na^+ and Cl^- contributions clearly changed, albeit in canceling directions. A longer model or different fluids might well result in changes in the BE_{total} to $BE_{unmeasured}$ relationship after H such that accounting for the ion changes would matter for trending post-H metabolic insult.

P31

TONGUE StO2 NOT USEFUL FOR PREDICTING DEATH IN HEMORRHAGE AND RESUSCITATION MODEL.
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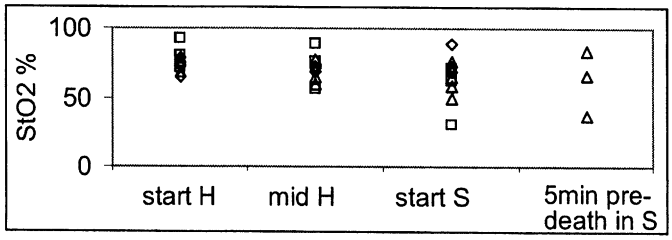
The StO_2 of various muscles has been reported to be potentially useful in hemorrhage and resuscitation monitoring. We decided to investigate the usefulness of monitoring StO_2 of the tongue. **Methods:** 11 anesthetized dogs were hemorrhaged (H, 90min MAP=35-40mmHg), hypotensively stabilized (S, 120min saline vehicle 1mL/kg/hr or enalaprilat [enal] 0.01mg/kg/hr ± HBOC or HSD for MAP=40-45mmHg), resuscitated (R, 60min lactated Ringer's for MAP=75-80mmHg), then only monitored (M, 60min no fluids), and euthanized. **Results:** 1 of 2 HBOC, 1 of 3 HSD, and 2 of 3 enal only dogs died at the start of S (□ died ≤5min into S). Only the remaining HBOC and 2 HSD dogs survived through S (◇survived). The remaining enal only and 3 saline vehicle dogs in S (△ died 32-55min into S).



Correlation coefficients (r) for tongue StO_2 during H versus cardiac output (CO) = 0.28±0.75 (SD), versus carotid flow = 0.46±0.46, versus BD = -0.38±0.56, and versus sublingual PCO_2 = -0.38±0.50. **Conclusions:** Tongue StO_2 failed to distinguish between those animals that would survive and those that would not and failed to trend with CO, carotid flow, systemic metabolic insult (BD), or even with an indicator of metabolic insult in the same tissue (sublingual PCO_2). Tongue StO_2 does not appear to be useful information. (Funding: IA Space Grant, Eagles)

P32

NOVEL FLUIDS FOR RESUSCITATION OF HEMORRHAGIC SHOCK ON THE BATTLEFIELD.
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Objective: A survey was conducted to identify strategies under development for use as resuscitation fluid that potentially might reduce complications and improve chances for survival from hemorrhagic shock. Of particular interest are strategies that can be utilized by combat medics on the battlefield.

Methods: Information on novel resuscitation fluids was supplied by more than 50 researchers in response to a public call for information by the U.S. Army Medical Research and Materiel Command and in response to direct invitation by the Life Sciences Research Office. The survey was conducted in association with an expert panel assembled to review and prioritize proposed treatment regimens and develop a framework for future scientific reviews. Provisions were made to review but not reveal proprietary data by executing confidentiality agreements.

Results: Several parallel approaches are under consideration for resuscitation fluid that include one or more of the following strategies: improve oxygen delivery (e.g., hemoglobin-based oxygen carriers); support metabolism (e.g., ethyl pyruvate); favorably alter rheology and coagulation (e.g., lyophilized platelets); promote cardiovascular stability (e.g., agents targeting the Bezold-Jarisch reflex); and modulate immune response (e.g., human recombinant interleukin-6). More than 20 researchers proposed measuring markers of inflammation and immune function to assess effects of resuscitation fluid treatment.

Conclusion: Development of one or more of these novel strategies to treat hemorrhagic shock may lead to improved effectiveness of resuscitation fluids, earlier after the wounding event, reducing the number of military casualties who are killed-in-action.
Project funded by the U.S. Army Medical Research and Materiel Command and the U.S. Office of Naval Research.

P33

IN VIVO LEUKOCYTE-ENDOTHELIUM INTERACTIONS IN RAT MESENTERIC MICROVESSELS AFTER ISCHEMIA/REPERFUSION AND SEPSIS. NK Nakagawa*, RA Nogueira*, S Sinosaki*, SR Shiwa*, LF Poli de Figueiredo, M Rocha e Silva and P Sannomiya*. Research Division, Heart Institute (INCOR), LIM-11, University of São Paulo Medical School, São Paulo, Brazil.
Leukocyte-endothelium interaction is known to be a remarkable event at the beginning of a systemic inflammatory response syndrome. The aim of this study was to evaluate leukocyte-endothelium interactions in superfused mesenteric post-capillary venules after hemorrhagic shock (HS)/reperfusion and cecal ligation and puncture (CLP). **Methods:** Anesthetized Wistar rats (200-250g) were submitted to: a) 0 hour: HS (MAP~40 mmHg lasting 1 hour) and reperfusion with lactated Ringer's solution (LR, 3x shed blood volume) + reinfusion of 25% of shed blood; b) 24 hours: CLP; c) 48 hours: partial cecal resection and peritoneal lavage (REL); d) 72 hours: intravital microscopy of the mesentery (venules diameter: 15-25μm). **Results:** Data are presented as mean values ± SD; * p<0.01 vs. SHAM. **Conclusions**: The double hit model promoted a severe inflammatory response with a similar magnitude than CLP alone. This inflammatory process was overcome by cecal resection and peritoneal lavage. However, reperfusion was associated with the maintenance of increased cell migration to perivascular tissue up to 72 hours.

Groups	n	Rolling cells/10 min	Adherent cells/100μm venule lenght	Migrated cells/ 5.000μm²
SHAM	4	100±13	3±1	2±1
+CLP	6	215±25*	15±1*	15±1*
+CLP+REL	3	106±13	5±1	5±1
HS+LR	3	175±10*	12±0*	13±1*
+CLP	3	207±16*	16±1*	16±1*
+CLP+REL	4	102±12	4±1	15±1*

Supported by PRONEX, FAPESP and UNICID.

P34

CHANGES IN *EX VIVO* Na^+, K^+-ATPASE (NKA) ACTIVITY IN HEMORRHAGE (H) AND RESUSCITATION (R).
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OBJECTIVE: To examine the effects of H and R on tissue NKA activity and the blood levels of endogenous ouabain-like NKA inhibitor ([I]). METHODS: Anesthetized male Wistar rats were bled to MAP 40 mmHg in a modified Wiggers protocol. In H groups, tissues were harvested at 50% of predicted peak shed blood volume (early shock, ES) and at 25% return of peak shed blood volume (late shock, LS). In R, rats were resuscitated with saline to MAP 80 mmHg prior to harvest. NKA activity was measured *ex vivo* as the liberation of inorganic phosphate (μmol/mg tissue/hr) in microsomal preparations of heart left ventricle (vent), skeletal muscle (mus), and red blood cells (RBC). Plasma [I] (μmol/mg tissue/hr) was measured as inhibition of a standardized kidney NKA assay. RESULTS: Vent: H caused elevation of NKA in ES > LS; R caused a fall in ES+R but further increase in LS+R. Mus: H caused equivalent increases in both ES and LS; R caused a significant decline only in ES+R. RBC: NKA was more elevated in ES then LS, and the decline with R more significant in ES+R. [I]: Equal falls in [I] were seen in both ES and LS, and with R there was further decline in LS+R. CONCLUSIONS: NKA activity increases with H, but relative effects in ES vs. LS vary by tissue. The decline in [I] with H is consistent with the increase in NKA and with previously known elevations of [I] in hypervolemia. R tends to decrease tissue NKA with the notable exception of vent in LS+R. [I] does not, however, increase in response to R after H.

	Control (n = 19)	ES (n = 15)	ES+R (n = 7)	LS (n = 19)	LS+R (n = 9)
vent	1.4±0.2	5.8±0.4*	4.1±0.4*‡	3.7±0.3*†	5.3±0.6*
mus	1.5±0.2	3.4±0.2*	1.8±0.1‡	3.9±0.3*	2.7±0.3
RBC	0.4±0.2	2.5±0.1*	0.9±0.2‡	1.8±0.1*†	1.2±0.4
[I]	0.46±0.03	0.20±0.01*	0.18±0.03*	0.23±0.03*	0.13±0.01*‡

$p < 0.05$: *H vs. Control, †LS vs. ES, ‡ R vs. H

P35

COMPARISON OF PLASMA VOLUME EXPANSION MEASURED BY INDOCYANINE GREEN (ICG) OR HEMATOCRIT IN HEMORRHAGED RESUSCITATED SWINE. P. Nascimento*, S. Vaid*, S. Hoskins*, J. Espana*, M. Kinsky*, D. Deyo* and G. Kramer. Resuscitation Research Laboratory, Dept. of Anesthesiology UTMB, Galveston, TX, 77555 (www.utmb.edu/rrl/).
Objective: To compare 2 methods of plasma volume (PV) measurement, ICG and hematocrit (Hct) dilution, in hemorrhaged pigs resuscitated with albumin over a 24-hr period. Methods: Six splenectomized pigs (31 ± 6 kg) were sedated with fentanyl and diazepam and hemorrhaged over 10 minutes by 19.5 mL/kg (30% of estimated blood volume) and then resuscitated with 19.5 mL/kg of 6% human albumin. PV was measured by ICG dilution (PV_{ICG}) at baseline (BL), immediately after hemorrhage (H10) and at 1 (R1), 6 (R6), 12 (R12) and 24 hrs (R24) after starting the resuscitation. From R1 to R24, PV was also calculated as a function of red blood cell volume (RBCV) and Hct [PV_{Hct} = (RBCV/Hct) x (1-Hct)]. RBCV was determined after hemorrhage from PV_{ICG} and Hct, and from loss of RBCs due to sampling.
Results: PV_{ICG} (mean ± SE) at BL, H10, R1, R6, R12 and R24 were 51.8 ± 3.3, 38.7 ± 5.2, 62.2 ± 3.5, 58.6 ± 2.9, 57.2 ± 4.3 and 56.0 ± 6.7 mL/kg, respectively. Table 1 shows ΔPV_{ICG} compared to ΔPV_{Hct}. Values are mean ± SE and ΔPV % change from value at H10 before resuscitation.

	R1	R6	R12	R24
ΔPV_{ICG}	72% ± 13	64% ± 15	64% ± 16	75% ± 19
ΔPV_{Hct}	75% ± 9	69% ± 9	71% ± 10	67% ± 22

Conclusion: PV (mL/kg) can only be directly measured by tracer dilution, but ΔPV (%) is also precisely measured by Hct dilution when loss of RBC is minimal or measured.

(Supported by Biopure Grant PV-04)

P36

HYPOTENSIVE RESUSCITATION FLUIDS IN A MODEL OF CONTROLLED HEMORRHAGIC SHOCK IN RATS

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OBJECTIVES: Initial care of hemorrhaged military casualties is difficult due to remote locations, delayed evacuation and limited medical supplies. Hypotensive resuscitation should reduce re-bleeding and fluid requirements, while supporting the casualty and preserving organ function until evacuation to medical facilities. The current study compared survival and fluid usage between Hextend (HX, Abbott) and 5% hypertonic saline (HS, Baxter, limited to 9ml/kg) followed by lactated Ringers (LR, Baxter) using male Sprague-Dawley rats. METHODS: Femoral arterial and venous cannulas were implanted under isoflurane, exteriorized at the neck and the animals were allowed to awaken. Under computer control, blood was withdrawn to lower mean arterial pressure (MAP) to 40 mmHg for 30 min, followed hypotensive resuscitation where fluid, either HX or HS, was used to support MAP at 60 mmHg for 240 min. Subsequent full resuscitation restored MAP to 80 mmHg to 10 min and then the animals were held with no additional fluid infusion for 24 hr. Blood pressure, heart rate, shed blood volume (SBV) and resuscitation fluid volume (RFV) were recorded every 5 sec using a data-acquisition program (LabView). Arterial samples, taken during control period, at the end of resuscitation (335 min) and at 24 hr, were analyzed for blood gases, electrolytes, metabolites, osmolarity. Tissue water content was also determined. Negative (no hemorrhage) and positive (hemorrhage, no resuscitation) groups were also included. CONCLUSIONS: The model allowed precise, repeatable hemorrhage and prolonged controlled resuscitation in awake animals with a hemorrhage of 55% of blood volume and an LD70 without resuscitation. Survival improved after hypotensive resuscitation with either HX or HS relative to no resuscitation but HX required significantly less volume and achieved better normalization of acidosis, hyperglycemia and lactacidemia. HS caused a modest and probably unimportant increase in Na and Cl of 5-10 mM. Hypotensive resuscitation with Hextend appears to be a successful resuscitation strategy in the rat model.

P37

BLUNTING OF HEMORRHAGIC SHOCK INDUCED SUSCEPTIBILITY TO INTRAPERITONEAL BACTERIAL CHALLENGE BY IL-6 IS ACCOMPANIED BY REVERSAL OF HS-INDUCED ORGAN APOPTOSIS.

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Resuscitated hemorrhagic shock (HS) is a global ischemia-reperfusion (I-R) injury resulting in systemic inflammation and multiorgan dysfunction syndrome (MODS). Recently, increased apoptosis in critical organ systems in sepsis has been proposed to play a pivotal role in MODS and to increase susceptibility to subsequent infections. Exogenous IL-6 has been shown to have anti-apoptotic properties in animal models of liver I-R injury and to decrease post-resuscitation inflammation in rodent and porcine models of controlled HS. To assess if the beneficial effects of IL-6 are accompanied by reduced susceptibility to infection, mice (n=12) were subjected to a controlled HS protocol (30 mm Hg for 3 hr; resuscitation with 2 x shed blood volume of Ringers solution) or sham protocol (n=12) followed 24 hours later by intraperitoneal challenge with *Staphylococcus aureus*. Liver bacterial counts 24 hr after challenge in HS mice (77,000 ± 36,000 CFU/g) were increased 6 fold compared to sham controls (12,000 ± 9,000; $p<0.01$). In HS mice that received IL-6 (3 mcg/kg) at the start of resuscitation (n=12), liver bacterial counts were reduced 57% compared to placebo-treated HS to levels statistically indistinguishable from IL-6-treated sham mice (n=12). To assess the contribution of apoptosis to the HS-induced increase in liver bacterial burden, mice were subjected to the HS protocol or sham protocol; the HS mice were randomized to receive IL-6 (3mcg/kg) or placebo at initial resuscitation. All mice were sacrificed 24 hours after the start of resuscitation ($n\geq4$ for each group). The liver of each mouse was harvested and fixed; liver sections stained and analyzed using the terminal deoxyuridine end labeling (TUNEL) assay. The number of TUNEL-positive cells in twenty-five 400x fields was counted in each liver by an experienced histologist blinded to the treatment each mouse received. HS increased the number of TUNEL-positive liver cells 8 fold from 0.43 ± 0.1 cells per 400x field in the sham mice to 3.31 ± 1.41 cells ($p<0.05$); IL-6 resuscitation completely reversed the HS-induced increase in TUNEL-positive cells to 0.39 ± 0.1 ($p<0.05$). Thus, IL-6 at the start of resuscitation protects the liver from HS-induced apoptosis; reduced liver apoptosis may explain the ability of IL-6 to blunted HS-induced increased susceptibility bacterial challenge.

P38

GREEN TEA POLYPHENOL EXTRACT ATTENUATES ISCHAEMIA/REPERFUSION INJURY OF THE GUT

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Various studies have clearly demonstrated that green tea catechins possess potent antioxidative properties, and the preventive effects against various oxidative diseases have been reported. The aim of this study was to investigate the effect of green tea extract on the tissue injury caused by ischemia/reperfusion (I/R) of the gut. I/R injury of the intestine was caused by clamping both the superior mesenteric artery and the celiac trunk for 45 min followed by release of the clamp allowing reperfusion for 1 h or 4 h. This procedure results in splanchnic artery occlusion (SAO)-shock. Rats subjected to SAO developed a significant fall in mean arterial blood pressure, and only 10% of the animals survived for the entire 4 h reperfusion period. Surviving animals were sacrificed for histological examination and biochemical studies. Rats subjected to SAO displayed a significant increase in tissue myeloperoxidase activity and malondialdehyde levels, significant increases in plasma tumour necrosis factor (TNF)-α levels and marked injury to the distal ileum. Increased

immunoreactivity to nitrotyrosine was observed in the ileum of rats subjected to SAO. Staining of sections of the ileum obtained from SAO rats with an ICAM-1 antibody and with anti-P-selectin antibody resulted in diffuse staining. Administration of green tea extract (20 and 10 mg kg^{-1} i.v.) at 15 min prior to the onset of gut reperfusion significantly reduced in a dose dependent manner the (i) fall in mean arterial blood pressure, (ii) mortality rate, (iii) infiltration of the reperfused intestine with polymorphonuclear neutrophils (iv) lipid peroxidation, (v) production of TNF-α and (vi) histological evidence of gut injury. Administration of green tea extract also markedly reduced the nitrotyrosine formation and the up-regulation of ICAM-1 and P-selectin during reperfusion. These results demonstrate that the green tea extract significantly reduce I/R injury of the intestine.

P39

ENHANCED PROTECTION PROVIDED BY ISCHEMIC PRECONDITIONING AND ALLOPURINOL AGAINST HEPATIC ISCHEMIA/REPERFUSION: ROLE OF ADENOSINE AND NITRIC OXIDE. W.Y. Lee* and S.M. Lee. Sungkyunkwan Univ., Suwon 440-746, South Korea.

This study was designed to examine the combined effect of ischemic preconditioning (IPC) and allopurinol (allo), a xanthine oxidase inhibitor, on hepatic I/R injury, and determine the role of adenosine and nitric oxide (NO), generated by IPC, or by the combination of IPC and allo (allo+IPC). Rats were subjected to 1.5 h of ischemia followed by 5 h of reperfusion. IPC was achieved by 10 min of ischemia and 10 min of reperfusion prior to sustained ischemia. Allo (50 mg/kg, i.p.) was administered 18 h and 1 h before sustained ischemia. In addition, to study the role of adenosine signaling during IPC, rats were pretreated with adenosine deaminase (ADA), adenosine A_2 receptor antagonist 3,7-dimethyl-1-[2-propargyl]xanthine (DMPX) and NO synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME). The hepatic I/R increased serum aminotransferase activities, and decreased hepatic content of reduced glutathione. These changes were inhibited by IPC, and further by allo+IPC. The pretreatment of ADA, DMPX or L-NAME attenuated the beneficial effect of allo+IPC. The activity of mitochondrial glutamate dehydrogenase decreased in I/R group; a decrease that was prevented by both IPC and allo+IPC. This protection elicited by allo+IPC were reduced by pretreatments with ADA and DMPX, but not with L-NAME. During IPC, the hepatic nitrite+nitrate level increased in both IPC and allo+IPC groups. This increase in allo+IPC group was restored by pretreatments with ADA, DMPX and L-NAME. At the end of ischemia during the IPC period, the hepatic content of xanthine increased significantly; an increase that was inhibited by allo pretreatment. Hepatic adenosine/xanthine concentration ratio was increased by allo pretreatment indicating that allo increase the availability of adenosine for producing NO. Our findings suggest that allo enhances the protective effect of IPC against postischemic hepatic injury and the protection is provided by adenosine, adenosine A_2 receptor and NO production. *(supported by KRF-2003-015-E00222)*

P40

PARG ACTIVITY MEDIATES INTESTINAL INJURY INDUCED BY SPLANCHNIC ARTERY OCCLUSION AND REPERFUSION

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Poly (ADP-ribosyl)ation, an early posttranslational modification in response to DNA damage, is catalyzed by poly (ADP-ribose) polymerase (PARP-1) and catabolized by poly(ADP-ribose) glycohydrolase (PARG). The aim of this study was to investigate the role of PARG on the modulation of the inflammatory response caused by splanchnic ischemia and reperfusion. SAO shock in rats as well as in wild-type (WT) mice was associated with a significant neutrophils infiltration in the ileum and a production of tumor necrosis factor-alpha (TNF-α). Reperfused ileum tissue sections from SAO-shocked WT mice as well as rats showed positive staining for P-selectin and ICAM-1 which was mainly localized in the vascular endothelial cells. The genetic disruption of the PARG gene in mice or the pharmacological inhibition of PARG by PARG inhibitors significantly improved histological status of the reperfused tissues that are associated with reduced the expression of P-selectin and ICAM-1, the neutrophils infiltration into the reperfused intestine and the TNF-α production. These results suggest that PARG activity modulates the inflammatory response in ischemia/reperfusion and participates in end (target) organ damage under these conditions.

P41

THE SELECTIVE PPARγ ANTAGONIST GW9662 ABOLISHED THE PROTECTION AFFORDED BY LPS-PRETREATMENT IN A RAT MODEL OF RENAL ISCHEMIA/REPERFUSION INJURY

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Background. We have recently reported that (i) pre-treatment of rats with endotoxin (lipopolysaccharide, LPS) and (ii) selective agonists of the nuclear receptor proliferator-activated receptor-γ (PPARγ) protect the kidney against ischemia/reperfusion (I/R) injury. Here we investigate the hypothesis that the renoprotective effects of LPS-pretreatment are due to an enhanced formation of endogenous ligands of PPARγ.
Methods. Rats were pre-treated with LPS (1 mg/kg, i.p., 24 h

prior to ischemia) in the absence (control) or presence of the selective PPARγ antagonist GW9662 (1 mg/kg, i.p., 24 h and 12 h prior to ischemia, ie just prior to LPS and 12 h after LPS). Twenty-four hours after injection of LPS, rats were subjected to 60 min bilateral renal ischemia, followed by 6 h reperfusion. Serum creatinine was used as an indicator of renal dysfunction. Serum aspartate aminotransferase and γ-glutamyl-transferase were used as indicators of renal injury. Creatinine clearance was used as an indicator of glomerular function and fractional excretion of sodium as an indicator of tubular function.
Results. I/R caused marked glomerular and tubular dysfunction as well as renal injury. Pre-treatment with LPS significantly attenuated all markers of renal injury and dysfunction caused by I/R. Most notably, the PPARγ antagonist GW9662 abolished the protective effects of LPS.
Conclusions. We document here for the first time that endogenous ligands of PPARγ may importantly contribute to the protection against renal I/R injury afforded by LPS pre-treatment in the rat.

P42

THE CARDIOPROTECTIVE EFFECTS OF ENDOTOXIN BUT NOT ISCHEMIA ARE ABOLISHED BY THE PPAR-γ ANTAGONIST GW9662

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Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors. The cyclopentone prostaglandin 15-deoxy^{12,14} PGJ_2 (15*d*-PGJ_2), which is a metabolite of the prostaglandin D_2 has been suggested to function as an endogenous ligand, with a high affinity for PPAR-γ. We investigated whether the cardioprotective effects of preconditioning with ischemia or endotoxin are due to endogenous ligands of PPAR-γ. Male Wistar rats were anesthesised with sodium thiopentone. The trachea was cannulated and the animals were ventilated with a Harvard ventilator (inspiratory oxygen concentration: 30%; 70 strokes/min, tidal volume: 8-10 ml/kg). The right jugular vein and the right carotid artery were also cannulated. A para-sternal thoracotomy was performed and the left anterior descending coronary artery was isolated. Two cycles of ischemic preconditioning followed by regional myocardial ischemia-reperfusion resulted in a 67% reduction in infarct size (P<0.05). The PPAR-γ antagonist GW9662 (1 mg/kg) did not affect the cardioprotective effects afforded by ischemic preconditioning. When compared to vehicle-treated animals, 16 h pre-treatment with LPS (1 mg/kg i.p.) resulted in a 34% reduction in myocardial infarct size (P<0.05). GW9662 abolished the cardioprotective effects afforded by LPS. This finding demonstrates that the delayed cardioprotective effects of endotoxin in the rat are largely due to endogenous PPAR-γ agonists.

P43

HYPERTONIC SALINE ABROGATES MESENTERIC ISCHEMIA/REPERFUSION INDUCED GASTRIC DYSFUNCTION. E. Gonzalez*, R. Kozar, J. Suliburk*, D. Mercer, F. Moore. University of Texas Health Science Center at Houston, Houston, TX 77030.

Objective: Mesenteric ischemia/reperfusion (I/R) induced by superior mesenteric artery occlusion (SMAO) causes gastric dysfunction [i.e. increased gastric residual volume (GRV) and luminal alkalinization]. We have shown that 4 ml/kg of 7.5% hypertonic saline (HS) resuscitation (resus) after SMAO prevents small bowel injury in part by local induction of anti-inflammatory heme-oxygenase-1 (HO-1). We hypothesized that HS would have similar beneficial effects in the stomach.
Methods: Rats were assigned to Sham/no resus, Sham/HS, SMAO/no resus or SMAO/HS. All animals underwent internal jugular line placement and midline celiotomy. SMAO clamps were placed in the assigned groups for 60 minutes and resuscitation was given 5 minutes prior to clamp removal. Animals were sacrificed at 6 hours of reperfusion. GRV and luminal pH were measured and gastric mucosa was harvested for analysis of HO-1 protein expression by Western immunoblot. Data are reported as mean ± SEM (n ? 5/group; ANOVA). Means with different letters are significantly different ($p < 0.05$).
Results:

Model	GRV (ml)	Luminal pH	HO-1 (AU)
Sham/No Res	0.30 ± 0.05 a	1.66 ± 0.16 a	0.32 ± 0.02 a
Sham/HS	0.36 ± 0.04 a	1.50 ± 0.18 a	0.41 ± 0.03 a
SMAO/No Res	1.70 ± 0.27 c	6.58 ± 0.20 b	0.35 ± 0.01 a
SMAO/HS	0.60 ± 0.08 d	3.10 ± 0.09 c	0.55 ± 0.03 b

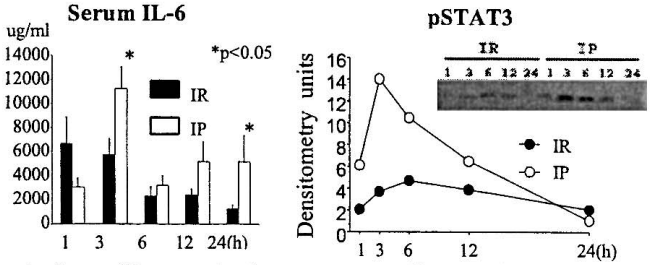
SMAO/no resus increased GRV and luminal pH, but had no effect on HO-1. SMAO/HS decreased GRV and luminal pH. This was associated with an increase in HO-1 protein expression.
Conclusion: HS resuscitation abrogates gastric dysfunction induced by SMAO and increased local HO-1 protein expression. Similar to the small bowel, this may represent a novel mechanism of gastric protection that warrants further investigation. Supported by NIGMS P50 GM 38529 and T32 GM 08792.

P44

INCREASED IL-6 / STAT 3 ACTIVATION IN HEPATIC ISCHEMIC PRECONDITIONING T. Matsumoto*, P. Efron*, S. Tschoeke*, T.Uchida*, R. Ungaro*, C. Tannahill*, K. O'Malley*, S. Fujita*, L. Moldawer, A. Hemming*, D. Foley*, Dept. of Surgery, Univ. of FL Coll. of Med., Gainesville, FL 32610

Introduction: Ischemic preconditioning (IP) has been shown to protect the liver from ischemia/reperfusion injury (IR). The intracellular mechanisms by which IP mediates protection are not well defined. We hypothesized that IL-6 and STAT3 modulate the protective effects of IP during total hepatic IR injury. **Methods:** Twenty-one days after splenic transposition, C57BL/6 female mice (6-8 week old) underwent 75 min of total hepatic ischemia followed by 1, 3, 6, 12 or 24 h of reperfusion

with or without prior IP (10 min of ischemia followed by 15 min of reperfusion). Mice were observed for survival at 24 h. Serum alanine aminotransferase (ALT), serum IL-6, and hepatic phosphorylated STAT3 (pSTAT3) protein levels were evaluated at above time points. Statistical analyses were performed by one-way ANOVA, Kaplan-Meier survival analysis and the Logrank test. **Results:** Survival at 24 h was greater in the IP vs. non-IP group (71% vs. 36%, $p<0.05$). ALT was decreased in IP compared to non-IP ($p<0.05$). Both serum IL-6 and hepatic pSTAT3 were increased in IP compared to non-IP mice.



Conclusions: IP not only decreases mortality and hepatocellular injury but also increases levels of serum IL-6 and pSTAT3 in mice undergoing hepatic I/R. Modulation of the IL-6/STAT3 signaling pathway may be one mechanism to reduce hepatic I/R injury. Further elucidation of this pathway may provide a pharmacological alternative to IP.

P45

p38 MAPK INHIBITION DECREASES ISCHEMIA INDUCED PROINFLAMMATORY MYOCARDIAL INJURY J. Pitcher*, M. Wang, B. Tsai*, A.Kher*, D.R. Meldrum. Indiana University, IN 46228

Objective: Understanding the myocardial inflammatory response to ischemia is an important part of achieving the elusive clinical goal of long enduring, and perfect, myocardial protection. p38 mitogen-activated protein kinase (MAPK) has been implicated in oxidant stress-induced myocardial TNF-α production; however, it is unknown whether p38 MAPK mediates the following important events in both myocardial apoptosis and functional depression: $MAPKAPK_2$, caspase 1 and caspase 11 activation, and TNF-α, IL-1β and IL-6 production.
Methods: Isolated rat hearts were perfused and subjected to an I/R insult, with and without p38 MAPK inhibition. Myocardial functional parameters were continuously recorded throughout the experiments. Myocardial tissue was then assessed for products of p38 MAPK activation, expression of TNF-α, IL-1β and IL-6, and activation of caspase-1 and caspase-11.
Results: Post-ischemic recovery of left ventricular developed pressure (LVDP), +dP/dt and –dP/dt was significantly increased by p38 MAPK inhibition. I/R resulted in marked elevation of left ventricular end-diastolic pressure (LVEDP) that was significantly reduced by p38 MAPK inhibition after 20 minutes of reperfusion. p38 MAPK inhibition decreased myocardial TNF-α, IL-1β and IL-6 production, and reduced active caspase-1 and caspase-11.
Conclusions: The p38 MAPK pathway indeed mediates the following important events in myocardial apoptosis and functional depression: $MAPKAPK_2$, caspase 1 and caspase 11 activation, and TNF, IL-1 and IL-6 production after myocardial ischemia. Single site (p38 MAPK) inhibition of these events may have important therapeutic implications in myocardial protection.

P46

CASPASE AND p38 MAPK ACTIVATION AFTER RENAL ISCHEMIA
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Objective: During shock, the low flow state results in acute renal failure (ARF). Renal apoptosis is the primary form of cell death, following brief periods of ischemia. Caspases and p38 MAPK have been shown to mediate apoptosis. We hypothesized that renal ischemia reperfusion (I/R) activates p38 MAPK and caspase-8, and leads to increased apoptosis.
Methods: Male rats underwent left kidney ischemia (n=4-6 /group) via temporary renal artery occlusion or a sham procedure (n=4-5 /group) followed by 0, 1, or 24 hours of reperfusion. Following I/R, the left kidney was assessed for activated p38 MAPK and caspase-8 (Western blot), as well as histone-associated DNA fragments (ELISA) as a measure of apoptosis. Data was analyzed with unpaired student's t-test, $p<0.05$ considered statistically significant.
Results: Ischemia alone increased p38 MAPK (81.1±6.7% vs. 17.5±3.1% sham), which was sustained after one hour of reperfusion (74.5±10.7% vs. 20.6±12.8% sham). Similarly, caspase-8 was increased following ischemia (49.2±4.1% vs. 18.2±4.5% sham) and one-hour reperfusion (58.0±3.1% vs. 37.8±6.3% sham). After 24 hours of reperfusion, apoptosis (DNA oligo- and mononucleosomes) was increased 24-fold in the ischemia group compared to the sham group.
Conclusions: These results suggest that I/R-induced renal apoptosis may be mediated by p38 MAPK and caspase-8 activation. Interventions targeted at these signaling pathways may reduce the incidence of ARF in conditions of low blood flow.

P47

ADRENOMEDULLIN (AM) AND ITS BINDING PROTEIN (AMBP-1) PREVENT ACUTE LUNG INJURY AFTER GUT ISCHEMIA/REPERFUSION. A. Dwivedi*, R. Wu, P. Das*, E. Nguyen*, H. Wang, K. Krisnasastry*, H.H. Simms, P. Wang. North Shore-Long Island Jewish Medical Center, Manhasset, NY 11030

An ischemic bowel remains a critical problem resulting in up to 80% mortality. Acute lung injury induced by ischemia and reperfusion (I/R) injury may be responsible for such high mortality. Our previous studies have shown that administration of a novel vasoactive peptide, AM, and its binding protein, AMBP-1, reduces

the systemic inflammatory response. However, it remains unknown whether AM/AMBP-1 has any protective effects on I/R-induced acute lung injury. To study this, intestinal I/R was induced by placing a microvascular clip across the superior mesenteric artery (SMA) for 90 min in adult male rats. Upon release of the SMA clamp, the animals were treated by either AM (12 μg/kg BW) in combination with AMBP-1 (40 μg/kg BW) or vehicle (1 ml normal saline) over a period of 30 min via a femoral vein catheter. The animals were euthanized 4 h later, and lung samples were assessed for granulocyte myeloperoxidase activity (MPO), water content, TNF-α, IL-6, IL-10 levels and morphological changes. Gene expression of the anti-inflammatory nuclear receptor, peroxisome proliferator-activated receptor-γ (PPAR-γ), was also measured. Results are as follows (mean±SEM; n =6-8/group):

	Sham	I/R-Vehicle	I/R-AM/AMBP-1
MPO (U/g protein)	2.2±0.1	6.4±0.3*	3.3±0.2*#
Water content (%)	75.0±1.1	81.7±0.7*	75.8±1.4#
TNF-α (ng/g protein)	1.8±0.1	3.1±0.3*	1.5± 0.4#
IL-6 (ng/g protein)	52.3±3.9	80.0±9.6*	53.6±7.7#
IL-10 (ng/g protein)	11.3±1.2	19.3±2.4*	12.5±2.7#
PPAR-γ/G3PDH (mRNA)	0.30±0.05	0.31±0.04	0.44±0.02*#

(One-way ANOVA: *P<.05 vs. Sham; # P<.05 vs. Vehicle)

Gene expression of the cytokines correlates with their protein levels (data not shown). Histological examination shows that AM/AMBP-1 restores the lung morphology to a level similar to that of the sham group. Our results demonstrate that administration of AM/AMBP-1 after intestinal ischemia prevents lung injury, downregulates inflammatory cytokines, and upregulates PPAR-γ expression. Thus, AM/AMBP-1 may be a novel treatment to attenuate acute lung injury after an episode of ischemic bowel. The beneficial effect of AM/AMBP-1 after I/R appears to be mediated by upregulation of PPAR-γ (NIH R01 HL076179).

P48

EFFECTS OF MESENTERIC VENOUS CONGESTION ON INTESTINAL BLOOD FLOW, OXYGEN METABOLISM AND REGIONAL pCO_2 GRADIENTS. RJ Cruz Jr, C Correia*, CMF Ribeiro*, T Harada*, LFP de Figueiredo, M Rocha e Silva. Heart Institute (InCor) / University of Sao Paulo, Brazil.

Mesenteric venous thrombosis is a life-threatening emergency with a mortality rate around 25%. In this study, we evaluated the systemic and regional pCO_2 gradients changes induced by mesenteric venous congestion. In addition, we sought to obtain evidence that systemic markers of splanchnic hypoperfusion can detect the initial changes in intestinal blood flow after superior mesenteric vein (SMV) occlusion. Methods: Fourteen dogs were subjected to 45 min of superior mesenteric artery (SMA-O, n=7) or vein occlusion (SMA-V, n=7), and observed for 120 min thereafter. Systemic hemodynamic was evaluated through a Swan-Ganz and arterial catheters, while gastrointestinal tract perfusion by superior mesenteric vein and serosal blood flows (SMVBF and SBF, ultrasonic flowprobe). Intestinal O_2-derived variables, mesenteric-arterial and tonometric-arterial pCO_2 gradients ($D_{mv\text{-}a}pCO_2$ and $D_{t\text{-}a}pCO_2$) were calculated. Results: Displayed in table (values are mean±SEM; * $p<0.05$ *vs.* basal; ¥ $p<0.05$ *vs.* SMA-O). The histopathologic injury scores were 2.7±0.5 and 4.8±0.2 for SMA-O and SMV-O groups, respectively.

	Groups	BASAL	Ischemia	R30	R120
MAP	SMA-O	129±8.6	137±7.7	126±4.9	130±11.3
(mmHg)	SMV-O	122±9.6	91±7.4*¥	92±8.5*¥	99±7.5*¥
CO	SMA-O	2.7±0.2	2.5±0.2	2.3±0.1	2.1±0.1*
(L/min)	SMV-O	3.3±0.3	2±0.2*	2±0.3*	1.5±0.3*¥
SMVBF	SMA-O	412±60	0*	245±39*	214±32*
(ml/min)	SMV-O	420±51	0*	77±9.5*¥	92±9.8*¥
$D_{t\text{-}a}pCO_2$	SMA-O	8.2±4.8	49±4.6*	35±6.1*	24±8.6*
(mmHg)	SMV-O	5.8±2.6	64±3.9 *¥	66±2.7*¥	67±1.3¥*
$D_{vm\text{-}a}pCO_2$	SMA-O	1.7±0.5	5.7±2*	5.2±1.5*	4.5±1*
(mmHg)	SMV-O	2.3±0.4	20.1±5*¥	11±2.1*¥	10±2.3*¥

Conclusion: Temporary SMV occlusion is associated with significant hemodynamic and metabolic disturbances. The $D_{t\text{-}a}pCO_2$ changes could be detected by systemic markers of splanchnic hypoperfusion in SMV-O group, but not in SMA-O.

P49

INCREASED ENOS-CAVEOLIN-1 COUPLING DECREASES ENOS ACTIVITY FOLLOWING TRAUMA AND SEPSIS S.A. Keller*, A. Karaa*, J.H. Ashburn*, M.G. Clemens, and T.T. Huynh Carolinas Medical Center, Charlotte, NC 28203 and the University of N.C. at Charlotte, Charlotte, NC 28223

Introduction: Recently, we found that femur fracture (FFx) and cecal ligation and puncture (CLP) altered the hepatic microcirculation in response to the vasoregulator endothelin-1 (ET-1). Here, we hypothesize that the hepatic hyperconstrictive response to ET-1 after sequential stress is due to an increase in constrictive forces, unmatched by vasodilatory forces; primarily via eNOS derived nitric oxide (NO). Methods: Male Sprague Dawley rats underwent sham, FFx, CLP, and FFx+CLP surgery. Liver tissue was harvested 24 hours after final surgery for RT-PCR, Western blot, immunoprecipitation, and nitric oxide synthase activity assay to assess hepatic vasoregulators. Results: ET-1 mRNA is upregulated after sepsis and sequential stress. Despite increased eNOS mRNA and protein expression after CLP and FFx+CLP, eNOS-caveolin-1 complex increased, and NOS activity and NO levels decreased, suggesting unmatched vasoconstriction.

Group	Sham	FFx	CLP	FFx+CLP
ET-1 mRNA (AU)	9±2	13±1	18±1*†	16±1*†
eNOS mRNA (AU)	6±0.6	8±0.4*	9±0.4*	9±0.2*†
eNOS protein (AU)	15±3	7±1	34±4*†	54±5*†‡
eNOS-Caveolin-1 (AU)	24±4	26±2	35±1*†	44±3*†‡
eNOS Activity (DPM)	35,703 ±6945	5,439 ±1356*	13,870 ±3705*	15,658 ±6092*

AU: Arbitrary Units DPM: Disintegration per minute
* P<0.05 vs Sham, †P<0.05 vs FFx, ‡P<0.05 vs CLP

Conclusions: Our data showed that despite increased levels of eNOS, simultaneous elevation in caveolin-1 leads to decreased eNOS activity and NO production. This may result in unmatched increases of ET-1 induced vasoconstriction lending in the observed hyperconstrictive response after trauma and sepsis.

P50

EFFECT OF ESTROGEN ON HEPATIC MICROCIRCULATION FOLLOWING ISCHEMIA/REPERFUSION (I/R).

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The effect of gender on hepatic I/R remains conflictive as well as the influence of estrogen. The present study was focused (i) on the gender related hepatic I/R injury, (ii) how this dimorphism is related to microcirculatory failure, and (iii) how the postischemic injury can be influenced by estrogen. Therefore female (n=8) and male (n=8) Sprague-Dawley rats were subjected to 90' ischemia following 60' reperfusion of the left liver lobe. Other 6 male rats were pre-treated by estradiol. Sinusoidal perfusion, the postischemic inflammatory response and Kupffer cell activity were examined by intravital microscopy, followed by analysis of serum AST, ALT, LDH levels. After I/R male rats revealed (i) an impairment of Kupffer cell activity ($p<0.05$) in contrast to female rats (male: 10.9±1.6% vs. female: 3.7±0.5%). In addition males showed (ii) a slight enhancement of sinusoidal perfusion failure (28.0±3.3% vs. 23.7±3.6) and of leukocyte-endothelium interaction within post-sinusoidal venules (847±126 cells/mm^2 vs. 634±142 cells/mm^2), as well as (iii) a massive parenchymal cell damage (AST and LDH: 3.300±661 U/L and 17.648±2229 U/L vs. 1.056±220 U/L and 7.274±1.773 U/L, $p<0.05$) compared with females. Pre-treatment with estrogen caused a normalisation of Kupffer cell function (2.1±0.4%, $p<0.05$) as well as an amelioration of sinusoidal perfusion (21.6±3.2%) and venular leukocyte-endothelium interaction (377±74 cells/mm^2, $p<0.05$). In contrast, no protective effect on postischemic parenchymal cell damage by pre-treatment with estrogen was found (4.023±1.015 U/L and 14.604±3.047 U/L). Ischemia and reperfusion of the liver generates a different gender-specific occurrence of (i) microvascular dysfunction, (ii) leukocyte inflammatory response, and (iii) hepatocellular damage, which appears to be estrogen related.

P51

MECHANISMS OF DIMETHYL SULFOXIDE AUGMENTATION OF IL-1β PRODUCTION

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Expression of the inflammatory cytokine IL-1β occurs in various inflammatory diseases and IL-1β production is regulated at multiple levels. There are conflicting reports about the effects of antioxidants on IL-1β production. We investigated the regulatory role of the anti-oxidant dimethyl sulfoxide (DMSO) on LPS-stimulated IL-1β gene expression in human peripheral blood mononuclear cells (PBMC) and *in vivo*. This study demonstrated that 1% DMSO increased LPS-stimulated (50ng/ml) IL-1β secretion in both a dose dependent and time depended manner without altering TNF or IL-6. DMSO also significantly decreases IL-8, indicating that the anti-oxidant exerts a specific effect on IL-1β gene expression. DMSO elevated IL-1β secretion by PBMC in response to exogenous superoxide anions. Despite the increase in IL-1β, there was no augmentation of nuclear factor kappa B with the addition of DMSO. The steady state mRNA coding for IL-1β following LPS stimulation was also increased. Cycloheximide studies demonstrated that the DMSO augmentation of IL-1β mRNA did not require *de novo* protein synthesis and studies with actinomycin D showed that DMSO did not increase the half life of IL-1β mRNA, suggesting that DMSO not change the stability of IL-1β mRNA. Experiments using a reporter vector containing the 5'-flanking region of the human IL-1β gene revealed that DMSO augmented LPS-induced IL-1β reporter activity. *In vivo,* treatment of mice with DMSO significantly increased plasma levels of IL-1β after an endotoxin challenge. These data indicate that DMSO directly increases LPS-stimulated IL-1β protein production through the mechanism of augmenting promoter activity and increasing mRNA levels.

P52

VASCULAR VOLUME EXPANSION AFTER HEMORRHAGE IS REDUCED BY BETA-BLOCKERS

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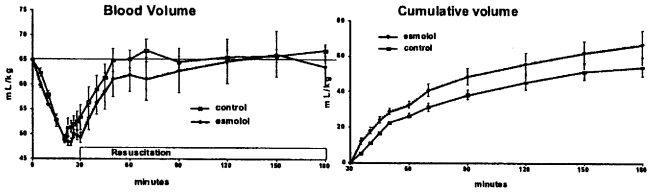
Blood loss and fluid shifts can compromise organ perfusion during major surgery. This is especially true in patients with coronary artery disease (CAD). Recent data suggests that beta-blockers decrease myocardial injury and death in CAD patients undergoing major surgery.[1,2] We, however, found that beta-blockers impair vascular volume expansion after a fluid bolus in normovolemic sheep.[3] Objective: We addressed how beta-blockers affect fluid balance and vascular volume during a hemorrhage under anesthesia. Methods: Female sheep, 30-45 kg, prepared with vascular catheters were anesthetized with isoflurane and ventilated. An infusion of esmolol (beta-blocker) (n=6) or control (no drug) (n=6) began 30 min before a 20 mL/kg hemorrhage. Hemodynamics, cumulative fluid and urine and hemoglobin samples were measured for 180 min study. Resuscitation with lactated Ringer's began 30 min after hemorrhage. Vascular volume expansion was calculated from serial hemoglobin samples. Results: Heart rate was significantly lower in beta-blocker versus control. Other hemodynamic indices were similar. In control, the vascular volume (mL/kg) was restored to baseline within 30 min of resuscitation, whereas it was delayed by 90 min in beta-blocker group. The cumulative volume (mL/kg) was 20% greater in beta-blocker group.



Conclusion: Beta-blockers modestly increased fluid requirements and blunt the response to vascular volume restoration during anesthesia and hemorrhage. References: 1.N Engl J Med. 1996 Dec 5;335(23):1713-20. 2.Eur Heart J. 2001 Aug;22(15):1353-8. 3.Anesth Analg 2004:98.SCA1-134.

P53

SYSTEMIC EFFECTS OF CEES (HALF SULFUR MUSTARD GAS) AFTER INTRATRACHEAL INSTILLATION. L.M. Hoesel, A.D. Niederbichler*, S.D. McClintock, J.V. Sarma, P.A.Ward Departments of Pathology and Surgery*, University of Michigan Medical School, Ann Arbor, MI 48109

Objective: Airway instillation into rats of 2-chlorethyl ethyl sulfide (CEES, half sulfur mustard compound), results in acute lung injury similar to what has been found in conflicts. Little is known about systemic effects of CEES after intratracheal instillation. **Methods**: Serum AST/ALT (parameters of liver function) and BUN/CREA (parameters of renal function) were measured in rats that underwent airway instillation of either CEES (2µl in 15% ethanol/PBS) or 15% ethanol/PBS as controls. **Results:** Both groups showed an increase in ALT and AST with a peak 6 hours after instillation and a return to normal values within 48 hours. However, CEES-treated rats displayed higher values of ALT and AST (1.6fold increase) at 4 and 6 hours after instillation, suggesting liver damage caused by CEES. In contrast, kidney parameters BUN and CREA did not change during the observation period of 24 hours in either group. **Conclusion:** These results show for the first time that intratracheal instillation of CEES may access the systemic blood circulation and exert harmful effects on liver function, but not on renal function. The administration of reducing agents, such as N-acetylcysteine (NAC) and glutathione (GSH), which have been shown to attenuate lung injury, may prevent evidence of liver damage. Possible protective impacts of intratracheal or intravenous administration of NAC/GSH on distal organ function and implications for future investigations will be discussed.

P54

INHIBITION OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) BY CYC-OXI-11 INCREASES SURVIVAL IN SEPSIS

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Administration of MIF antagonist, e.g. ISO-1 or anti-MIF antibody, during sepsis has been shown to be beneficial. Recently, we have developed several classes of MIF inhibitors and found that amino acid Schiff bases, namely L-Tryp-SB, are among the most potent inhibitors of MIF tautomerase activity. However, this class was only examined *in vitro* due to the stability of these compounds. To design a more potent, yet stable, inhibitor of MIF activity, we focused on improving the stability of the Schiff base by substituting the imine bond linkages with the oxime bond. Cyc-Oxi-11, one of 29 molecules synthesized around this new scaffold, has emerged as the most potent inhibitor of MIF activity ever described. Cyc-Oxi-11 is two-fold more potent than the labile L-Tryp-SB. It is also 30-fold more potent inhibitor of MIF tautomerase and proinflammatory activity *in vitro* compared to ISO-1. Cyc-Oxi-11 significantly inhibits binding of fluorescently tagged MIF to THP-1 cells, can bind to intracellular MIF and inhibits MIF induced NF-kB activation. In addition, Cyc-Oxi-11 abolished the ability of MIF to override glucocorticoid inhibition of TNF production from LPS-stimulated macrophages. In an experimental sepsis, administration of Cyc-Oxi-11 (3.5 mg/Kg) improve significantly the survival rate to 70% compared to 10% in control group. Taken together, the potent inhibitory effects of Cyc-Oxi-11 on MIF tautomerase activity correlate very well with blocking MIF cytokine activity in vivo and in vitro. These data identify Cyc-Oxi-11 as the most potent inhibitor of MIF proinflammatory activity in vivo and support that the inhibition of MIF's active site as a novel therapeutic intervention in human sepsis.

P55

PHARMACOKINETICS OF INTRAOSSEOUS DRUG DELIVERY DURING CPR

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Objective: Intravenous (IV) access can delay drug therapy in cases of vascular collapse in shock and cardiac arrest. The intraosseous (IO) route provides vascular access via the non-collapsible vessels in bone marrow. New adult IO devices are designed specifically for the adult tibia (T) and sternum (S). However, the pharmacokinetics of IO drug delivery during cardiopulmonary resuscitation (CPR) remain poorly defined. In the present study, we compared IO drug delivery in T versus S during CPR. Methods: Eight chloralose-anesthetized swine (30-45 kg) were subjected to cardiac arrest by KCl injection. CPR was initiated 8-min post-cardiac arrest via a Thumper (Michigan Instruments Inc.) at 100 compressions per min without ventilation. IO devices, Jamshidi (Baxter) or EZ-IO (VidaCare), were placed in the S and T. Evans blue, 5 mg/kg, and indocyanine green, 2.5 mg/kg, tracers were each co-administered with epinephrine, 2 mg/kg, as a bolus into the S and T, respectively. Arterial sampling was performed at 10-second intervals and analyzed by spectrophotometry to determine the arterial dose and the drug delivery time.
Results: Peak (maximal) tracer concentrations were at 48 ± 10 sec for S and 121 ± 30 sec for T. Half maximal concentrations were 20 ± 3 and 51 ± 7 sec for S and T, respectively. Experiments with bolus infusion via jugular vein resulted in peak arterial concentrations at ~30-

seconds with half max times of 12-seconds. The total dose delivered to arterial blood was similar for both S and T injections.
Conclusions: During CPR IO infusions via both S and T effectively delivered drugs, although sternal delivery was faster. Data suggest that IO sternum access is comparable to IV access for drug delivery during CPR.

P56

SELECTIVE ALPHA7 NICOTINIC ACETYLCHOLINE RECEPTOR AGONIST IMPROVES SURVIVAL IN A MURINE ENDOTOXEMIA AND SEPSIS

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Recently, we identified a pivotal role for α7 subunit nicotinic acetylcholine receptors (α7nAchR) on macrophages in mediating the inhibition of TNF by the vagus nerve (Nature, 2003, 421:384). Nicotine, a non-specific agonist on these receptors, inhibits the release of TNF and high mobility group box1 (HMGB1) and prevents lethality in experimental sepsis (Nat. Med., 2004, 10(11): 1216). Here we developed these studies by demonstrating the anti-inflammatory efficiency of GTS-21, a selective α7nAchR agonist. *In vitro*, GTS-21 dose-dependently and more efficiently then nicotine inhibited the release of TNF and also suppressed HMGB1 levels. Single intraperitoneal (i.p.) injection of GTS-21 [4 mg/kg], 30 min before endotoxin infusion [7.5 mg/kg, i.p.] inhibited significantly serum TNF in endotoxemic mice. GTS-21 treatment (i.p.) initiated 30 min before endotoxemia induction and continued twice daily, for 3 consecutive days, significantly increased survival from lethal endotoxemia (vehicle-treated survival = 20%; vs. GTS-21 [0.4 mg/kg] survival = 40%; vs. GTS-21 [4 mg/kg] survival = 90%, $p<0.005$). Mice, subjected to cecal ligation and puncture (CLP) were treated (i.p.) with GTS-21 twice daily, for 3 days, beginning 24 h after surgery. GTS-21 significantly improved survival in this preclinical model of sepsis (vehicle-treated survival = 42%; vs. GTS-21 [4 mg/kg] survival = 90%, $p<0.005$). The protective drug effect was associated with lower serum levels of HMGB1 in GTS-21 treated mice. Our results demonstrate the potential of selective α7nAchR cholinergic activation in the treatment of sepsis. This study was funded in part by NIH/NIGMS, DARPA and Critical Therapeutics Inc.

P57

RENAL ISCHEMIA/REPERFUSION (I/R) DECREASES RENAL CORTICAL AND MEDULLARY iNOS AND COX-2 SYNTHESIS AND BLOOD FLOW (BF).

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Objective: To determine if Renal I/R concomitantly decreases synthesis of the enzymes responsible for endogenous renal cortical and medullary vasodilator eicosanoids and NO and BF.
Methods: Anesthetized male Sprague-Dawley rats (350 gms) had laser Doppler fibers placed in the cortex (Cor,2mm) and Medulla (Med,4mm) and were then subjected to Sham or supra-renal aortic clamping for 15, 30 and 45 minutes followed by reperfusion for 60 minutes. The kidney cortex and medulla were analyzed by Western Blot (followed by densitometry) for iNOS and COX-2 content. Data is reported as densitometry units or percent change in BF compared to time zero (Mean ∀SEM, N>5). *-significant compared to Sham at $p<0.05$ by Anova.
Table 1. The Effect of Increasing time of I/R on Renal Blood Flow and Cortical and Medullary iNOS and COX-2 Content

Group	Sham	15/30-I/R	30/60-I/R	45/60-I/R
Med-COX-2	6751+/-94	6259+/-77*	6222+/-47*	6339+/-47*
Cor-COX-2	7719+/-122	7328+/-38	7452+/-129	8114+/-125
Med-iNOS	2074+/-92	1876+/-113	2252+/-172	1887+/-256
Cor-iNOS	8624+/-163	7559+/-42*	7085+/-58*	7162+/-115*
Med-BF	5.3+/-2.3	-23.2+/-7.1*	-31+/-4.1*	-52+/-8.0
Cor-BF	14.6+/-3.1	-9.3+/-5.1*	-28.4+/-9.1*	-60.3+/-6.2

Conclusion: Increasing time of I/R decreased medullary COX-2 synthesis and cortical iNOS synthesis concomitant with a progressive decrease in medullary and cortical blood flow. These data suggest that the cortex and medulla may have different endogenous vasodilators that contribute to maintaining blood flow. Preserving the enzymes responsible for intra-renal vasodilator NO and eicosanoid synthesis may contribute to preserving microvascular renal cortical and medullary blood flow and function following I/R injury.

P58

ANALYSIS OF SNPS IN IL-1β GENE PROMOTER AND THEIR EFFECT ON LPS RESPONSE.

Jiang JX, Wen AQ, Feng K, Zhu PF, Wang ZG. State Key Laboratory of Trauma, Burns and Combined Injury, Institute of Surgery Research, Third Military Medical University , Chongqing, 400042, PRC. Interleukin 1β(IL-1β) is one of the pivotal cytokines in the pathogenesis of sepsis. Gene polymorphisms have been shown to be important determinants for disease susceptibility, phenotype diversity and response to pharmacotherapies. Single nucleotide polymorphisms(SNP) in IL-1β gene in Chinese population were analyzed through direct sequencing of the important functional regions of IL-1β gene among 10 representative nationalities(Han, Miao, Dai, Mongolian, Tibetan, Korean, Zhuang, Uygur, Bulang,Li). Six SNPs were found in 5'flank and intron regions respectively. Results from bioinformatics analysis indicated that the three SNPs(-470 G? C, -511T? C and -31C? T) in 5'flank were located in the promoter area and related to the binding of nuclear transcription factors. Distribution frequencies and linkage disequilibrium of the three SNPs in the promoter of IL-1βgene were further analyzed among 259 healthy volunteers. It showed that the SNPs at -1470, -511 and -31 loci were common alleles (43.6%, 50.8% and 47%). There was strong linkage disequilibrium among the three SNPs, specially between -511 and -31 loci. Individuals with base mutation at all the three loci accounted for 32%. The genotype G/C - T/C - C/T was the commonest (29.4%). Furthermore, effect of the SNPs in the promoter of IL-1β gene on the production of IL-1βby human peripheral WBCs was examined in response to lipopolysaccharide stimulation. It was found that G? C base transversion at -1470 locus could reduce LPS-induced production of IL-1β, while T? C and C? T base transversion at -511 and -31 loci could significantly enhance LPS-induced IL-1β production. Base mutations at -511 and -31 loci could synergistically enhance the expression of IL-1β, while the base mutation at -1470 locus reduced the enhancement induced by -511C and -31T. In order to further elucidate

the functional significance of the above three SNPs, GL3 vectors containing point mutations at -1470,-511 and -31 loci in the promoter of IL-1βgene(-1672~+121) were constructed. The plasmids were then transfected into THP1 and ECV304 cells. The above functional effects of the three SNPs at -1470, -511 and -31 loci in the promoter of IL-1β gene on the target expression was further confirmed in response to LPS stimulation. It is suggested that the three SNPs at -1470, -511 and -31 loci in the promoter of IL-1β gene might be used as useful biomarkers for the assessment of inflammatory disease susceptibility.

P59

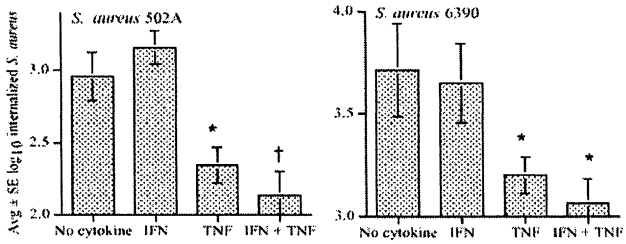
IMMUNMODULATION IN A "TWO-HIT"-MODEL OF COMBINED NEUROTRAUMA. M. Maegele*, N. Yücel*, S. Sauerland*, A. Niederbichler*, B. Bouillon*, U. Schäfer*, T. K. McIntosh*, A. Mautes*, E. Neugebauer. Univ. Cologne, Cologne (Germany), Univ. Saarland, Homburg (Germany), Univ. Pennsylvania, Philadelphia (USA), Univ. Michigan, Ann Arbor (USA)

Cytokine-mediated immuno-responses have been characterized following both isolated traumatic brain injury (TBI) and muskuloskletal trauma. However, little is known about possible interactive effects among these responses when both impacts occur simulanteously in the sense of a combined neurotrauma (cNT). Clinically, 50% of all traumatized patients sustain combined injuries including TBI. Materials and Methods: A new experimental "two-hit"-model of cNT incorporating TBI via moderate fluid-percussion and tibia fracture was used to investigate circulating cytokine interleukin-6 (IL-6) and -10 (IL-10) concentrations following cNT versus TBI only. 60 Sprague-Dawley (SD) rats were randomized to the following experimental groups: i.) SHAM (n=10), ii.) TBI only (n=25), and iii.) cNT, i.e. TBI + tibia fracture (n=25). Blood samples were drawn at 30', 6h, 24h, 48h, and 7d following trauma. Circulating IL-6 and -10 levels were determined via immunoassay (R&D Systems). Results: Circulating IL-6 and -10 levels following cNT were higher compared to TBI only and SHAM at all time points studied. Both responses followed a different pattern: IL-6 concentrations in cNT rose steeply in the acute phase reaching maximum at 6hrs post-injury (mean: 386 pg/ml in cNT vs. 147 pg/ml in TBI only), while circulating IL-10 concentrations in cNT increased slowly reaching maximum at 24hrs following impact (mean: 254 pg/ml in cNT versus 28 pg/ml in TBI only). Conclusion: Amplification of immuno-responses following cNT may contribute to increased susceptibility to early sepsis and single-/ multiorgan failure associated with whole body inflammation in cNT, as known from the clinical scenario. Supported by the Köln-Fortune Program, No. 80/2001 26800540.

P60

INFLAMMATORY CYTOKINES CAN ALTER BOTH EXPRESSION OF THE HEPARAN SULFATE PROTEOGLYCAN SYNDECAN-1 AND BACTERIA-ENTEROCYTE INTERACTIONS. M. Henry-Stanley, L. Erickson*, R. Garni and C. Wells. Univ. of MN, Minneapolis.

Inflammatory cytokines are produced in the intestines of trauma patients and there is evidence that cytokines can alter expression of the heparan sulfate proteoglycan syndecan-1 (S-1) on the mammalian cell surface. S1 is prominently expressed on normal human enterocytes and cultured HT-29 enterocytes, and S-1 may participate in enterocyte internalization of the opportunistic pathogen *Staphylococcus aureus*. Following overnight incubation with 10 ng/ml TNFα and/or 100 U/ml IFNγ, HT-29 cells had decreased expression of cell surface S1 (IFA for the S1 core protein) and increased levels of shed S1 in the extracellular milieu (ELISA for sCD138, with sample purification by either flash evaporation or anion exchange chromatography). These effects were most prominent following enterocyte treatment with both TNFα and IFNγ. The gentamicin protection assay was used to assess the effect of cytokine treatment on the internalization of two strains of *S. aureus* (502A and 6390) by HT-29 enterocytes and results showed that cytokine-induced alterations in S1 expression were associated with decreased internalization of both *S. aureus* strains (* and †: decreased versus no cytokine at P<0.05 and P<0.01).



Thus, inflammatory cytokines can alter enterocyte expression of the heparan sulfate proteoglycan S1, and S1 may play a role in enterocyte interactions with potentially pathogenic intestinal *S. aureus*. (Supported by NIH GM 066751.)

P61

RATIO OF INTERLEUKIN-6 (IL-6) BLOOD LEVEL IN PULMONARY AND PERIPHERAL ARTERY ON A PATIENT ON VENTILATOR AS AN INDICATOR OF BIOTRAUMA IN VENTILATOR-INDUCED LUNG INJURY H. HIRASAWA, S. ODA*, K. MATUDA and Y.HIRAYAMA* Chiba Univ. Graduate School of Medicine, Chiba, Japan

Objective: It has been claimed that injurious mechanical ventilation leads to the increase in inflammatory cytokine release in the lung and such adverse effect is referred to "biotrauma" as one type of ventilator-induced lung injury (VILI). Even though biotrauma is conceptually accepted, there are few papers showing the development of biotrauma in clinical settings. Therefore, we investigated the ratio of IL-6 blood level in pulmonary and peripheral artery to investigate the development of biotrauma under various ventilatory strategy. Method: 36 patients who were mechanically ventilated were divided into three groups according to tidal volume. Group A: ventilated with tidal volume larger than 12 mL/kg. Group B ventilated with tidal volume between 12-6 mL/kg. Group C ventilated with tidal volume less than 6 mL/kg. On those patients, IL-6 blood level in pulmonary artery (IL-6 PA) and in peripheral artery (IL-6 A) was measured simultaneously and the ratio (IL-6 A)/(IL-6 PA) (A/PA) was calculated. If A/PA is >1, it indicates that IL-6 is mainly released in the lung (biotrauma) and contrarily if A/PA is <1, it indicates that IL-6 is mainly released in the area other

than lung (no biotrauma). Results: A/PA is 3.1 ± 1.8 (Mean ± SD) in Group A, 1.0 ± 0.3 in Group B, and 0.9 ± 0.8 in Group C, respectively. Thus, A/PA in Group A is significantly larger than A/PA in Group B and Group C. Furthermore, A/PA is > 1.0 on the patients with ARDS compared to A/PA on the patients without ARDS. And A/PA is smaller < 1.0 on the patients without ARDS but with abdominal emergency. Those results indicate that aggressive or injurious mechanical ventilation with larger tidal volume may lead to the increase in the release of cytokine in the lung. Furthermore, patients with ARDS may release more cytokines compared to the patients without ARDS. Conclusions: Injurious mechanical ventilation may cause increased release of cytokine and may cause biotrauma as one type of VILI.

P62

CYTOKINE LONG-TERM PATTERN IN A POLYMICROBIAL SEPSIS MODEL

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Background: Severe sepsis remains a serious clinical problem with few therapeutic options to improve survival. Cytokines are thought to contribute to the underlying inflammatory pathogenesis. Aim of the present study was to analyze a cytokine pattern in order to determine possible treatment targets in a polymicrobial sepsis model. Methods: Groups of male BALB/c mice (n=8 per group and time point) were subjected to either a sublethal cecal ligation and puncture or sham operation. Mice were sacrificed on day 1, 2, 7, 10, 14 and 21 after CLP and serum was collected measure the concentrations of IL-1b, IL-2, IL-4, IL-5, IL-10, GM-CSF, IFN-g, TNF-alpha, IL-1a, IL-3, IL-6, IL-12p40, IL-12p70, IL-17, G-CSF, KC, MIP-1a and RANTES. Cytokines were measured by a multiplex assay (Bioplex-Multiarray system). Results: Mortality in the CLP group was 10%, while none of the sham mice died. Serum concentrations of 5 cytokines (IL-2, IL-17, G-CSF, GM-CSF, and MIP1a) were consistently elevated at each timepoint from day 1 to day 21 in CLP mice when compared to sham mice, and 2 other cytokines (TNFa and IL-6) were significantly elevated through the duration of the study starting on day 2. In contrast, 1 cytokine (RANTES) was persistently decreased in post-CLP mice compared to sham mice from day 2 to day 21 ($p<0.05$). Summary: Cytokines that were persistently elevated throughout the study (IL-2, IL-17, G-CSF, GM-CSF, and MIP1a) may play important roles in the pathogenesis of sepsis. In contrast, serum concentrations of RANTES were decreased after CLP throughout the study period. Conclusion: Knowledge of the time course of the cytokine response serves as a rational basis for the detection of sepsis; the development of algorithms to assess the severity of sepsis; and the development of strategies of cytokine manipulation for the treatment of sepsis.

P63

PULMONARY RESPONSES OF CYTOKINES FOLLOWING MAJOR SURGERY. K.Okawa*, M.Miyashita, K.Sasajima*, H.Maruyama, T.Matsutani, C.Kim*, S.Takeda*, A.Sakamoto*, T.Tajiri*. First Dept. of Surgery and Anesthesiol, Nippon Medical School, Tokyo, Japan.

Objective: To investigate the effects of major surgical injury on the lung, the relationship between pulmonary function and proinflammatory cytokines in bronchoalveolar lavage fluid (BALF) was studied.

Methods: A total of 19 patients with esophageal cancer who underwent thoraco-abdominal surgery were studied. Respiratory index (RI) was monitored until 3-postoperative day (POD). BAL was performed 0-POD and 1-POD. The supernatant was used for the assay of IL-8, IL-6 and TNF-α by ELISA. The cells were used for immunostaining of these cytokines and for mRNA by RT-PCR method.

Results: After surgery, 7 patients developed pulmonary complications and 12 patients had no complications. The percentage of polymorphonuclear (PMN) cells in BALF was significantly higher on 1-POD than that on 0-POD ($p<0.05$). IL-8 concentration in BALF was 210±57pg/ml on 0-POD and 359 ±69pg/ml on 1-POD. IL-8 level increased significantly on 1-POD than 0-POD ($p<0.05$). There were no significant differences in IL-6 and TNF-α concentrations on either 0-POD and 1-POD. Positive correlations were found between IL-8 concentration and RI on both 0-POD and 1-POD. Positive correlation was also found between IL-8 concentration in BALF and the PMN percentages on 1-POD. IL-8 level in patients with pulmonary complications (n=7) was significantly higher than that without complications on 1-POD (n=12) ($p<0.05$). Immunostaining of BAL cells revealed the presence of TNF-α,IL-8 and IL-6 in the cytoplasm of alveolar macrophages on POD-0 and POD-1. RT-PCR method showed that BAL cells expressed TNF-α,IL-8 and IL-6 mRNA.

Conclusion: These results suggest that among cytokines investigated IL-8 and PMN percentage in BALF are most likely related to impaired pulmonary functions and the development of pulmonary complications after major surgery.

P64

EXPRESSION OF INTERLEUKIN 16 IS ALTERED IN MURINE MODEL OF HEMORRHAGIC SHOCK

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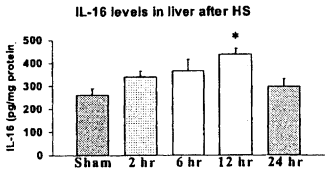
We previously reported increased expression of interleukin 16 (IL-16), a potent chemokinetic and chemoattractant cytokine for all CD4+ cells, in septic mice. Severe hemorrhagic shock (HS) has been shown to significantly alter expression of cytokines, eliciting an acute inflammatory response. However, alterations in IL-16 levels following HS have not been evaluated. To assess changes in IL-16 expression in a murine model of HS, 4-6 male C3H/HeN mice per time point were hemorrhaged (90

min at 35±5mmHg), euthanized 2, 6, 12, and 24 hours later and plasma, liver, and spleen were collected. Levels of IL-16 were analyzed by ELISA and Western Blot. IL-16 levels in liver increased following HS, peaking at 12hr (One-way ANOVA and Tukey's test: $P<0.05$ compared to sham). Western blot confirmed elevated levels of bioactive IL-16 in both liver and spleen 12hr after hemorrhage. Expression of IL-16 in liver and spleen 24hr after hemorrhage returned to levels comparable to sham. Although circulating levels of IL-16 were very low, there was a discernible increase 2 and 6 hrs after HS. Taken together, these data suggest that IL-16 may play a role in early proinflammatory changes in the leukocyte populations of the spleen and liver following shock.



(Supported by NIH GM46354; GAANN Training Grant P200A030100)

P65

MURINE PULMONARY FIBROBLASTS EXHIBIT SELECTIVE LPS TOLERANCE IN CXC CHEMOKINE PRODUCTION. JA Nemzek and M Osuchowski*. Unit for Laboratory Animal Medicine and Department of Pathology, U of Michigan, Ann Arbor, MI 48109.

While alveolar macrophages are a major source of pro-inflammatory cytokines, pulmonary fibroblasts also respond to lung insult. However, little is known about CXC chemokine production and regulation in pulmonary fibroblasts. To examine this, murine lung fibroblasts were obtained by collagenase digestion. Cultured cells were exposed to no stimulus, 100ng/ml lipopolysaccharide (LPS), or 20ug/ml lipotechoic acid (LTA). Cell media was analyzed for cytokines via ELISA. After LPS, the cells produced substantial amounts of KC and MIP-2α (17.3 ± 7.3 and 10.1 ± 3.8 ng/ml in 24 hours, respectively) but relatively low levels of LIX (1.1 ± 0.3 ng/ml). LTA resulted in similar profiles. Next, to study tolerance, cells were pre-exposed either to no stimulus or 100ng/ml LPS for 15 hours, followed by re-exposure to no stimulus, 10ug/ml LPS, or 20ug/ml LTA. Naïve fibroblasts produced TNF in response to LPS and LTA (2.8 ± 0.9 and 1.7 ± 0.3 ng/ml, respectively); however, tolerance was induced with pre-exposure to LPS (0.1 ± 0.07 and 0.09 ± 0.08 ng/ml, respectively). Similarly, MIP-2α levels produced after LPS (12.6 ± 3.7 ng/ml) or LTA (8.0 ±1.6 ng/ml) were significantly reduced with prior LPS exposure (6.6 ± 2.0 and 3.9 ± 1.3). However, fibroblasts did not develop tolerance in the LPS:LPS or LPS:LTA protocols with respect to KC or LIX. To study the effect of phosphotidylinositol 3 kinase (PI3K) on chemokine regulation and tolerance, the tolerance protocols were repeated in the presence of wortmannin. The PI3K inhibitor had no effect on TNF and chemokine responses. This suggests PI3kinase does not regulate LPS tolerance in pulmonary fibroblasts and is contrary to similar reports in macrophages. This study showed that pulmonary fibroblasts respond to inflammatory stimuli by producing CXC chemokines and develop LPS tolerance manifested by reduced TNF and MIP-2 α production. These results suggest that the regulation of CXC chemokines is not uniform and that the relative contribution of each CXC chemokine may vary with the stimulus.

P66

DEVELOPMENT OF A RAPID ASSAY FOR MEDIATOR DETECTION. D. Horton*, Daniel G. Remick. U Michigan, Ann Arbor MI 48109

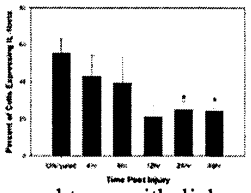
It is well documented in the literature that plasma levels of interleukin-6 (IL-6) obtained 6h after the induction of sepsis can be used as a predictor for subsequent mortality in the early phases of sepsis. In order to deliver appropriate, targeted therapies in a timely manner, it is necessary to rapidly determine circulating levels of inflammatory mediators, including IL-6. The objective of this study was to develop a rapid immunoassay to determine the plasma IL-6 levels from a small quantity of peripheral blood. A rapid immunochromatographic test strip was developed and pilot studies, using whole rabbit IgG as a plasma sample and antibodies against it, were initiated to determine whether the approach was feasible. The strip relies on monoclonal antibodies to qualitatively analyze the levels of protein in plasma. Polyclonal goat anti-rabbit IgG was covalently linked to blue latex particles (Duke Scientific Corporation, Palo Alto, CA). 3.6μg monoclonal mouse anti-rabbit IgG and polyclonal mouse anti-goat IgG were bound to nitrocellulose membranes and used as capture and control reagents, respectively. After drying the membrane for 1 h at room temperature, it was blocked for non-specific protein by soaking in phosphate-buffered saline containing 1% BSA and incubating at room temperature while shaking for 2 h. Membranes were then washed twice with Tween 20-PBS for 10 min. After drying for 1 h at room temperature, membranes could either be stored at room temperature or used for rapid immunodetection of whole rabbit IgG. For the immnodetection assay, each strip is rotated in a solution containing whole rabbit IgG mixed with goat anti-rabbit/blue latex particles conjugate and rotated for 10 minutes, after which development of color would be observed. The test would be considered positive if there are two distinct blue lines observed on the membrane. As our interest lies in treatment of CLP-induced sepsis based on plasma IL-6 levels, a rapid method of detecting IL-6 is necessary for a timely treatment regimen and this assay is the first step in the process to achieve that goal.

P67

BRONCHIAL GLAND SECRETION OF IL-1β IN SHEEP AFTER SMOKE AND BURN INJURY. A.S. Burke*[1], P. Enkhbaatar*[2], L.D. Traber[2], D.L. Traber[2], D.N. Herndon*[2], H.K. Hawkins*[2], R.A. Cox*[2]. (Spon: D.L. Traber) Shriners Hospitals for Children, Galveston, TX 77550: The University of Texas Medical Branch, Galveston, TX 77555.

Objective: This study examines the immunohistochemical (IHC) expression of IL-1β in bronchial serous glands in a sheep

model of smoke inhalation and burn (S+B) injury. IL-1β is a potent chemotactic peptide and modulator of acute inflammation. Methods: The main bronchi from the right lower lobes of uninjured sheep (n=4) and sheep at 4 (n=4), 8 (n=4), 12 (n-4), 24 (n=4) and 48 (n=4) hrs after S+B injury were fixed and processed for histological and IHC analysis per protocol. IHC staining for IL-1β was performed with an ABC-peroxidase-DAB procedure. Results: IL-1β stained cytoplasmic granules in serous cells. Staining was virtually absent in goblet cells. After injury, we observed less staining with apical localization of residual IL-1β in the serous cells. IL-1β staining was present in acinar lumens, gland ducts and bronchial lumens. Quantitatively, the mean percentage of gland cells staining for IL-1β decreased progressively from 55.0 ± 15.3 SD in the uninjured tissue to 25.2 ±8.6 SD and 24.5 ± 3.1 SD in tissue 24 and 48 hrs after injury, respectively, p<0.05* (Figure).



Conclusions: These data suggest that S+B injury promotes serous cell Secretion of IL-1β after S+B injury. Secretion of IL-1β may contribute to the focal inflammatory response, transendothelial migration of leukocytes and transepithelial migration of neutrophils into the acinar lumen after injury. Pharmacological intervention in this process might benefit S+B injured patients.

P68

MIP-2 AND KC DIFFERENTIALLY CONTRIBUTE TO THE NEUTROPHIL ACTIVATIONAL/PHOSPHO-PROTEIN STATUS RESULTANT FROM HEMORRHAGE

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Neutrophil (PMN) priming via an initial injury (i.e. hemorrhagic shock (Hem)) predisposes the trauma patient to a dysfunctional immune response to a secondary inflammatory/infectious challenge. Acute lung injury (ALI)/dysfunction observed in patients/experimental animals may be a consequence of this form of destructive priming. Resident pulmonary cells mediate PMN recruitment to the lung by the production of chemokines that signal through a common CXC-type 2 receptor (CXCR2). Our laboratory has shown that systemic antibody neutralization of mouse PMN chemokines, keratinocyte-derived chemokine (KC) and macrophage inflammatory protein-2 (MIP-2), CXCR2 antagonism and local pulmonary silencing with KC or MIP-2 siRNA, differentially attenuates indices of lung inflammation and injury, as well as PMN apoptotic, chemotactic and respiratory burst capacity. Possible explanations for the divergent effects of chemokines that share a common receptor might be altered ligand-receptor affinity and/or the utilization of a divergent downstream signaling network. To address the latter hypothesis we sought to determine, if PMNs isolated from mice 12 h after Hem of 90 min. at 35±5mmHg or Sham-Hem, exhibited differences in protein phosphorylation (serine, tyrosine and/or threonine phophorylation), following antibody neutralization of KC or MIP-2 (25ug or 5ug/mouse respectively during Hem rx.). Increases in phosphorylated vs. unphosphorylated or total protein were assessed via Pro-Q Diamond phospho-protein and SYPRO Ruby protein gel stains (Molecular Probes, Eugene, OR) of electrophoresed neutrophil lysates. Not surprisingly, PMNs from all Hem mice showed an increase in phospho-proteins above Sham-Hem level. A decrease in phospho-protein levels was observed in PMN lysates from anti-MIP-2 treated mice as compared to anti-KC or a non-specific IgG control. These finding support our hypothesis that MIP-2 and KC differentially contribute to the activation status of blood PMNs following Hem, although signaling through a common receptor. (NIH HL73525)

P69

HMGB1 DISRUPTS ENTEROCYTE BARRIER INTEGRITY BY IMPAIRING ENTEROCYTE COMMUNICATION AND INHIBITING PHOSPHORYLATION OF CONNEXIN-43 (Cx43).

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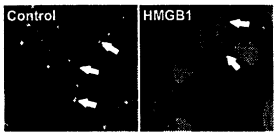
Children's Hospital of Pittsburgh and New York University

Introduction: Sepsis is characterized by the release of soluble mediators including HMGB1, causing intestinal barrier failure. The mechanism[s] by which HMGB1 causes enterocyte disruption are unknown. We have demonstrated that enterocytes communicate via gap junctions (GJs) comprised of Cx43 which function when phosphorylated at the cell surface. We now hypothesize that barrier integrity requires cell communication, and that HMGB1 causes barrier dysfunction by dephosphorylating Cx43 and inhibiting GJs. **Methods:** Cx43 and phospho-Cx43 were detected in T84 and IEC-6 enterocytes by immuno-electron microscopy, confocal and SDS-PAGE. TER was measured in T84 cells±the GJ inhibitor oleamide (50μM, 1h) or HMGB1 (100 ng/ml) on 0.3μm filters for 7 days. GJ communication was determined using fluorescence recovery after photobleaching of IEC-6 cells with the tracer calcein-AM. **Results:** Enterocytes express Cx43 on the plasma membrane between adjacent cells. Inhibition of GJs decreased TER of monolayers versus controls (ΔTER: ctrl=92±3.1 vs. Oleamide = -84±9.6 Ωcm^2, $p<0.05$), revealing a role for GJs for barrier integrity. HMGB1 caused a dose-dependent de-phosphorylation and internalization of Cx43 (Figure, arrows= pCx43), leading to reduced enterocyte communication (%transfer ctrl:59±5 vs.HMG 30±4, $p<0.05$) and a decrease in barrier integrity (-81±9Ωcm^2, $p<0.05$). Strikingly, pre-incubation of HMGB1 treated enterocytes with the phosphatase inhibitor pervanadate (10μM) restored gap junction communication and phosphorylation of Cx43, reversing the disruption in enterocyte barrier integrity. **Conclusions**: Enterocyte barrier integrity requires GJs. HMGB1 causes dephosphorylation of Cx43 and reduces enterocyte barrier function and communication. This suggests a novel role for HMGB1 in sepsis related intestinal barrier dysfunction.



P70

CROCETIN AND AMINO ACIDS INFLUENCE THE PROINFLAMMATORY CYTOKINE RESPONSE.

A. M. Thomas*, X. Tan, J. DeSousa*, R. Yang*, N. Qureshi and C. Van Way III. Departments of Surgery & Basic Medical Sciences, School of Medicine, University of Missouri, 2411 Holmes Street, Kansas City, MO 64108. The exact mechanisms

involved in hemorrhagic shock have not yet been defined. There is, however, some indication that the mechanisms occurring during hemorrhagic shock may be similar to those of septic shock. In addition, LPS can cause major complications in patients undergoing hemorrhagic shock. We have previously shown that proteasomal activity is essential for LPS-induced shock. We have now studied the effects of several compounds used in resuscitation fluids for hemorrhagic shock on the purified proteasomal activity. We observed a dose-dependent inhibition of the purified 20S proteasome's chymotrypsin-like activity with crocetin and glutamine. On the other hand, dipeptivin (alanine-glutamine dipeptin, 1:1.6) showed an induction of this activity. We also studied the role of these compounds on LPS-induced inflammatory cytokines. Crocetin, glutamine, dipeptivin, arginine.HCl, arginine glutamate salt, and ornithine.HCl showed an inhibition of TNF-α levels in peritoneal murine macrophages. In contrast, dipeptivin showed an induction of TNF-α?in these macrophages. Similarly, with the exception of dipeptiven, these compounds also down-regulated LPS-induced TNF-α, IL-1, iNOS, and IL-6 gene expression in murine macrophages. Finally, we have carried out an Affymetrix Chip gene-array analyses of liver RNA samples prepared from 6 different rat groups, normal control, SHAM control, shock control, lactated ringer's control, crocetin treated, and dipeptivin treated rats to investigate pathways that are either up-regulated or down-regulated during hemorrhagic shock. These studies will provide insights into the mechanisms involved in hemorrhagic shock and will lead to novel therapeutic strategies for shock. (Supported by GM50870, ONR N00014-01-0151 and AHA).

P71

REGULATION OF TNF-α PRODUCTION BY MAPK SIGNALLING PATHWAYS: ROLE OF PHOSPHODIESTERASE INHIBITION.

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University of California San Diego, San Diego, CA 92103-8896

Using an in vitro whole blood model as well as isolated peripheral blood mononuclear cells (PBMC), we have previously shown that Pentoxifylline (PTX),a non-specific phosphodiesterase inhibitor, significantly reduces LPS-induced TNF-α synthesis. The relationship between phosphodiesterase inhibition and the MAPK signal transduction pathways, particularly p38 and ERK 1 / 2 in regulating LPS-induced TNF-α production remains unclear.

Whole blood collected form healthy volunteers was pre-incubated with either the MAPK p38 inhibitor SB203580 (10 μM) or MEK 1 inhibitor PD98059 (50 μM) prior to stimulation with LPS (100 μg) or concomitant PTX (2 mM) + LPS. Serum was separated form whole blood and TNF-α was measured (pg/ml) by ELISA. Data are presented as mean ± SEM (n=4). PBMC isolated from whole blood (5x106 cell/ml) were stimulated with LPS (10 μg/ml) or PTX (20 mM) + LPS. Proteins were separated by SDS-PAGE, transferred to membranes, and immunoblotted with phosphorylated p38 or ERK 1 / 2 antibodies.

SB203580 pre-treatment decreased TNF-α production by 79% compared to LPS stimulation alone (1097 ± 497 vs. 5240 ± 747). PTX treatment reduced TNF-α production by 85% (828 ± 191). An additive effet was observed with SB203580 pre-treatment in addition to PTX (80 ± 83). PD 98059 pre-treatment did not alter TNF-α production in LPS stimulated cells (3380 ± 200 vs. 3452 ± 264) or PTX + LPS treated cells (485 ± 101 vs. 504 ± 91). There was no difference in p38 or ERK 1 / 2 phosphorylation in the PTX treatment group compared to LPS stimulation alone. These results indicate that: a) LPS-induced p38 MAPK activation participates in the regulation of TNF-α production; b) TNF-α production appears to be independent of ERK 1 / 2; c) phosphodiesterase inhibition by PTX decreases TNF-α production, at least in part, by a p38 MAPK-dependent mechanism

P72

LYSOPHOSPHATIDYLCHOLINE REDUCES THE MULTIPLE ORGAN INJURY AND DYSFUNCTION CAUSED BY ENDOTOXEMIA IN THE RAT

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William Harvey Research Institute, Centre for Experimental Medicine, Nephrology and Critical Care, London, UK

Background: Reduced serum levels of lyso-phosphatidylcholine (LPC) are associated with poor outcome in patients with sepsis [1]. Recently, LPC was shown to reduce mortality in septic shock in the mouse [2]. **Objective:** Here we investigate the effects of LPC on the multiple organ injury and dysfunction associated with acute severe endotoxemia. **Methods:** Rats received either *E. coli* lipopolysaccharide (LPS, 6 mg/kg i.v.) to induce endotoxemia, or vehicle (saline, 1 ml/kg i.v.). LPC (10 mg/kg i.v.) or vehicle (2% BSA in PBS, 1 ml/kg i.v.) was administered 1, 2 or 4 h after LPS. **Results:** Endotoxemia for 6 h resulted in an increase in serum levels of urea and creatinine (indicators of renal dysfunction), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (markers for hepatic injury), lipase (indicator of pancreatic injury), and creatine kinase (CK, an indicator of neuromuscular injury). Therapeutic treatment of rats with LPC at 1 or 2 h after the induction of endotoxemia attenuated rises in serum levels of urea, creatinine, ALT, AST, lipase, and CK. The protective effect was no longer observed when LPC was administered at 4 h after the induction of endotoxemia. LPC did not affect the characteristic biphasic fall in blood pressure or the increase in heart rate caused by endotoxemia. **Conclusions**: LPC reduces the organ injury and dysfunction, but not the hypotension, caused by endotoxemia in the anesthetised rat. This finding supports the view that LPC may be useful in the therapy of the organ injury and dysfunction associated with endotoxemia, and other diseases associated with local or systemic inflammation.

[1] Drobnik W, et al., J Lipid Res 2003; 44:754-761.
[2] Yan JJ, et al., Nat Med 2004;10:161-167.

P73

ADMINISTRATION OF IFNγ AND anti-IL-10 DOES NOT IMPROVE BACTERIAL CLEARANCE OR MORTALITY OF POST-CLP IMMUNOSUPPRESSION IN MICE.

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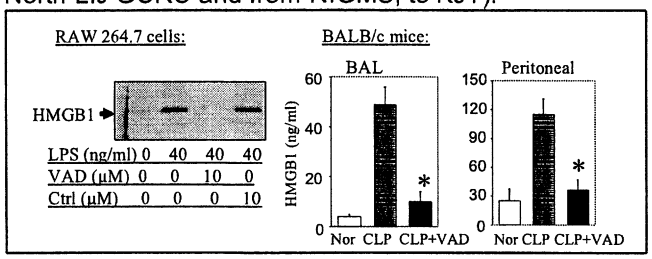
Objective: Mice challenged with Pseudomonas several days after non-lethal CLP have lower serum IFNγ, higher serum IL-10, and impaired bacterial clearance when compared to non-injured mice. We sought to determine the functional significance of the altered IFNγ and IL-10 responses in this model of post-injury immunosuppression. **Methods**: Male mice (C57BL/6, 6-8 wks) were subjected to sublethal CLP (25g needle) or sham CLP and allowed to recover for 5 days before challenge with Pseudomonas aeruginosa (5 x 10^7 cfu iv). One CLP group was treated with IFNγ and a neutralizing antibody directed against IL-10. In separate experiments, IL-10 knockout mice were treated with IFNγ and challenged with Pseudomonas. **Results**: Mice subjected to CLP had higher bacterial cfu's in lung tissue, higher serum IL-10, and lower serum IFNγ when compared to sham controls 6 hrs after Pseudomonas challenge ($p<.05$). Administration of IFNγ and neutralization of IL-10 in post-CLP mice resulted in higher serum IFNγ & IL-12 and lower IL-10 ($p<.05$) but did not affect bacterial clearance or mortality after Pseudomonas challenge. Further studies showed non-injured IL-10 knockout mice had moderately better clearance of Pseudomonas than wild-type mice but also had higher mortality. IL-10 KO's also had a high Gram+ cocci background in lungs. Administration of IFNγ to IL-10 KO's did not improve clearance of Pseudomonas or mortality but did reduce the growth of the Gram+ organisms. **Conclusions**: Reversal of the CLP-induced alterations in circulating IFNγ and IL-10 did not improve mortality or clearance of a Pseudomonas challenge, suggesting these cytokines do not directly affect the immune response to Pseudomonas. However, IFNγ may play a role in immune function against gram positive organisms.

P74

CASPASE INHIBITOR (Z-VAD-FMK) REDUCES HMGB1 RELEASE IN MACROPHAGES EXPOSED TO LPS AND IN SEPTIC MICE. R. Yuan*, H. Wang, E. Miller#, K.J. Tracey and H. Yang. Laboratory of Biomedical Science and Department of Surgery#, Institute for Medical Research at North Shore-LIJ, Manhasset, NY 11030.

Sepsis induces apoptosis and prevention of apoptosis by caspase inhibitor improves survival in sepsis induced by cecal ligation and puncture (CLP, Hotchkiss et al, 2000). Previously we showed that HMGB1 is a late mediator of lethal sepsis (Yang et al, 2004). Here we examined whether caspase inhibitor also affects HMGB1 release *in vitro* and *in vivo*. In macrophage-like RAW 264.7 cells, Z-VAD-FMK (VAD), a broad-spectrum caspase inhibitor, inhibited LPS-induced HMGB1 release (Fig.). In animal studies, BALB/c mice underwent CLP and received either VAD or control peptide (Z-FA-FMK, 0.5 mg/mouse)injected intraperitoneally at 90 min and were sacrificed at 24 hours after CLP. Besides reducing sepsis-induced apoptosis in the spleen and thymus in CLP mice (data not shown), VAD treatment significantly attenuated sepsis-induced HMGB1 levels in bronchoalveolar (BAL) and peritoneal lavage fluid compared to control animals (Fig. *: $P<0.05$ *vs.* CLP, n=7 per group). Similar pattern of inhibition in serum HMGB1 was observed by VAD treatment (control group = 98 ± 30 *vs.* VAD group = 80 ± 33 ng/ml, P=N.S.). Thus, our data indicate that caspase inhibitor VAD may exert protection against lethal sepsis by inhibiting cell apoptosis and by reducing HMGB1 release. (Supported by grants from the North-LIJ GCRC and from NIGMS, to KJT).

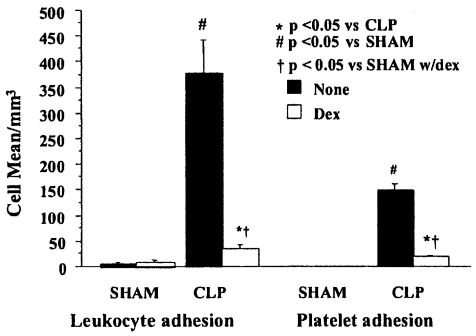


P75

EFFECT OF CORTICOSTEROIDS IN CEREBRAL MICROCIRCULATION AFTER CECAL PERFORATION: V. Vachharajani*, S. Vital*, L. Scott., D.N. Granger Louisiana State University Health Sciences Center, Shreveport, LA 71130.

Background: The effect of corticosteroids in the treatment of sepsis is controversial and its mechanism of action is unclear. We have demonstrated increased leukocyte (LA) and platelet adhesion (PA) in post-capillary venules of the cerebral microcirculation in lean as well as obese mice after cecal ligation and perforation (CLP) compared to sham (Vachharajani et al, Microcirculation 2005). **Objective:** To assess the effect of dexamethasone (dex) upon leukocyte and platelet adhesion in cerebral microcirculation in mice after CLP. **Methods**: Wild type (WT) C57BL/6 mice were subjected to SHAM and CLP surgery with or without 4mg/kg dex. Post-capillary venules were assessed for LA and PA 4-hours after injury, utilizing intravital microscopy. Ongoing study is evaluating the same in ob/ob mice. **Results**: As depicted in figure 1, the LA and PA in treated mice reduced significantly in mice treated with dex, although the reduction was not all the way down to that of SHAM surgery mice.

Figure 1:



Conclusion: Corticosteroids attenuate inflammatory response in cerebral microcirculation after CLP via reduction in LA and PA. This suggests a potential role for corticosteroids in the treatment of septic encephalopathy.

P76

MITOCHONDRIAL FUNCTION AND MITOCHONDRIAL PROTEOME IN LPS TREATED RATS. I. Miller[1], M. Gemeiner[1], B. Gesslbauer[2], K. Staniek[1], H. Nohl[1], S. Haindl[3], S. Bahrami[3], H. Redl[3], A. V. Kozlov[3].
[1]) University of Veterinary Medicine Vienna, [2]) University Graz, [3]) Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria

It has been shown previously that endotoxic shock affects the mitochondrial function. The objective of this study was to investigate a possible correlation between mitochondrial function altered by endotoxin and the mitochondrial proteome. Sprague Dawley rats were challenged with LPS (8 and 20 mg/kg i.p.), the control group received equal volumes of saline. 16 hours later animals were sacrificed and mitochondria were prepared from liver and heart. Using the standard polarographic procedure (Clark electrode) we have found that LPS influences respiration in state 3 and ADP/O ratio. Proteomic experiments were carried out by DIGE technology (differential image gel electrophoresis) which offers the advantage to separate three different fluorescently labelled samples on one gel. The variability of the mitochondrial protein pattern was determined in both animal groups and found similar. The majority of the approx. 1200 spots detected showed minor to moderate variation; however, there was also a number of protein spots with more than 50 % variability. One of them, a protein of higher abundance, was identified as mitochondrial aldehyde dehydrogenase and found in two different isoforms, on the gels visible as spot chain with shifting positions. We assume that also some of the other spots with higher variation coefficients may represent different isoforms or modifications. Changes in mitochondrial protein patterns of treated and untreated rats were not very pronounced. Nevertheless, a couple of spots found in liver mitochondria seem upregulated in LPS-stimulation and are presently under closer investigation.

P77

KETAMINE ATTENUATES LIPOPOLYSACHARIDE (LPS) INDUCED EARLY GASTRIC DYSFUNCTION: ROLE OF STRESS INDUCIBLE PHOSPHOSPROTEINS. JW Suliburk*, EA Gonzalez*, DW Mercer. Univ. of Texas at Houston 77030

Into: Endotoxic shock causes gastric dysfunction manifested by gastroparesis, increased gastric residual volume and gastric luminal alkalinization. Ketamine has powerful anti-inflammatory properties, but this mechanism is still unkown. Because ketamine attenuates LPS induced inflammation, we hypothesized that ketamine would disrupt the early signaling events of LPS induced inflammation by altering phosphorylation of stress inducible phosphoproteins JNK, p38 and IkB. **Methods**: Adult rats received saline or ketamine (70 mg/kg, ip) 1 hour prior to LPS (20 mg/kg, ip) or saline. Animals were sacrificed at 15, 30 and 60 minutes post LPS, gastric mucosa harvested and gastric volume and pH recorded. Gastric mucosa JNK, p38 and IkB phosphoproteins were analyzed with a multiplexed suspension immunoassay. Data are mean ± SEM (n≥ 5; ANOVA). * $p < 0.05$ vs sal/sal, ** $p < 0.05$ vs sal/LPS.

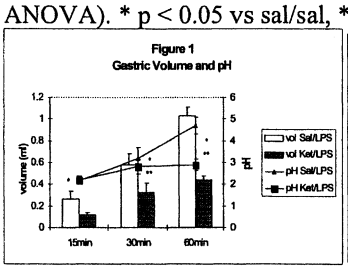


Table 1		Sal/Sal	Sal/LPS	Ket/Sal	Ket/LPS
15m	JNK	1.6 ± .1	3.9 ± 0.2*	2.2 ± 0.6	4 ± 0.7*
	P38	1.7 ± .2	4.4 ± 0.6*	1.8 ± 0.2	4.2 ± 1*
	IkB	0.9 ± .04	1.5 ± 0.1	1 ± .01	1.6 ± 0.1
30m	JNK	1.5 ± .2	2.5 ± 0.2*	2.3 ± 0.5	3 ± 0.1*
	P38	2 ± 0.3	4.1 ± 0.9*	2.4 ±0.7	4.2 ± 1*
	IkB	1 ± .07	2.8 ± 0.9*	0.9 ±0.8	2 ± 0.6*
60m	JNK	1.6 ± 0.2	2.7 ± 0.2*	2.1 ± 0.5	3 ± 0.2*
	P38	1.9 ± 0.2	4.2 ± 1*	3 ± 0.6	5 ± 0.7*
	IkB	0.9 ± 0.1	3.3 ± .7*	1.1 ± .02	3 ± 0.8*
Values are mean fluorescent intensity.					

Results: Ketamine attenuated LPS induced increases in gastric luminal fluid and pH (Fig 1). Control animals receiving saline or ketamine and no LPS had gastric volumes of 0.1 ml and luminal pH of 2 at all time points (not shown). LPS upregulated phosphorylation of JNK, p38 and IkB as early as 15 minutes following LPS (Table 1). Ketamine did not effect the early phosphorylation of these proteins. **Conclusion**: Endotoxin causes gastric dysfunction and upregulates stress inducible phosphoproteins within minutes following LPS. While ketamine attenuates gastric dysfunction, its salutary effects do not appear related to alterations in phosphorylation of JNK, p38 or IkB. Supported by NIGMS 38529 and 08792.

P78

INFLUENCE OF NOS INHIBITION ON MMP PRODUCTION DURING ENDOTOXEMIA E. Robinson*, C. Seaworth*, D. Mercer The University of Texas Health Science Center at Houston, Houston, TX 77030

Introduction: Matrix metalloproteinases (MMPs) are endopeptidases that degrade the extracellular matrix and contribute to lipopolysaccharide (LPS) induced gastric injury. MMPs are closely modulated by their activators, membrane type-1 MMP (MT1-MMP) and endogenous inhibitors, the tissue inhibitors of metalloproteinases (TIMPs.) Prior studies in our laboratory have demonstrated that LPS increases the production of gastric inducible nitric oxide synthase (iNOS) and that iNOS contributes to LPS induced gastric injury. While nitric oxide has been shown to modulate MMP production in cell lines, the differential effect of selective versus non-selective NOS inhibition on LPS induced gastric MMP production is unknown. The purpose of these studies was to determine the effects of selective and non-selective NOS inhibition on LPS induced gastric MMP production. **Methods:** Sprague-Dawley rats were treated with L-NAME (5 mg/kg SC) a non-selective NOS inhibitor, or Aminoguanidine (AG 45 mg/kg IP) a selective iNOS inhibitor, or vehicle followed by saline or LPS (20 mg/kg IP) and sacrificed 24 hours following LPS administration. Gastric injury was determined by computerized planimetry. Gastric mucosa was harvested for determination of MMP

production by gelatin zymography and by Western analysis for MMP-2, MMP-9, MT1-MMP, TIMP-1 and TIMP-2. (n≥ 4/group; ANOVA) **Results:** Gastric injury after LPS administration was increased with L-NAME and decreased with AG. AG decreased LPS induced MT1-MMP and MMP-2 protein production while L-NAME had no effect. Both AG and L-NAME attenuated the LPS induced increase in TIMP-1 protein production but neither agent had any effect on TIMP-2 or MMP-9 protein levels. **Conclusions:** NOS inhibitors modulate gastric MMP and TIMP production after LPS administration. The ability of selective iNOS inhibition to ameliorate LPS induced gastric injury may be due in part to its inhibition of MMP-2 activation.

P79

PERITONEAL INFLAMMATORY REACTION AFTER SEPTIC AND NON SEPTIC ABDOMINAL SURGERY

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Objective: Bacterial peritonitis has a natural mortality rate of 80-100%. Progress in surgical techniques, new developments in intensive care medicine and antibiotic therapy reduced this rate to 30%. In this study we measured and compared the systemic and peritoneal inflammatory reaction in aseptic (control) and septic abdominal surgery treated with continuous peritoneal lavage (CPL). **Methods:** 20 pts (12? , 8? , mean age 50,4 yrs) with major abdominal surgery (gastric-=4, pancreas-=2, small bowel-=3, large bowel-: 7, gall bladder-resection= 4) and 20 pts(13? , 7? , mean age 65 yrs) with diffuse bacterial peritonitis operated and treated with continuous peritoneal lavage (CPL) participated in a pilot study. Blood sampling was performed every day and the peritoneal exudate was investigated until the drainage was removed. Endotoxin (Limulus Amebocyte Test), IL-6 (Elisa) and CRP (nephelometer), bacterial cultures as well as count and differentiation of leucocytes in the blood and peritoneal exudates were performed. **Results:** An uncomplicated clinical outcome was associated with lower levels of all inflammation markers. Endotoxin and IL-6 exudate levels were 10-200 x higher than their values in blood and increased postoperatively during elective abdominal surgery whereas levels were high (> 500x) in peritonitis and decreased continuously during an uneventful postoperative course with CPL. Polymorphonuclear leucocytes decreased in exudates, the rate of lymphcytes increased with a constant left blood shift in elective abdominal surgery. During CPL Endotoxin-, IL-6 plasma and exudate levels as well as positive bacteria cultures decreased but there was an increase of bacterial contamination in the drainage system after the 3rd postoperative day.

Conclusion: Even during elective surgery there is a severe peritoneal inflammatory reaction and seems to be more severe and prolonged than the systemic acute phase reaction. CPL helps to reduce it. Count and differentiation of peritoneal exudates are helpful to monitor the postoperative course and could be an indicator for local complications.

P80

THE EFFECTIVENESS OF ENDOTOXIN REMOVAL WITH POLYMYXIN B IMMOBILIZED FIBER IN THE SEPTIC SHOCK PATIENTS.

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Background and Objectives: Immobilized polymyxin B fibers (PMXs) which were developed to remove endotoxin selectively, have been available clinically in Japan. But there were no randomized controlled trials in clinical cases. Here, we evaluated effectiveness of PMXs in the septic shock patients.

Patients and Methods: A prospective, randomized, clinical trial of PMXs in the patients of septic shock caused by abdominal infection (alimentary tract perforation, anastomotic leakage, etc). After informed consent, patients were randomly assigned to a group with or without PMX at entry center. Primary endpoints were mortality from all causes at Day 28 after enrollment and at hospital discharge. Secondary endpoints were lengths of ICU and hospital stay and changes of hemodynamic state at Day 1 after enrollment.

Results: Total 17 patients (9 PMX treated, and 8 no PMX treated) were enrolled. The mortality at Day 28 was 2/9 in treated vs. 2/8 in no treated. The hospital mortality was 3/9 in treated vs. 6/8 (2 were death from cancer) in no treated. The lengths of ICU and hospital stay were not different (ICU: 11.9±17.0 days in treated vs.10.0 ±11.7 days in no treated; Hospital 71.0± 76.1 days in treated vs. 82.4± 74.6 days in no treated). Hemodynamic improvement at Day 1 was 5/9 in treated and 5/8 in no treated.

Conclusions: PMXs tended to reduce hospital mortality and may improve survival rate in the septic shock patients from abdominal infection. But more cases are needed to evaluate the efficacy of this new technique.

P81

HEAT STRESS INCREASES SUSCEPTIBILITY TO LIPOPOLISACCHARIDE IN MICE.

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Objective: Elevated blood concentration of lipopolysaccharide (LPS) and proinflammatory cytokines in patients with heat stroke has been reported. However, the effect of heat stress on host response to LPS is not clear. The purpose of this study is to elucidate the effects of heat stress upon the susceptibility to LPS toxicity.

Method: Various doses of LPS was intraperitonially (ip) administered immediately after one hour of heat stress (Ambient temperature of 42 degrees C, No restriction of water intake) in C3H/HeN mice (H group). Another group of mice had LPS ip

without heat stress (C group). After analyzing the cumulative mortality, LD_{50} was determined in both groups. Plasma concentrations of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were measured at 2, 6, 12, 24, 36 and 48 hours after LPS ip in C3H/HeN mice with or without heat stress (H or C group, respectively).

Fig. 1. Cumulative Mortality

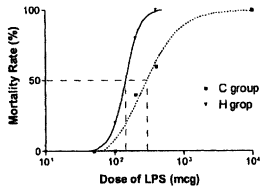
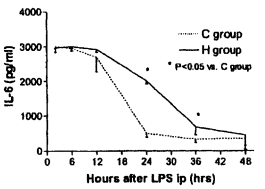


Fig. 2. Plasma IL-6 after LPS ip



Results: LD_{50} of LPS was 141 mcg in mice exposed to heat stress, whereas 258 mcg in control group (Fig. 1). There was no significant difference in plasma TNF-α between C group and H group. But plasma IL-6 was significantly elevated ($p < 0.05$, vs. C group) 24 and 36 hours after LPS administration in H group (Fig. 2).

Conclusion: Our data suggests that heat stress increases susceptibility to LPS toxicity and sustains the high level of plasma IL-6 in mice.

P82

CHOLINESTERASE INHIBITORS IMPROVE SURVIVAL IN MURINE ENDOTOXEMIA

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Cholinergic agonists, including acetylcholine and nicotine inhibit the release of TNF and other pro-inflammatory cytokines and suppress systemic inflammation through activation of peripheral, alpha7 nicotinic acetylcholine receptor-dependent mechanism (Nature, 2000, 420:853; Nature, 2003, 421:384; Nat. Med., 2004, 10(11): 1216). The goal of this study was to explore the efficacy of an alternative mechanism of cholinergic activation, inhibition of acetylcholine degradation by cholinesterase inhibitors, to suppress systemic inflammation. We tested the anti-inflammatory activities of galantamine, huperzine A and physostigmine in murine endotoxemia, a standard model of systemic inflammation. Single intraperitoneal (i.p.) injections of galantamine [0.1 - 4.0 mg/kg] 1h before endotoxin administration (6 mg/kg, i.p.) dose-dependently inhibited serum TNF levels in endotoxemic mice. Galantamine [4 mg/kg, i.p.] also exerted significant protection against lethality when injected once, either 1h (galantamine-treated survival=100%, vehicle-treated survival = 5%, n=20) or 6h (galantamine-treated survival=70%, vehicle-treated survival = 0%, n=20) before endotoxemia induction. Huperzine A [0.4 mg/kg, i.p.] administered 1h before endotoxin markedly prevented lethality (huperzine A-treated survival=70%, vehicle-treated survival=0%, n=20). Physostigmine [0.2 mg/kg, i.p.] injected 30 min before endotoxemia induction also exerted significant protection (physostigmine-treated survival=80%, vehicle-treated survival=5%, n=20). These results indicate that activation of cholinergic neurotransmission by cholinesterase inhibitors may be a novel approach for prevention and treatment of systemic inflammation. This study was funded in part by NIH/NIGMS and DARPA.

P83

POLYMORPHISMS OF HSP-70 (HSPA1B AND HSPA1L LOCI) DO NOT INFLUENCE RISK OF INFECTION OR OUTCOMES IN THE SURGICAL ICU

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Background: Heat shock proteins (HSP) are induced in various stress conditions and have many cytoprotective effects, including forming immune complexes, stabilizing intracellular proteins, and facilitating protein biosynthesis. The HSP-70 gene exhibits polymorphisms at the HSPA1B and HSPA1L loci which reportedly influence cytokine levels and clinical outcomes. These HSP variations also have been linked to TNF-β polymorphisms associated with poor outcomes. This study further evaluated the role of HSP polymorphisms in critically ill patients. Methods: 76 consecutive SIRS+ SICU patients were prospectively enrolled. Genomic DNA was isolated from whole blood samples. Specific fragments including the polymorphic sites were amplified by PCR, and restriction digestions were performed. Genotypes were determined by electrophoresis and confirmed by direct sequencing. Plasma cytokine levels were assayed in a subset of patients by ELISA. Results: HSP polymorphisms showed no significant relationship to infection rates and mortality. No organ specific dysfunctions were related to genotype (data not shown). No linkage of HSP genotype to TNF genotype could be demonstrated (data not shown). The HSPA1B AG polymorphism was associated with higher levels of TNF-α and IL-6 compared to the GG genotype.

	HSPA1L			HSPA1B		
	CT	TT	p	AG	GG	p
Infection rate (%)	50	42	NS[1]	47	29	NS[1]
D28 mortality (%)	9	16	NS[1]	10	14	NS[1]
Mean TNF-α (pg/ml)	115	232	NS[2]	101	10	.01[2]
Mean IL-6 (pg/ml)	52	826	NS[2]	390	77	.08[2]

[1] Yates corrected chi-square test [2] Student's t-test

Conclusions: These data suggest that polymorphisms of the HSPA1L or HSPA1B loci do not influence the predilection for infection or outcome in the surgical ICU.

P84

EFFECTS OF ENTEROCOCCUS ON INFLAMMATORY MEDIATORS IN A MOUSE PERITONITIS MODEL

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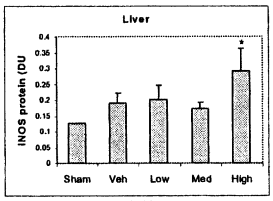
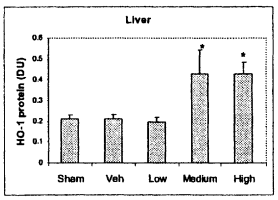
Objective: *Enterococcus faecalis* can be an important pathogen in intra-abdominal infections due to its associated

morbidity and incidence of antibiotic resistance. We previously utilized a mouse model of enterococcal peritonitis to demonstrate mortality; however, the effect of intra-abdominal infection with enterococci on the inflammatory response has not previously been assessed. This study characterizes a mouse peritonitis model and the effects of *E. faecalis* on early inflammatory mediators. **Methods:** Mice were intraperitoneally injected with saline (sham), 25% sterile rat fecal extract (SRFE) (Veh), or SRFE with *E. faecalis* at low (LD), medium (MD) or high (HD) dose ($2.5x10^8$, $5x10^8$, or $1x10^9$ CFU). Mice were sacrificed 5h after bacterial injection and serum and liver harvested. iNOS and HO-1 protein levels were measured via Western immunoblot and serum cytokines measured with a multiplex suspension immunoassay. Data are mean ± SEM, n=3; ANOVA *p<0.05. **Results**: HD was associated with a significant increase in iNOS levels in liver compared to sham. HO-1 was significantly increased in both MD and HD compared to sham, vehicle and LD. Pro-inflammatory IL-6 and anti-inflammatory IL-10 were significantly increased in HD enterococcus when compared to sham (not shown). **Conclusion**: Enterococcal peritonitis in a mouse model results in systemic inflammatory changes with increased pro and anti-inflammatory mediators and upregulation of hepatic iNOS and HO-1. Further studies evaluating therapies for intra-abdominal sepsis and enterococcal infections can be assessed using this model. Supported by NIGMS 08792.





P85

QUEST FOR MORTALITY PREDICTORS: PROFILING THE CYTOKINES DURING PROGRESSION OF SEPSIS.
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Failure to improve survival in septic patients using existing therapeutic approaches may be due to the multiple, complex immunological mechanisms underlying sepsis-related deaths. A critical imperative is identification of factors optimally reflecting the dynamic changes of inflammation during sepsis which accurately predict survival or mortality. Sepsis triggers dramatic production of inflammatory mediators within first three days of the disease. In previous work, IL-6 emerged as a promising outcome predictor in septic peritonitis. In this study we profiled the progression of sepsis. Outbred, adult female ICR mice (n=40) underwent cecal ligation and puncture (CLP) with an 18-gauge needle and were treated with imipenem and fluids every 12 h for 5 days. Body weight and mortality was followed for 2 weeks. Blood (20 µl) was obtained by a tail vein-clip after 6h and every 24 hours after surgery. A validated, sequential ELISA technology was developed allowing us to measure 18 total cytokines from this small volume. This permits determining multiple cytokine levels without sacrificing the mice to correlate levels with subsequent mortality. Three-day survival was 63% and 14-day survival was 50%. Cytokine plasma levels were analyzed to predict mortality within 18-48 hours prior to death. Several accurate predictors of mortality were identified: IL-6 at concentration >30 ng/mL predicted mortality with 99% specificity (SP) and 89% sensitivity (SN) and IL-1RA >35 ng/mL offered 98% SP with 93% SN. KC >37 ng/mL had 100% SP and 87% SN. MIP-2 level >23 ng/mL had 96% SP and was 80% sensitive. For IL-6R, a decrease rather than an increase correlated with mortality. IL-6R <1300 pg/mL offered 93% SP with 61% SN. TNF, TNF SR I and II were also significantly different although less accurate predictors, whereas IL-1B, eotaxin and IL-10 remained unrelated. These data indicate that selected cytokines can accurately predict CLP-induced mortality in heterogeneous mice model within 18-48h of death. Inflammatory markers accurately correlating with progression and outcome of sepsis will define the immunological status of a patient allowing for an individually tailored treatment.

P86

THE LUNG AS AN INFLAMMATORY ORGAN DURING LATE SEPSIS
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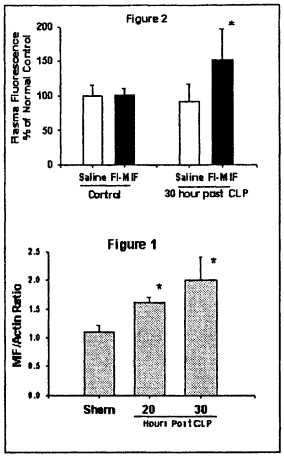
Acute lung injury during sepsis is common and the lung may influence the progression of sepsis by mechanisms other than poor gaseous exchange. Macrophage migration inhibitory factor (MIF), an important cytokine in sepsis and the acute respiratory distress syndrome, is a known cardiac depressant. Due to the intimate relationship between pulmonary blood flow and the heart, we hypothesized that cardiac dysfunction during sepsis is linked to the release of lung-associated MIF. Polymicrobial sepsis, induced by cecal ligation and puncture (CLP) caused a significant increase in total lung MIF at 30hr post CLP, to approximately double the level of the 20hr sham operated animals (Fig.1). Bronchoalveolar lavage fluids from the septic rats, also showed significant increases in MIF concentration over the same period (MIF at 20hr: 22±7.2; 30hr 45.4 ± 23.2 ng/ml; p=0.03). At 30hr post CLP, blood sampled simultaneously from the vena cava and carotid artery revealed that the mean concentration of MIF in the post-lung samples (135.1± 32.5ng/ml) was 25.1±16.1% higher than the pre-lung value (p=0.005). Labeled MIF (FL-MIF) instilled into the lungs at 30 hr post CLP was evident within the plasma 30 min later, although not in plasma of normal animals similarly treated (Fig.2). Intratracheally instilled MIF (2.0µg) induced increased alveolar KC, MIP2, and TNFα at 3hr, and plasma KC and MIP2, as well as increased MIF within the cardiomyocytes at 6hr post instillation. The data suggest that MIF released from the lung may be responsible, at least in part, for the cardiac dysfunction observed in the late stages of sepsis.



P87

SUPPRESSION OF HMGB1 RELEASE BY STEAROYL LYSOPHOSPHATIDYLCHOLINE: AN ADDITIONAL MECHANISM FOR ITS THERAPEUTIC EFFECTS IN EXPERIMENTAL SEPSIS.

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"Severe sepsis" refers to an overwhelming systemic inflammatory response to infection, but the underlying causes of its lethality are still unclear. Inefficient pathogen clearance by innate immune cells (e.g., macrophages, monocytes, and neutrophils) can lead to excess production of various pro-inflammatory cytokines (e.g., TNF, IL-1, and HMGB1). Recently, Yan et al. demonstrated that stearoyl lysophosphatidylcholine (LPC) is protective in experimental, lethal sepsis by stimulating neutrophils to destroy ingested bacteria in an H_2O_2-dependent mechanism (***Nature Medicine, 2004, 10: 161-167***). However, stearoyl LPC also confers protection against lethal endotoxemia, implying an additional, bactericidal-independent mechanism for its protective effects. **OBJECTIVES.** To gain further insight into its protective mechanisms, we evaluated the effect of LPC on the release of HMGB1, a newly identified "late" mediator of lethal sepsis. **METHODS.** *In vivo*, Balb/C mice were subjected to endotoxemia (*by intraperitoneal injection of lipopolysaccharide, LPS*) or sepsis (*by cecal ligation and puncture, CLP*), and the effects of LPC (20 mg/kg, i.p.) on circulating HMGB1 levels were evaluated. *In vitro*, thioglycollate-elicited murine peritoneal macrophages or human peripheral blood mononuclear cells (HuPBMCs) were stimulated with LPS (200 ng/ml) in the absence, or presence of LPC (1, 5 and 30 μM), and the levels of HMGB1 in the culture medium were determined. **RESULTS.** Repeated administration of stearoyl LPC, significantly attenuated circulating HMGB1 levels in animal models of endotoxemia and sepsis. Similarly, stearoyl LPC dose-dependently decreased LPS-induced HMGB1 release from macrophages and monocytes. **CONCLUSIONS.** Thus, stearoyl LPC confers protection against lethal experimental sepsis partly by facilitating the elimination of the invading pathogens, and partly by attenuating the excess accumulation of a "late" proinflammatory cytokine, HMGB1.

(This research was supported in part by the National Institutes of

P88

THE ROLE OF BID PROTEIN IN SEPSIS INDUCED APOPTOSIS.

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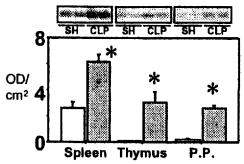
Programmed cell death (or apoptosis, Ao) can be induced by cell death receptor (extrinsic) or mitochondrial (intrinsic) pathway. Recent studies suggest that there is cross talk between these two pathways. In this respect, FasL/Fas-mediated Ao is induced via two signaling known as Fas type I and II pathways. In type I cells, upon receptor cross-linking caspase 8 is recruited to the DISC and activates down stream caspases culminating in Ao. Alternatively, in type II cells, Ao appears to be mediated through caspase 8 induced mitochondrial damage by activating BID, a cytoplasmic pro-apoptotic protein. BID is a specific substrate of and cleaved by caspase 8 into tBID, which is in turn translocated to mitochondrial membrane where it induces Ao. While much is known about the action of BID in various cell types in vitro, its role in lymphoid Ao seen in sepsis is unclear. Therefore, the aim of this study was to determine the contribution of BID protein to Ao during sepsis. To study this, BID deficient (BID-/-) and C57BL/6 (background, B6) mice were subjected to sham (SH) or cecal ligation & puncture (CLP). 24h post-surgery, thymus, spleen, lung, liver and Peyer's patches (P.P.) were harvested for tBID and/or cytochrome C detection by Western blot or fixed for Ao analyzed by histochemical and TUNEL staining. The results show that activated/cleaved BID



Apoptosis index[1]	Thy	Spl	P.P.	Lung	Liver
B6/SH	+/-	+/-	+/-	+/-	+/-
B6/CLP	+++	+++	+++	+	+
BID/SH	+/-	+/-	+/-	+/-	+/-
BID/CLP	+	+	+	+	+

* P<0.05, Student's t test, n=3-4.
1. Determined by TUNEL or active caspase 3 staining.

or tBID is markedly increased in mitochondria fractions of Thy, Spl and P.P. from septic B6 mice, which is correlated with increased cytochrome C levels in cytosolic fractions (suggesting mitochiondrial damage) and increased Ao seen during sepsis. Furthermore, BID-/- mice exhibit significantly reduced Ao in the Thy, Spl and P.P. but no change in the lung and liver compared with B6 mice after sepsis. Taken together, these data support not only the significant role of BID to sepsis induced lymphoid Ao, but the existence of a bridge between extrinsic apoptotic signals (e.g., FasL-Fas) and the intrinsic mitochondrial pathway via BID-tBID activation during sepsis. (NIH GM53209)

P89

INSULIN POTENTIATES LIPOPOLYSACCHARIDE (LPS)-INDUCED HYPOGLYCEMIA AND INFLAMMATORY RESPONSE

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Objectives: Controversy exists whether the beneficial effects of strict glycemic control in critically ill patients are secondary to the independent effects of insulin or glycemic control. The purpose of this study was to evaluate the effects of insulin on glucose and serum cytokine levels in rats receiving LPS. We hypothesized that insulin would blunt the inflammatory response to LPS. **Methods:** Rats were given subcutaneous saline or insulin (0.1-1U/kg) one hour before intraperitoneal saline or LPS (20 mg/kg). Rats were sacrificed 5 hours later and serum cytokine levels measured using a multiplexed suspension immunoassay. Serum glucose levels recorded at 1, 3, and 6 hours. Data are mean ± SEM (n≥3; ANOVA). **Results:** Higher doses of insulin (0.5 U/kg, 1.0 U/kg) but not low dose (0.1 U/kg) augmented LPS-induced changes in both pro-inflammatory (TNF-α, IL-1a, IL-1β, IL-6) and anti-inflammatory (IL-2 and IL-4) serum cytokine levels when compared to rats receiving LPS alone. In the absence of LPS, insulin had no effect on cytokine levels. Higher doses of insulin resulted in significantly lower glucose levels in rats receiving LPS as compared to rats receiving either LPS or insulin alone. Glucose levels were inversely correlated with levels of all of the above-measured pro- and anti-inflammatory cytokines. **Conclusion:** At higher doses, insulin potentiates both the hypoglycemic and the pro- and anti-

inflammatory response to endotoxemia. Caution should be used in using high doses of insulin to achieve glycemic control in septic patients until the inflammatory response can be better characterized. Further studies are necessary to determine whether these effects are independent of glycemic control and whether they persist over time. (Supported by NIGMS 08792).

P90

A2A RECEPTOR DEFICIENCY PREVENTS ORGAN DYSFUNCTION AND IMPROVES SURVIVAL IN SEPTIC SHOCK INDUCED BY CECAL LIGATION AND PUNCTURE. G. Haskó, B. Csóka*, C. Ledent*, E. Deitch, Z. Németh*. UMDNJ-New Jersey Medical School, Newark, NJ 07103 and Free University of Brussels Campus Erasme, Belgium.

Objective: Adenosine plays an important role in the modulation of immune responses via adenosine A2a receptors expressed on immune cells. High amounts of adenosine are released during tissue damage associated with sepsis. Thus, we hypothesized that A2a receptors may be involved in the regulation of immune function and organ damage in sepsis. **Methods:** Sepsis was induced in mice by cecal ligation and puncture (CLP). To determine the role of A2a receptors, we utilized A2a receptor knockout mice (KO) and their wild-type counterparts (WT), as well as CD-1 mice treated with the A2a receptor antagonist ZM241385. Survival after CLP was recorded for 7 days. For the detection of tissue damage, liver enzymes, blood urea nitrogen and histological changes were evaluated. The immunological status of animals was assessed by measuring cytokine levels, and bacterial and lymphocyte counts from blood and peritoneal lavage fluid. **Results:** A2a receptor KO mice showed an improved survival rate, which was associated with attenuated organ dysfunction. Bacterial counts in blood and peritoneal lavage fluid were lower in A2a KO mice than in their WT counterparts. Decreased levels of IL-10, IL-18 and MIP-2 were found in A2a KO mice as compared to WT animals, while IL-12 concentrations were comparable. Pharmacological blockade of A2a receptors decreased mortality, IL-10 and MIP-2 levels, while not affecting IL-12 concentrations. **Conclusion:** A2a receptor activation by endogenous adenosine contributes to mortality, and organ and immune dysfunction in sepsis induced by CLP. Thus, A2a receptor blockade may be therapeutically useful for the treatment of septic shock.

P91

HEMOCONCENTRATION AND INCREASED CYTOKINE PROFILES IN NON-RESUSCITATED SEPSIS. M. Law,* J. Siddiqui and D. Remick. U.Michigan, Ann Arbor, MI 48109)

Models of sepsis exist that utilize various resuscitation protocols. We investigated the effects of antibiotic treatment and fluid resuscitation on the immunopathology of the cecal ligation and puncture (CLP) murine sepsis model. Adult female mice were divided into two groups, resuscitation and impenim (RES, n=10), and non-resuscitation (NON, n=9). Mice were weighed daily, 20 µl of blood was obtained via tail snip at 6, 24, 48, 72, and 96 hours for analysis of immune cell profiles, while plasma was collected for cytokine screening by protein array. Survival rates between days 2.5 and 6 were much higher for RES (75%) than for the NON- (15%). The table shows cytokines (pg/ml) and blood cells that were significantly increased in the NON compared to the RES at 24 hours post CLP. Other measured cytokines were not significantly different. The major difference between the two groups was observed at the 24 hour time point. There were also increased numbers of peripheral blood neutrophils (NE), lymphocytes (LY) and red blood cells (RBC). A portion of the enhanced inflammatory response may represent simple hemoconcentration.

	RES	NON
TNFα	42	200
TNFSRII	1048	2109
MIP-2	629	4510
IL-2	140	554
IL-4	37	216
IL-18	142	367
NE x10^6	2.5	3.1
LY x10^6	2.0	2.7
RBC x K	10	30

These data show that the administration of fluids and antibiotics results in a decreased inflammatory response, with subsequent improvement in survival of the septic insult.

P92

EXPRESSION OF EXTRACELLULAR SUPEROXIDE DISMUTASE (EC-SOD) AND ITS PROTECTIVE ROLE DURING SYSTEMIC INFLAMMATION. J. Ueda, Jie Du, B.M. Evers and H. Saito (Sponsor: E.R. Sherwood). University of Texas Medical Branch, Galveston, TX 77555.

Objective: Extracellular superoxide dismutase (EC-SOD) may have a role to protect tissues from oxidative damages during systemic inflammation and sepsis. The present study was designed to understand the expression of EC-SOD in the lungs and to examine the role of this enzyme during systemic inflammation.

Methods: Systemic inflammation was induced in C57BL/6 mice (2 to 4-mo-old, male) by lipopolysaccharide (LPS, 20 mg/kg, i.p.) injection. Mice were sacrificed at various time-points and lung tissues were subjected to Western blot analysis on SOD expression. Oxidative damage was assessed by immunohistochemical analysis of 3-nitrotyrosine in the lung tissues. LPS-mediated lethality was compared in transgenic mice overexpressing human EC-SOD and wild-type control mice.

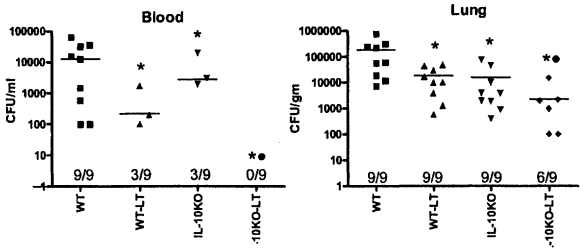
Results: Protein level of EC-SOD, but not other types of SOD (CuZn-SOD and Mn-SOD), was decreased significantly in the lungs during the LPS-mediated systemic inflammation. EC-SOD transgenic mice showed a significantly lower mortality rate compared to the wild-type control mice (12.5% vs. 70.6%, $p < 0.02$ by Mantel-Cox log-rank analysis).

Conclusion: These data suggest that EC-SOD has a protective role during systemic inflammation. Significant loss of EC-SOD in the lungs may be causally involved in elevated oxidative damage and mortality during systemic inflammation.

P93

RAPID CLEARANCE OF *PSEUDOMONAS AERUGINOSA* BY ENDOTOXIN (LPS) TOLERANT MICE. T. VARMA*, E. MURPHEY, C LIN*, AND E. SHERWOOD. University of Texas Medical Branch, Galveston, TX 77555-0591

Introduction: LPS-tolerance mimics many of the immunological alterations commonly present in critically ill patients including ↓IFNγ,↓IL-12 &↑IL-10. However, little is known about the ability of LPS tolerant mice to respond to infection with opportunistic pathogens such as *Pseudomonas aeruginosa*. **Material and Methods:** LPS tolerance was induced in female wild type (WT) and IL-10 knockout (IL-10KO) mice. Acute bacterial clearance and cytokine production were studied following challenge with *P. aeruginosa* (8 x 10^7 cfu, i.p.). **Results:** LPS-tolerance could be induced in wt and IL-10 KO mice as indicated by decreased production of pro-inflammatory cytokines such as TNFα, IL-6 and IL-1β. LPS-tolerant wt mice cleared bacteria from their blood and lungs more effectively (see figure) than control wt mice despite showing decreased production of IFNγ and IL-12 in blood and spleen. Control IL-10 KO mice produced increased amounts of IFNγ and IL-12 compared to wt mice and cleared bacteria more effectively. LPS-tolerant IL-10 KO mice were by far the most efficient in clearing *P. aeruginosa* from blood and lungs. This is despite producing IFNγ and IL-12 at levels that were markedly decreased compared to non-tolerant IL-10KO mice and comparable to levels observed in control wt mice. **Conclusions:** We observed that: 1) LPS-tolerance could be induced in wt and IL-10 KO mice; 2) LPS tolerance caused enhanced clearance of *P. aeruginosa* despite suppression of IFNγ and IL-12 production; 3) LPS tolerant IL-10KO mice exhibit a remarkable ability to clear bacteria after systemic challenge.



P94

***IN VIVO* DELIVERY OF FAS siRNA DIFFERENTIALLY REGULATES THE EXPRESSION OF FAS IN THE LIVER NON-PARENCHYMA AND SPLEEN FOLLOWING CLP.** D.E. Wesche*, B. Galen*, M.E. Garber*, C.S. Chung, and A. Ayala. Department of Surgery, Rhode Island Hospital and Brown University, Providence, RI 02903.

Clinical studies have shown the critically ill patients have a decrease in lymphocytes, partcularly T cells, which may impair their ability to fight off the lethal effects of sepsis. Experimental studies suggest that this dysregulated lymphocyte apoptosis may occur through stimulation with endotoxin or the Fas/FasL death receptor pathway druring sepsis. Prior studies from our lab have shown that T and B cells of the spleen and intestine undergo apoptosis via the Fas pathway, and not endotoxin. Studies that block the Fas pathway, using FasL -/- mice, Fas siRNA, or FasFP have shown an improvement in septic survival. The direct link between increased immune cell apoptosis and the development of multiple organ failure is unknown. Here, we hypothesize that liver failure is due to a loss of lymphocytes, which impairs the innate/adaptive immune cell cross-talk leading to uncontrolled infection. To examine this, the expression of Fas and FasL in the liver non-parenchymal population, I.e. endothelial cells, Kupffer cells, NK and NKT cells, CD4+ and CD8+ T cells. For comparative purposes, the spleen was also analyzed. Flow cytometric analysis revealed an increase in Fas and FasL expression in liver CD8+ T cells with CLP. FasL expression increased somewhat in other non-parenchymal cells of the liver and did not change in the spleen with CLP. There was, however, an increase in Fas receptor in the spleen. Lastly, *in vivo* hydrodynamic administration of Fas siRNA in mice decreased the levels of Fas on the liver CD8+ T cells and hepatocytes, but not Kupffer cells. While the mechanism of Fas siRNA uptake *in vivo* remains to be established, we show that apoptotic targets expressing Fas are present in the lymphoid and hepatocellular compartment of the liver and on lymphocytes of the spleen (NIH GM53209, GAANN P200A030100).

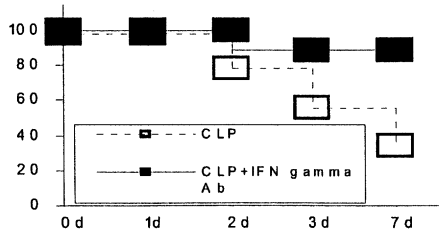
P95

INHIBITION OF IFN-γ ATTENUATES LETHALITY AFTER CLP: IMPLICATION OF HMGB1. K. Yin, E. Gribbin* and H. Wang. Dept. of Cell Biology, UMDNJ-SOM, Stratford, NJ 08084 and Northshore University Hospital, Manhasset, NY 11030.

We have previously shown that inhibition of IFN-γ decreased bacterial load by accelerating peritoneal fibrin deposition and tissue repair in the cecal ligation and puncture (CLP) model of peritonitis. Circulating inflammatory mediators such as IL-6 were also reduced by IFN-γ inhibition. In the present study, we show that administration of IFN-γ antibody (1.2 mg/kg, i.v.) attenuated mortality after CLP. Administration of this antibody was able to reduce mortality both when given immediately after CLP (n = 26 for both groups) or 24h after CLP surgery (n = 11 for both groups) compared to CLP rats given irrelevant antibody. Mortality in sepsis has been closely associated with increased release of High Mobility Group Box-1 (HMGB1). Furthermore, it has been reported that IFN-γ stimulates the release of HMGB1 from macrophages *in vitro*. Our studies showed that inhibition of IFN-γ activity (*in vivo*) reduced the levels of HMGB1 in peritoneal fluid, 24h after CLP as compared to CLP rats given irrelevant antibody. In addition, CLP rats which survived 4 days had very low levels of HMGB1 compared to rats which had died. These results suggest that the attenuation of mortality in IFN-γ

antibody treated rats was at least in part, mediated by a decrease in HMGB1 release.

Figure below shows increased survival of CLP rats given IFN-γ antibody 24h after CLP surgery.



P96

NOREPINEPHRINE POTENTIATES ENDOTOXIN-INDUCED PROINFLAMMATORY CYTOKINE RELEASE.

M. Zhou, W. Dong*, P. Das*, R. Wu and P. Wang. Division of Surgical Research, North Shore-Long Island Jewish Medical Center, Manhasset, NY 11030.

The sympathetic nervous system is activated in sepsis, resulting in an increased release of norepinephrine (NE). We have previously shown that gut-derived NE upregulates proinflammatory cytokines by activating α_{2A}-adrenoceptor (AR) on Kupffer cells (KCs). However, it remains unknown whether NE can potentiate endotoxin-induced cytokine release and organ injury in sepsis. To study this, NE (80μM) was infused intravenously into male rats (300g) for 2 h at a rate of 13μl/min. At 30 min after the initiation of NE infusion, LPS (7.5mg/kg) was injected intraperitoneally, and 4h thereafter plasma and peritoneal fluids were collected. In addition, an α_{2A}-AR antagonist, BRL 44408-maleate (BRL) was infused with NE+LPS to determine whether or not inhibition of α_{2A}-AR is beneficial. TNF-α (pg/ml x10^2) and IL-6 (pg/ml x10^3) levels were determined by ELISA and liver injury was assessed by ALT (IU/L) assay. In an additional experiment, KCs were isolated from normal rats and stimulated with either of the following: NE (20nM); LPS (100ng/ml); NE plus LPS; NE plus LPS with MAP kinase inhibitors, PD98059 (10 μM, inhibiting P44/42) or SB 203580 (10 μM, inhibiting P38). The plasma results (means±SE, n=4-6/group) are shown below.

	Vehicle	LPS	NE+LPS	BRL
TNF-α	0.17±0.05	1.64±0.42	24.84±8.13*#	1.87±0.08+
IL-6	0.11±0.02	9.78±1.16*	18.04±2.81*#	12.58±3.57*
ALT	38.4±1.6	63.1±14.5	165.4±39.2*#	72.3±9.0+

(ANOVA *P<0.05 vs. Vehicle; #P<0.05 vs. LPS; +P<0.05 vs. NE+LPS)

Similar results were also observed in peritoneal fluids. As shown above, NE significantly potentiated LPS-induced TNF-α, IL-6 release and ALT levels. Administration of the α_{2A}-AR antagonist, BRL, significantly attenuated the synergetic effect of NE on LPS-induced TNF-α production. Similarly, KC cultures showed that NE significantly enhanced LPS-induced TNF-α and IL-6 release, but such effects were attenuated by inhibiting P44/42 and P38 MAP kinases. In summary, our results indicate that NE significantly potentiates LPS-induced proinflammatory cytokine production and liver injury. The synergetic effect of NE and LPS appears to be mediated by α_{2A}-AR through MAP kinase signaling. Thus, specific α_{2A}-AR antagonists may be a novel and useful therapy for treating inflammatory conditions such as sepsis (NIH R01 GM053008).

P97

INVOLVEMENT OF ENDOPLASMIC RETICULAR (ER) STRESS AND CASPASE-12 ACTIVIATION IN LEUKOCYTE APOPTOSIS IN THE EARLY COURSE OF POLYMICROBIAL SEPSIS.

T.M. Rachel*, M. Perl, CS. Chung , YP. Chen*, A. Ayala; Department of Surgery, Rhode Island Hospital and Brown University, Providence, RI, 02903.

Apoptosis of leukocytes is suggested to be an important pathogenic factor in immune dysfunction following sepsis. Caspase-12 activation in the ER has been recently linked to oxidative stress induced programmed cell death. However, so far the role of caspase-12 in leukocyte apoptosis in sepsis remains unstudied. Thus, we tested the hypothesis that activation of the ER/caspase-12 cell death pathway contributes to the apoptotic processes seen in leukocytes following sepsis. Male C3H/HeN mice were subjected to cecal ligation and puncture or sham procedure. Splenocytes (SPL), thymocytes and peritoneal macrophages (PM) were isolated 4 hours later. Cell lysates were analyzed for active caspase-12 by fluorometric assay and results were confirmed by western blot. Incubation of SPL, PM and thymocytes with 0.25 μM of Thapsigargin for 4 hours displayed an increase of active caspase-12, indicating their susceptibility to oxidative stress mediated apoptosis. SPL and PM displayed a significant increase of activated caspase-12 4hrs following CLP when compared to sham animals. Active caspase-12 levels in thymocytes from septic animals did not differ from those of their sham counterparts. These findings indicate a cell type specific activation of the ER/caspase-12 pathway in splenocytes and peritoneal macrophages early in sepsis. However, the exact role of caspase-12 activation in the induction of leukocyte apoptosis under septic conditions remains to be elucidated. (NIH GM53209)

P98

GENDER DIMORPHISM IN α-AGONIST THERAPY AND AORTIC CYTOKINE EXPRESSION DURING SEPSIS

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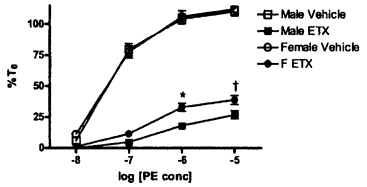
Objectives: Females have a lower incidence of multiple organ failure and better survival from sepsis. Gender disparities following acute injury may be related to differences in the inflammatory response. We hypothesized that females have better aortic responsiveness to phenylephrine (PE) following endotoxemia, and these improvements correlate with differences in aortic tissue cytokine expression. Methods: Thoracic aorta (n=12-16/group) were isolated from adult male and female Sprague-Dawley rats 6 hours after treatment with endotoxin (*Salmonella typhimurium* LPS, 20 mg/kg i.p.) or vehicle (1.0 cc saline i.p.). Arteries were suspended in perfused organ baths for measurement of

isometric force transduction, and contractile responses to KCl (80 mmol/L) and PE (0.01-10 μmol/L) were generated. Arteries were also assessed for expression of TNF-α, IL-1β, and iNOS mRNA (RT-PCR). Stats: two-way ANOVA or unpaired t-test ($P<0.05$ statistically significant). Results: Endotoxemia decreased maximal aortic contractility (T_0) in both males and females. However, septic females had increased responses to PE (Fig). Aortic expression of iNOS, TNF, and IL-1 were increased during endotoxemia with significantly greater increases in males than in females. Conclusions: Septic females have greater responses to phenylephrine therapy, which may be partly related to less upregulation of vascular tissue inflammatory cytokines. This suggests a role for gender-directed treatment of severe sepsis.



P99

ENDOTHELIN AND LPS EFFECTS ON REACTIVE OXYGEN AND NITRIC OXIDE PRODUCTION BY ENDOTHELIAL CELLS. D Gopalakrishna*, A Karaa*, SH Lee* and MG Clemens. Dept of Biology, UNC Charlotte, Charlotte NC 28223.

Objectives: It has been shown that microcirculation is hypersensitized to Endothelin1 (ET-1) following endotoxin (LPS) treatment leading to increased vasoconstriction. This may be related to decreased activation of endothelial nitric oxide synthase (eNOS) by ET-1. eNOS can also be uncoupled from NO production to produce superoxide (O_2^-). Production of reactive oxygen species (ROS) by eNOS would further contribute to the hyperconstriction and injury caused by ET-1 following LPS. We therefore tested whether ET-1 affects ROS production by vascular endothelial cells and whether this effect is altered by LPS. Method: Human Umbilical Vein Endothelial Cells (HUVEC) were subjected to a 6 hour LPS treatment (250ng/ml) followed by a 30 min with ET-1 (10nM). Conversion of tritiated L-Arginine to L-Citrulline was used to measure eNOS activity. Nitro blue tetrazolium (NBT) reduction to insoluble formazan was used to estimate ROS. Results: Stimulation of HUVECs with ET-1 increased NO synthesis by 1.4 fold. LPS pretreatment alone had no significant effect on basal NO production; however ET-1 stimulation of LPS treated HUVECs failed to increase NO production. Western blot for eNOS protein showed no decrease in eNOS protein levels. Stimulation of control HUVECs with ET-1 tended to decrease ROS, but this decrease was not significant. LPS alone resulted in a 2.6 fold increase in ROS production ($p<0.05$) that was not significantly affected by ET-1 stimulation. Conclusion: These results demonstrate LPS produces a significant impairment in ET-1 induced NO production by eNOS but not eNOS protein levels. This is associated with an increase in ROS production. ET-1 tended to decrease ROS in control cells but not in LPS-treated HUVECs. Thus increased ROS in the absence of adequate NO production may contribute to vascular dysregulation following inflammatory stress. Supported by DK38201

P100

GW9662 INHIBITION OF PPARγ ABROGATES THE PROTECTIVE FUNCTION OF ENTERAL GLUTAMINE. N Sato*, MA Childs*, FA Moore, L Zou*, BC Kone*, S Schultz*, RA Kozar. University of Texas-Houston, Houston, TX 77401

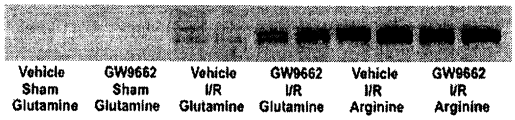
Enteral glutamine (glut) and arginine (arg) differentially modulate the molecular events regulating gut injury and inflammation following gut ischemia/reperfusion (I/R). We have demonstrated that glut was protective via induction of the anti-inflammatory mediator PPARγ while arg was harmful via induction of the pro-inflammatory mediator iNOS. We now hypothesize that GW9662, a specific inhibitor of PPARγ, would abrogate the protective effects of enteral glut. *Methods*: Rats received IV GW9662(1mg/kg) or vehicle 30 min prior to laparotomy. Jejunal sacs were filled with 60 mM glut or arg followed by 60 min of superior mesenteric artery occlusion and 6 hrs of R. Jejunum was harvested for histology (mucosal injury, Chiu score 0-5), myeloperoxidase (MPO, index of gut inflammation) and iNOS protein. *Results*: Injury and inflammation were

	Injury	Inflammation	iNOS
Groups	**Chiu Score**	**MPO**	**Protein**
Sham glut	0.8 ± 0.3 a	1.3 ± 0.2 a	31 ± 2 a
Sham glut+blocker	0.6 ± 0.2 a	1.3 ± 0.2 a	29 ± 1 a
IR glut	1.8 ± 0.2 a	2.3 ± 0.3 b	72 ± 4 b
IR glut + blocker	3.1 ± 0.2 b	4.6 ± 0.9 c	118 ±10 c
IR arg	4.0 ± 0.4 b	9.0 ±1.2 d	213 ± 8 d
IR arg + blocker	3.8 ± 0.3 b	9.3 ± 0.6 d	209 ± 12d

**Means with different letters are significantly different, ANOVA*

significantly increased by GW9662 in I/R glut + blocker animals and correlated with a significant increase in iNOS expression. GW9662 had no effect on arg-induced injury, inflammation, or iNOS. *Conclusion*: GW9662, a specific inhibitor of PPARγ, abrogates the protective effect of enteral glut resulting in gut injury and inflammation and is associated with increased expression of iNOS. Induction of PPARγ represents a novel mechanism for enteral glutamine protection during gut I/R.

Representative immunoblot of iNOS protein expression:

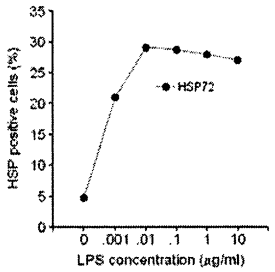


P101

LPS AND FORMYL PEPTIDES INDUCE HEAT SHOCK PROTEIN 72 EXPRESSION ON THE SURFACE OF HUMAN NEUTROPHILS (PMN)

N. Hashiguchi,* M. Hirsh, Y. Chen,* L. Yip,* D.B. Hoyt, and W.G. Junger. University of California San Diego, Department of Surgery, Division of Trauma, San Diego, CA, 92103-8236.

PMN are central to the pathogenesis of organ dysfunction in trauma patients by causing tissue damage. Effective clearance of inflamed PMN reduces the risk of organ failure. Recently, we have shown that heat shock protein 72 (HSP72) on the surface of PMN contributes to PMN clearance by recruiting γδT cells that recognize HSP72. Here we studied the mechanisms that lead to HSP72 expression on the surface of human PMN. The gram-negative bacterial product lipopolysaccharide (LPS) elicited robust HSP72 expression on the cell surface of human PMN. HSP72 expression was dose-dependent and reached highest levels at LPS concentrations of 10 ng/ml (Fig). Stimulation of PMN with LPS in combination with the bacterial peptide fMLP doubled HSP72 expression. The expression of HSP72 on the cell surface of PMN from trauma patients was elevated in patients without severe complications, while PMN of patients with complications such as sepsis lacked surface HSP72 expression. These findings suggest that HSP72 could be essential for the protection of patients from post-traumatic complications such as sepsis and organ failure. Thus, LPS-induced HSP72 expression may be an important signal that allows γδT cells to protect host organs like the lungs by clearing them from inflamed PMN.



Supported by Grants GM51477, GM-60475 from NIH and a linkage grant from NATO.

P102

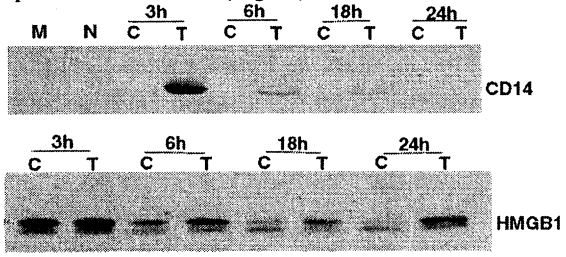
HMGB1 IS UPREGULATED AS PART OF THE ACUTE PHASE RESPONSE IN THE LIVER.

M. Scott, H. Liao*, T. Billiar. Department of Surgery, University of Pittsburgh, Pittsburgh, PA 15213.

Introduction: Remote tissue injury stimulates the hepatic acute phase response (APR) characterized by the production of acute phase proteins such as serum amyloid A (SAA) and lipopolysaccharide binding protein (LBP) by the liver. HMGB1 is an early inflammatory danger signal and we hypothesized that induction of the hepatic APR would also upregulate expression of HMGB1.

Methods: C57BL/6 mice were injected in the left hind limb with 0.2mL turpentine (T) or saline (control, C). Serum and liver were collected at 0, 3, 6, 18, and 24h after injection. SAA and LBP levels were determined by ELISA. Western blotting determined relative levels of CD14 in serum and HMGB1 in the cytosolic fraction of whole liver lysates.

Results: Induction of the hepatic APR by remote tissue injury was confirmed by 100-fold increases in SAA levels and a 3-fold increase in LBP levels compared to no increases in control-treated animals. Soluble CD14 is produced as part of the inflammatory response in the liver. Levels were strongly increased by 3h after turpentine injection and were still detected until 18h (Figure)(M=mol.wt. marker, N=normal unmanipulated mouse). At all time points measured HMGB1 levels were increased in liver cytosol of mice injected with turpentine compared with controls (Figure).



Conclusion: Early upregulation of HMGB1 and soluble CD14 may serve to enhance the systemic immune response as part of the acute phase response in the liver.

P103

FIBRONECTIN DIFFERENTIALLY MODULATES MMP-9 PRODUCTION.

M.A. Rahat*, B. Marom*, N. Lahat*, H. Bitterman. Carmel Medical Center, Faculty of Medicine, Technion, Haifa 34362, Israel.

OBJECTIVE: The extracellular matrix (ECM) is degraded by the inflammatory microenvironment, and in turn the degraded products may affect the behavior of the local inflammatory monocytes/macrophages (MØ). In this study, the effect of the ECM protein fibronectin (FN) on MØ secretion of matrix metalloproteinase 9 (MMP-9), which enables their migration, was delineated. METHODS: MØ (the monocytic cell line U937) were incubated on a plastic substrate, or on plastic coated with either native FN or FN fragmented by commercial MMP-9, with or without the addition of TNFα. RESULTS: Native and fragmented FN were constitutively secreted by the MØ, although in minute amounts, and addition of TNFα both induced MMP-9 and increased FN fragmentation. Native and fragmented FN oppositely affected MMP-9 secretion: without TNFα native FN enhanced MMP-9 by 50% ($p<0.001$) and fragmented FN was antagonistic, while in the presence of TNFα native FN inhibited MMP-9 secretion by 2-fold ($p<0.01$) and fragmented FN acted antagonistically. The high levels of the integrin FN receptors α4β1 and α5β1were not altered, but were activated by TNFα and mediated the effects of both native and fragmented FN. In the absence of TNFα, native FN-elevated MMP-9 secretion was mediated by JNK and ERK1/2, and TNFα-induced MMP-9 secretion was mediated by JNK on all matrices. In addition, in the

presence of TNFα ERK1/2 inhibited native FN signaling while Rho kinase mediated the effects of the fragmented FN. CONCLUSIONS: The migration of MØ through inflammatory tissues is regulated in part by the opposite effects of native and fragmented ECM proteins, where accumulated degraded products accelerate migration.

P104

IL-6 INHIBITS THE INDUCTION OF IGF-I BY GH IN CWSV-1 HEPATOCYTES

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GH stimulates IGF-I and serine protease inhibitor 1 (Spi 2.1) expression via the JAK/STAT signaling pathway. Sepsis results in hepatic "GH resistance" as evidenced by the reduction in plasma IGF-I despite a 2-4 fold increase in GH. This study examines the effects of IL-6 on GH-inducible gene expression (IGF-I, Spi 2.1), GH receptor (GHR), and GH signaling. CWSV1 cells ± IL-6 (10 ng/ml, 24h) were incubated with rhGH (500 ng/ml) for 24 h. IGF-I and Spi 2.1 mRNA were measured by Northern blot and normalized to 18s mRNA. Data are means ± SE for test groups, *p<0.05, ‡p=0.001 vs. Control + GH by ANOVA, Turkey-Kramer test.

Group	IGF-I mRNA	Spi 2.1 mRNA
Control	0.30 ± 0.02	0.48 ± 0.07
IL-6	0.37 ± 0.04	0.41 ± 0.06
Cont. +GH	1.10 ± 0.08	1.37 ± 0.14
IL-6 + GH	0.78* ± 0.07	0.72‡ ± 0.09

IL-6 inhibits the induction of IGF-I and Spi2.1 mRNA by GH. Next, CWSV1 cells ± IL-6 (10 ng/ml, 24h) were harvested at 5, 10, 15, 30, 60, 90 and 120 min after GH stimulation. Total and phosphorylated GHR, JAK2, STAT5, and ERK1/2 were measured by immunoprecipitation and immunoblot analysis. IL-6 had no effect on the relative abundance of GHR or signaling proteins. GH stimulated the phosphorylation (2- to 5- fold) of GHR, STAT5, and ERK1/2. However, the time course of GHR, JAK2, and ERK1/2 phosphorylation were not altered by IL-6. IL-6 attenuated the GH induced phosphorylation of STAT5 only at a single time point (90 min). We conclude that IL-6 inhibits the induction of IGF-I and Spi2.1 mRNA by GH, without influencing the abundance or phosphorylation state of GHR or the associated proteins of the JAK/STAT or MAP kinase signaling pathways. An inhibitory effect of IL-6 on IGF-I mRNA synthesis or degradation following GH stimulation represents a potential mechanism for this observation.

(Supported by GM-55639, RNC; T32-GM64332 TAA)

P105

TREATMENT OF CD8⁺T/NK CELL-DEFICIENT MICE WITH IMIPENUM IMPROVES RESISTANCE TO CLP-INDUCED INJURY. E. R. Sherwood, V. T. Enoh* and C.Y. Lin*. Univ Texas Medical Branch, Galveston, TX 77555-0591

Introduction: CD8 knockout mice treated with anti-asialoGM1 (CD8KO/αAsGM1 mice) exhibit 40-50% survival after CLP compared to 100% mortality in wild type mice. We hypothesized that treatment of CD8KO/αAsGM1 mice with antibiotics would further enhance survival. **Methods:** Wild type C57BL/6J (WT) and CD8KO/αAsGM1 mice were exposed to CLP (1 cm cecum ligated/20 g needle puncture) and treated with saline or imipenum (25 mg/kg) IP every 6 hours. Survival, bacterial burden, temperature, acid-base balance and pro-inflammatory cytokine expression were measured. **Results:** Saline-treated WT and CD8KO/αAsGM1 mice had $>10^5$ bacterial CFU/ml in blood and peritoneal fluid at 18 hours after CLP while those treated with imipenum showed >99% decreases in bacterial counts. All WT mice treated with saline or imipenum died by 30 hours after CLP and exhibited significant ($p<0.05$) hypothermia, metabolic acidosis and increased plasma levels of IL-6, KC and MIP-2 compared to CD8KO/αAsGM1 mice. Long-term survival was significantly ($p<0.05$) improved in CD8KO/αAsGM1 mice treated with saline (40%) or imipenum (100%) compared to WT mice. Comparison of saline- and imipenum-treated CD8KO/αAsGM1 mice at 36 hours post-CLP showed that bacterial CFU were not detectable in blood or peritoneal fluid from mice treated with imipenum but saline-treated mice had $>10^5$ CFU/ml in both compartments and exhibited significant ($p<0.05$) hypothermia, metabolic acidosis and increased serum levels of IL-6 and MIP-2 compared to imipenum-treated mice. **Conclusions:** Imipenum treatment markedly decreased bacterial burden but did not improve survival in wild type mice. Therefore, mortality in WT mice appears unrelated to bacterial dissemination and may be due to gut ischemia. CD8KO/αAsGM1 mice exhibited a significant improvement in survival after imipenum treatment. CD8KO/αAsGM1 mice appear resistant to gut ischemia but exhibit delayed mortality caused by bacterial dissemination if not treated with antibiotics.

P106

MICE DEFICIENT IN CD8⁺ T AND NK CELLS ARE RESISTANT TO HEMODYNAMIC DETERIORATION FOLLOWING CECAL LIGATION AND PUNCTURE. W. Tao, V.T. Enoh*, C. Lin* and E. Sherwood. University of Texas Medical Branch, Galveston, TX 77555-0591

Introduction: The present study was designed to assess hemodynamics and organ dysfunction in wild type (WT) and CD8 knockout/anti-asialoGM1-treated (CD8KO/αAsGM1) mice after CLP. **Methods:** Arterial pressure was measured by carotid artery cannulation and left ventricular pressure-volume loops were obtained by insertion of a 1.4F conductance catheter into the left ventricle. Parameters of global perfusion as well as hepatic, renal and pulmonary function were also measured. **Results:** CD8KO/αAsGM1 mice exhibited significantly ($p<0.05$) higher mean arterial pressure, systemic vascular resistance and cardiac output compared to wild type mice after CLP (see table). The CLP-induced hemodynamic derangements in wild type mice were associated with severe metabolic acidosis and elevated serum creatinine levels. Myocardial function was better preserved in CD8KO/αAsGM1 mice as indicated by significantly ($p<0.05$) improved left ventricular pressure development over time, time varying maximum elastance, end systolic pressure volume relationship and preload recruitable stroke work. The impairment in

myocardial function was associated with induction of TNFα, IL-1 and IL-6 mRNAs in the hearts of wild type mice, which were markedly attenuated in CD8KO/αAsGM1 mice. **Conclusions:** This study shows that cardiovascular collapse and systemic hypoperfusion occurs after CLP in wild type, but not CD8KO/αAsGM1 mice. The improvement in hemodynamics in CD8KO/αAsGM1 mice is associated with an attenuated pro-inflammatory response.

Parameter	Sham	WT	CD8KO/αAsGM1
MAP (mmHg)	86 ± 4	51 ± 5*	82 ± 4**
CO (ml/min)	7.2 ± 1.0	5.1 ± 0.4*	5.9 ± 0.7
SVR (mmHg/ml/min)	12.2 ± 1.4	7.9 ± 0.6*	14.1 ± 1.6**
dp/dt/EDV (mmhg/sec/µl)	1049 ± 223	287 ± 23*	662 ± 78**
ESPVR (mmHg/µl)	19.5 ± 2.3	4.9 ± 0.4**	9.4 ± 1.1**
PRSW (mmHg/µl)	112 ± 21	24 ± 3*	75 ± 9**

P107

PRO-INFLAMMATORY ALTERATIONS IN THE LUNG CELL ADHESION MOLECULE NETWORK DUE TO SHOCK WAVE-INDUCED INJURY

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Hemorrhagic pulmonary contusion induced by exposure to shock wave (SW) is associated with pulmonary oxidative stress, pulmonary edema, and increase in capillary permeability for the inflammatory leukocytes (LKC). Recent observations suggest that redox signaling regulates barrier functions of the endothelium (Edt) adhesion junctions and increase LKC/Edt interaction in inflammatory events. In this report we examined interplay of endothelial cadherin (VE-Cdh), intercellular adhesion molecules (ICAM-1), and CD11b adhesion molecules during LKC sequestration in the injured lung. Lung injury was induced by exposure of animals to shock wave (SW) with peak overpressure at 122±8 kPa. SW produces immediate pulmonary trauma associated with alveolar influx of blood. Sections of inflammatory lesions (IL) were assessed with histology, 3D-immunofluorescence (IMF) image analysis, and immunoblotting for ICAM-1 and CD11b adhesion molecules, myeloperoxidase (MPO), and VE-Cdh proteins. IMF imaging of MPO and CD11b in phagocytes and VE-Cdh in Edt reveal that transmigration of inflammatory cells in IL occurred during 3-24 hrs postexposure. MPO and 3-nitrotyrosines (3NTyr) showed close spatial depositions in IL suggesting a presence of oxidative alterations in alveolar septa. Spatial distribution of IMF for 3NTyr was inversely correlated with that one of VE-Cdh in IL (Pearson, r=0.31). Accumulation of ICAM-1 protein in IL and ICAM-1 expression on capillary Edt occurred in the same time frame. Spatial IMF of ICAM-1 was inversely correlated with IMF of VE-Cdh in IL. IMF of CD11b in LKC in IL was highest at the membrane protrusions of the leading edges and correlated with IMF of ICAM-1 in Edt. These observations are consistent with hypothesis that extracellular MPO released from LKC promotes redox signaling in the alveolar Edt and thus, can affect cell adhesion molecule network.

P108

HMGB1 INTERACTS WITH BOTH TLR2 AND TLR4

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University of Colorado Health Sciences Center, CO 80262

High mobility group box 1(HMGB1), originally described as a DNA-binding protein, can also be released extracellularly and functions as a late mediator of inflammatory responses. Although recent reports indicated that the receptor for advanced glycation endproducts (RAGE), as well as Toll-like receptors (TLR2 and TLR4), were involved in cellular activation by HMGB1, there has been little evidence of direct association between HMGB1 and these receptors. We hypothesize that HMGB1 is bound by a heteromeric receptor composed of both TLR2-TLR4. **Methods**: We used fluorescence resonance energy transfer (FRET) and immunoprecipitation to directly investigate cell surface interactions of HMGB1 with TLR2, TLR4, and RAGE. **Results**: In non-permeabilized RAW 264.7 macrophages FRET (intensity and area) between HMGB1 and TLR2 or TLR4 transiently increased from zero to maximum between 5-15 minutes, demonstrating association of HMGB1 with TLR2 and TLR4, but not RAGE. Co-immunoprecipitation also found interaction between HMGB1 and TLR2 (max at 5-15 mins) as well as TLR4 (max at 0-5, decreasing after 15 mins), but not with RAGE. Total cell contents of both TLR2 and TLR4 began to decrease after HMGB1 stimulation. **Conclusions**: These studies provide the first direct evidence that HMGB1 can interact with both TLR2 and TLR4, and also supply an explanation for the ability of HMGB1 to induce cellular activation and generate inflammatory responses that are similar to those initiated by LPS. The time course of co-immunoprecipitation (total cell contents) of TLR2 and TLR4 with HMGBP1 suggests that each of these receptors may associate with HMGB1 separately. Further analysis with triple FRETs and biochemistry may help resolve the stoichiometry of the binding receptor.

P109

HMGB1 IMPAIRS ENTEROCYTE MIGRATION BY DISRUPTING THE ACTIN CYTOSKELETON AND INHIBITING RHO-GTPASE.

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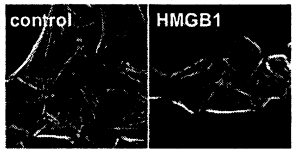
Background: Necrotizing enterocolitis (NEC) is characterized by persistent mucosal defects and systemic sepsis. We have shown that experimental NEC is associated with increased expression of HMGB1 in the intestinal mucosa and decreased migration of enterocytes along the crypt-villus axis. Enterocyte migration requires the formation of actin stress fibers and focal adhesions through the activity of Rho-GTPase, which is increased by vav3, and decreased by p190 Rho-GAP. We thus hypothesize that HMGB1 impairs enterocyte migration by disrupting the actin cytoskeleton through inhibition of Rho-GTPase activity. **Methods:** Enterocyte migration was measured

as the ability of IEC-6 cells to move into a scraped wound. Stress fibers were assessed by confocal microscopy. Rho-GTPase activity was determined in a pull down assay using rhotekin-glutathione beads. Focal adhesion kinase (FAK), vav3 and p190RhoGAP expression were assessed by SDS-PAGE. **Results:** HMGB1 caused a dose dependent inhibition of enterocyte migration (Ctrl 300±50, HMGB1 100ng/ml 80±30, 500ng/ml 35±20, 1μg/ml 75±35, $p<0.05$ ANOVA (μm/h)) *in vitro*, a disruption of actin stress fibers (Figure) and a dose-dependent inhibition of Rho-GTPase activity. There was no effect of HMGB1 on the expression or phosphorylation of FAK or vav3. Strikingly however, HMGB1 significantly increased p190 expression, leading to an increase in GTP displacement and accounting for the observed decrease in Rho-GTPase activity and stress fibers. **Conclusion:** HMGB1 inhibits enterocyte migration through disruption of the cytoskeleton by activation of p190GAP and inhibition of Rho. These findings provide insights into the mechanism[s] by which HMGB1 could lead to impaired intestinal restitution in the pathogenesis of necrotizing enterocolitis.



P110

AGED STORED BLOOD EFFECT ON ENDOTHELIAL CELL BARRIER FUNCTION.

H. Smith*, J. Janowski*, J. Reichner*, W. Biffl, Rhode Island Hospital/Brown University, Providence RI 02903.

Transfusion of aged stored red blood cells (RBCs) is associated with hyperinflammatory acute lung injury (ALI). The neutrophil (PMN) is implicated as the primary effecter cell in ALI. Plasma from aged stored RBCs has a direct pro-inflammatory effect on PMNs, however, the direct effect on endothelial cells is less defined. We hypothesize that stored blood also has a direct effect on endothelial cells, decreasing EC barrier function. **METHODS**: Human lung microvascular ECs were grown to confluence on gold-plated electrode arrays. Electrical resistance of the monolayer, an index of EC barrier function, was measured for 20 hours in real time using an electric cell-substrate impedance sensor system (ECIS). Plasma fractions from fresh RBCs (Day 1), stored RBCs (Day 42), leukocyte-reduced stored RBCs (Day 42 LR), medium, or thrombin were added, with or without fresh PMNs. Reported values are % change of resistance normalized to initial baseline resistance. **RESULTS**: ECs show an initial decrease in resistance in the presence of human plasma with variable recovery (Fig. 1), an effect that is more pronounced

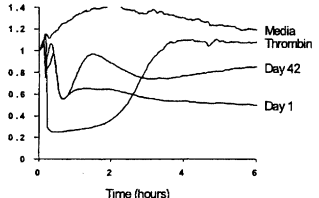


Fig. 1 Stored Plasma on EC

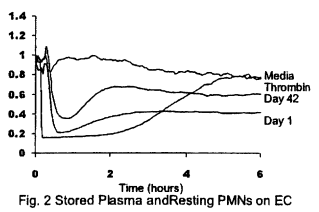


Fig. 2 Stored Plasma andResting PMNs on EC

in combination with resting PMNS (Fig. 2). Electrical resistance with Day 42, but not Day 1, rebounded to near baseline levels. Day 42 LR showed an intermediate response (data not shown).

CONCLUSIONS: Fresh, but not stored, RBC plasma significantly decreases endothelial resistance. This unforeseen finding is expected to be related to enhanced adhesion molecule expression, cytoskeleton rearrangement, or inflammatory mediator release and requires further study.

P111

GRAM POSITIVE AND GRAM NEGATIVE MURINE PNEUMONIA INDUCE DISTINCT CHANGES IN LYMPHOCYTE APOPTOSIS AND NEUTROPHIL MIGRATION.

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Background: Gram positive and gram negative bacteria initiate the host inflammatory response via different mechanisms. We sought to determine how this affects the host white cell distribution. **Methods:** FVB/N mice were intratracheally injected with *Pseudomonas aeruginosa* or *Streptococcus pneumoniae*, both with 90% 8d mortality. Mice (n=4-9/group) were sacrificed at 12h and 24h, and blood was taken for complete blood count with manual differential. Lymphocyte apoptosis was analyzed by flow cytometry for active caspase 3. Lungs from each group were stained for myeloperoxidase. Groups were compared to unmanipulated mice (UM) by ANOVA. **Results:** The WBC counts were decreased in both models, but differentials were strikingly disparate. Flow cytometry in mice given pseudomonas showed increased lymphocyte apoptosis (57% active caspase 3+) compared to pneumococcus (24%) and UM mice (4%, $p<.01$ for all comparisons). Lung myeloperoxidase staining showed extensive infiltration of neutrophils in pseudomonas pneumonia. In contrast, lungs from mice given pneumococcus had only mild neutrophilic infiltration. **Conclusions:** Although both have similar mortalities and WBC counts, pneumococcus pneumonia is characterized by a predominance of neutrophils while pseudomonas pneumonia, despite having twice as much lymphocyte apoptosis, has a predominance of lymphocytes. This can be explained by massive neutrophil migration to the lung in pseudomonas pneumonia.

	UM	Pneumo 12h	Pneumo 24h	Pseudo 12h	Pseudo 24
WBC	5.0	3.0	1.9*	2.3*	1.6*
%Neutro	14.5	54.4*	56.8*	10.3	7.0
%Lympho	81.5	42.4*	41.6*	84.8	88.0

*p<.001 vs. UM

P112

INDUCTION OF CARDIOVASCULAR CHANGES IN CHRONIC INFLAMMATION MODEL

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Epidemiological and clinical evidence indicate inflammatory processes play a pivotal role in a number of conditions

associated with aging, including osteoporosis and cardiovascular disease. Previously we have reported that chronic administration of endotoxin induces bone loss, especially within trabecular-rich regions of the skeleton. The *purpose* of this study was to investigate the cardiovascular changes induced by chronic endotoxemia. Time-release pellets designed to deliver either 0, 3.3, or 33.3 μg of LPS/d for 90 days, were implanted subcutaneously in the dorsal region of the neck in 3-month old male Sprague-Dawley rats (n=24). At the end of the study, the hearts were harvested and placed in formalin for histological and immunohistochemical study. Trichrome staining demonstrated that both groups receiving LPS had increased fibrous tissue and roughened borders within the arteriole intima, consistent with small vessel disease. Animals receiving LPS also had increased staining for troponin, suggestive of myocardial damage. The number of mast cells present in the myocardium tended (p=0.06) to be increased in the animals receiving the higher dose of LPS. Inflammatory mediators, cyclooxygenase (COX)-2, tumor necrosis factor (TNF)-α, and interleukin (IL-1)β, were up-regulated in the heart in both LPS groups compared to controls. Based on these findings, we conclude that inflammation-induced changes associated with coronary artery disease occurred, lending support to the utility of this model for future mechanistic studies of the role of chronic inflammation in osteoporosis and atherosclerosis.

P113

THROMBIN-ACTIVATABLE CARBOXYPEPTIDASE ACTIVITY IS A SIGNIFICANT SOURCE OF C5a REGULATION SECRETED BY INFECTED ALVEOLAR MACROPHAGES. S. Price*, I. Ben-David*, J. Weinberg*, G. Stempfle*, L. Bird*, L. D'Alecy*, S Whitesall*, and J. Younger. Dept. of Emergency Medicine, Univ. of Michigan.

Secreted basic carboxypeptidases (CP) include CP-N and CP-R (the latter secreted as pro-CP-R which is activated by thrombin) and are important regulators of C5a, bradykinin, and fibrin function during inflammation. CP-N has been regarded as the CP responsible for deactivating C5a to C5a-*des arg*. We examined constitutive and inducible CP activity in the MH-S murine alveolar macrophage (Mϕ) cell line. Supernatants from Mϕ infected for 4 hours with *Klebsiella pneumoniae* were assayed for CP activity, defined as the rate of cleavage of the dipeptide hippuryl-arginine (μmol/min) as shown below:

	No Thrombin	Thrombin	P value
Uninfected	0.94 ± 0.63	3.63±0.29	n.s.
Infected	0.73 ± 0.27	4.85±0.51	< 0.01
P value	<0.01	<0.01	

To confirm that hip-arg proteolytic activity was biologically relevant, thrombin-treated supernatants from infected cells were incubated with the mC5a C-terminal peptide PVQLGR from which arginine cleavage was independently quantified by HPLC. RTPCR of normal mouse lung confirmed the presence of pro-CP-R and CP-N transcripts. Resting Mϕ express constitutive CP activity that can be increased ~3.8-fold by thrombin. Acute Gram-negative infection increases thrombin-inducible CP activity to ~6.6 fold constitutive levels. These data suggest that, depending on thrombin availability at the site of injury, CP-R represents a major potential reservoir for C5a-regulation during Gram-negative bacterial infection.

P114

TLR4 SIGNALING MODULATES TISSUE AND CELLULAR INFLAMMATORY RESPONSE TO ROS
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Reactive oxygen species (ROS) are involved in LPS-induced Toll-like receptor 4 (TLR4) signaling. While ROS contribute to tissue inflammatory response to ischemia reperfusion injury, the role of TLR4 in ROS-induced inflammatory response is unclear. Using isolated hearts and primary coronary artery endothelial cells, we tested the hypothesis that TLR4 modulates inflammatory factor expression following hydrogen peroxide stimulation. **Methods and results**: Hearts isolated from TLR4 defective (C3H/HeJ) and control (C3H/HeN) mice were perfused with Krebs-Henseleit buffer. Hearts were stimulated with hydrogen peroxide (0.25 mM/L, for 30 min followed by 1 h buffer perfusion), and TNF and MIP-2 in myocardial homogenate were analyzed by ELISA. Hydrogen peroxide stimulation induced myocardial production of TNF and MIP-2. However, hearts isolated from C3H/HeJ mice had significantly lower levels of these two inflammatory factors in comparison to hearts isolated from C3H/HeN mice. To examine whether TLR4 is involved in endothelial inflammatory response to ROS, we treated human coronary artery endothelial cells (HCAEC) in culture with hydrogen peroxide (0.25 mM/L) for 4 h in the presence or absence of TLR4-blocking antibody (10 μg/mL). TNF and IL-8 levels in culture medium were elevated following hydrogen peroxide stimulation. TLR4-blocking antibody attenuated cellular inflammatory response. To examine whether hydrogen peroxide and LPS induce a similar gene program, we extracted RNA from HCAEC following a 1 h treatment with hydrogen peroxide or LPS (200 μg/ml) and analyzed gene expression with microarray. While hydrogen peroxide and LPS upregulated a group of common genes, including multiple proinflammatory genes, hydrogen peroxide induced the expression of numerous distinct genes. **Conclusion:** TLR4 signaling modulates the production of inflammatory factors by the myocardium and HCAEC in response to hydrogen peroxide. This innate immunity receptor appears to play an important role in the inflammatory response to ROS.

P115

HYPOXIA REDUCES THE OUTPUT OF MATRIX METALLOPROTEINASE-9 (MMP-9) IN MONOCYTES BY INHIBITING ITS SECRETION AND INCREASING ITS MEMBRANAL ASSOCIATION. H. Bitterman, M.A. Rahat*, B. Marom*, L.W. Cerem, N. Lahat*. Carmel Medical Center. Faculty of Medicine, Technion, Haifa 34362, Israel

Cellular hypoxia, which characterizes ischemia, tissue injury, and inflammation, recruits monocytes and macrophages,

immobilizes them at the hypoxic site and alters their function. To migrate across the basement membrane and the extracellular matrix (ECM), and as part of their inflammatory functions, monocytes and macrophages secrete proteases, including matrix metalloproteinase-9 (MMP-9), whose expression is induced by pro-inflammatory cytokines (e.g. TNFα). OBJECTIVE: In this study we evaluated the yet unknown effects of hypoxia on the secretion of monocyte MMP-9. RESULTS: exposure to hypoxia (<0.3% O_2 for 48 hours) reduced the levels of secreted TNFα-induced proMMP-9 by 3-fold ($p<0.01$) in both the monocytic cell line U937 and in primary human monocytes. Addition of TNFα induced MMP-9 transcription, but no change in MMP-9 mRNA steady-state was observed between normoxia and hypoxia. Hypoxia inhibited the trafficking of proMMP-9 via the secretory vesicles and increased the intracellular accumulation of proMMP-9 by 47% and 62% compared to normoxia ($p<0.05$), as evaluated by zymography of cellular extracts or by confocal microscopy, respectively. Secretion of proMMP-9 was inhibited by the addition of cytochalazin B or nocodazole, which inhibit the polymerization of actin and tubulin fibers, or the addition of the Rho kinase inhibitor Y27632, suggesting the involvement of the cytoskeleton and the Rho GTPases in the process of the enzyme secretion. Furthermore, attachment of proMMP-9 to the cell membrane increased after hypoxia via its interactions with the surface molecules CD44 and RECK.
CONCLUSIONS: Hypoxia reduces the secreted amounts of proMMP-9 post-translationally by utilizing two mutually nonexclusive mechanisms, mostly inhibition of cellular trafficking and, to a lesser extent, attachment to the cellular membrane.

P116

DIFFERENT MECHANISMS MEDIATE SYNERGISTIC PRODUCTION OF TNF-α AND NITRIC OXIDE FROM MOUSE MACROPHAGES IN RESPONSE TO BACTERIAL LPS AND PEPTIDOGLYCAN. X. Tan, J. Shen*, C. J. Papasian*, D. C. Morrison*, N. Qureshi and J. J. Gao* Shock/Trauma Research Center, Department of Basic Medical Sciences, University of Missouri, Kansas City, MO 64108 LPS and peptidoglycan (PepG), two important bacterial cell wall components that possess potent immune-stimulating capacity, have been shown to synergize with each other in inducing *in vivo* production of TNF-α and nitric oxide (NO), two key mediators of septic shock. However, the molecular mechanisms responsible for the synergistic induction of these two mediators are not clearly understood. In this study, we have demonstrated that LPS and PepG cause synergistic induction of TNF-α and NO via different mechanisms using *in vitro* cultured RAW264.7 macrophages. While LPS and PepG synergistically induce TNF-α protein production, the mRNA levels of TNF-α was not enhanced synergistically. However, the half-life of TNF-α mRNA is significantly longer in macrophages treated with both LPS and PepG compared to those in macrophages treated with LPS or PepG individually. Contrary to TNF-α, synergistic induction of NO by LPS and PepG was associated with proportionately elevated mRNA levels of inducible nitric oxide synthase *(iNOS),* an enzyme that catalyzes the production of NO. These results suggest that the synergistic induction of TNF-α by LPS + PepG is most likely controlled at the post-transcriptional level, whereas NO induction is controlled, at least partially, at the transcription level of the *iNOS* gene. In addition to the above findings, our data also indicate that treatment of RAW 264.7 macrophages with LPS and PepG did not elevate expression of the receptors for these two agents (TLR4 and TLR2, respectively), suggesting this synergy between them is not due to the alteration of their cellular surface receptor numbers. (*Supported by Grant AI54962*)

P117

EXTRACELLULAR ADENOSINE AUGMENTS MACROPHAGE IL-10 PRODUCTION BY A POST-TRANSCRIPTIONAL MECHANISM. Z. Nemeth*, C. Lutz, B. Csoka*, E. Deitch, G. Hasko. UMDNJ-New Jersey Medical School, Newark, NJ 07103

Objective: Extracellular levels of the endogenous purine mediator adenosine are increased in patients with sepsis. These high concentrations of adenosine may contribute to the macrophage dysfunction observed in sepsis, because adenosine induces a macrophage phenotype resembling that found in septic patients. For example, both septic macrophages and macrophages exposed to adenosine exhibit an increased production of IL-10. The intracellular mechanisms of how adenosine augments IL-10 production are not known. **Methods:** RAW 264.7 macrophages were stimulated to produce IL-10 by LPS in the presence or absence of adenosine. IL-10 protein levels were measured by ELISA and IL-10 mRNA was analyzed using real-time PCR. IL-10 promoter activity and the function of IL-10 mRNA 3'-untranslated region (3'-UTR) were assessed by transfection with luciferase constructs and luciferase assay. Wild-type and mutant RNA probes corresponding to portions of the IL-10 3'-UTR were produced by in vitro transcription and translation. Binding of proteins to these RNA probes was studied using gel-shift experiments or by UV-crosslinking. **Results:** Adenosine increased IL-10 production by RAW cells without affecting IL-10 promoter activity and IL-10 mRNA levels, indicating a translational effect. This translational effect appeared to involve the 3'-UTR of the IL-10 mRNA, because adenosine increased luciferase activity in cells transfected with constructs containing the IL-10 3'-UTR downstream from the luciferase gene. Adenosine enhanced binding of proteins to the IL-10 3'-UTR, which was dependent on a region containing the GUAUUUAUU nonamer. **Conclusion:** Extracellular adenosine augments IL-10 production by macrophages via a post-transcriptional mechanism.

P118

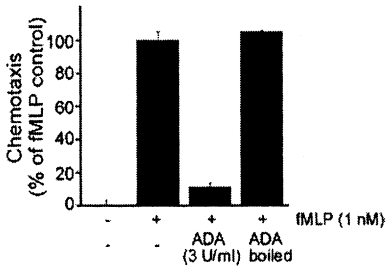
THE PROTEASOME REGULATES THE CpG-DNA INDUCED INFLAMMATORY RESPONESE J. Shen*, J. Gao*, G. Zhang, X. Tan, D. C. Morrison*, C. Papasian* and N. Qureshi Shock/Trauma Research Center, Department of Basic Medical Sciences, School of Medicine, University of Missouri-Kansas City, MO 64108 We have previously shown that

proteasome, a giant proteasome, is a central regulator of the inflammatory signal transduction triggered by LPS. The objective of the present study was to identify whether the proteasome also regulates CpG-DNA (bacterial DNA), induced inflammatory signal transduction. We sought to determine the effects of lactacystin, a well –established proteasome inhibitor, on the CpG-DNA-induced macrophage tumor necrosis factor-a (TNF-α) secretion and expression of multiple genes such as TNF-α; interleukin-1β; and inducible nitric-oxide synthase. Our data indicated that lactacystin blocks the CpG-DNA-induced cytokine production and expression of all of inflammation related genes selected in our panel. In addition, Lactacystin dysregulated CpG-DNA triggered mitogen-activated protein kinase phosphorylation in macrophages. Thus, these data suggest that proteasome pathway also plays an important role in regulating CpG-DNA induced signal transduction in inflammatory mediator cells and may, therefore be an important potential therapeutic target in the treatment of bacterial sepsis. Supported by GM50870 (N.Q.) and AI054962 (N.Q.)

P119

NEUTROPHIL (PMN) CHEMOTAXIS REQUIRES ATP RELEASE AND P2 AND A3 RECEPTOR ACTIVATION
Y. Chen*, R. Corriden*, N. Hashiguchi*, L. Yip*, P.A. Insel*, and W.G. Junger. Univ. of California San Diego, Dept. of Surgery/Trauma and Pharmacology, San Diego, Ca 92103-8236

Excessive PMN activation and extravasation cause host tissue injury and organ damage following sepsis, burns, and trauma. The bacterial peptide fMLP is one of the most powerful chemoattractants that guide PMN to sources of infection and inflammation. Here we studied the underlying mechanisms of PMN chemotaxis. HPLC analysis revealed that fMLP stimulation of PMN induces ATP release into the extracellular space, reaching 350 nM within 3 min. Exogenous ATP increased chemotaxis through P2 receptor activation, while apyrase, an enzyme that digests ATP, decreased chemotaxis. Released ATP was converted to adenosine, to a concentration of ~ 40 nM within 3 min after fMLP stimulation. Adenosine deaminase (ADA), an enzyme that hydrolyzes adenosine blocked chemotaxis, an effect that was lost by boiling ADA (Fig). Fluorescent microscopy revealed that the A3 member of adenosine receptors relocates to the leading edge of migrating PMN. Inhibition of A3 receptors with MRS-1191 reduced chemotaxis, while inhibition of the A1 or A2 adenosine receptors had no effect. We conclude that ATP release, its conversion to adenosine, and P2 and A3 receptor activation appear to be important components of PMN chemotaxis towards fMLP and perhaps other chemotactic agents. Supported by NIH Grants GM51477, GM-60475, and HL69785.



P120

PI3-KINASE/AKT PATHWAY ACTIVATION ACTS AS A SWITCH FOR C5A-INDUCED EFFECTS ON NEUTROPHIL IL-8 GENERATION DURING INFLAMMATION.
N.Riedemann, N. Tabriz*, T. Barkhausen*, M. Van Griensven, D. Kendoff*, H. Pape, P. Ward, C. Krettek*. Medizinische Hochschule Hannover, 30625 Hannover, Germany

Objective: We recently demonstrated that Pi3-kinase activation is involved in C5a-induced MIF generation in neutrophils. We now set to investigate the effects of C5a on Pi3-kinase/AKT-pathway activation in human neutrophils and its effects on pro-inflammatory mediator generation. Methods: Freshly isolated human neutrophils were treated with various concentrations of C5a, LPS and C5a+LPS in the absence and presents of the potent Pi3-kinase inhibitor LY294002. Phosphorylation of various kinases of the AKT pathway in neutrophils were detected by Western blot and cell supernatants were analyzed with flow cytometric bead assay for generation of various mediators. Results: C5a activated the Pi3-kinase/AKT pathway in neutrophils via phosphorylation of PDK1, AKT and GSK3ß during the first 5-15 minutes of incubation. The negative regulator of Pi3-kinase activation, PTEN, was not activated by C5a. In the absence of Pi3-kinase inhibitor C5a resulted in strong reduction of LPS-induced IL-8 generation. Under conditions where the Pi3-kinase/AKT pathway was blocked, C5a strongly enhanced IL-8 generation as well as LPS-induced IL-8 generation in neutrophils.
Conclusions: Contact of neutrophils with C5a results in strong inhibition of LPS-induced IL-8 generation. We demonstrate for the first time that this C5a-induced impairment of an important neutrophil innate immune function is mediated by fast activation of the Pi3-kinase/AKT signaling pathway, which controls inhibitory versus stimulatory effects of C5a.

P121

APOPTOSIS AND BCL-2 PROTEIN FAMILY EXPRESSION IN NEUTROPHILS DURING EXPERIMENTAL HUMAN ENDOTOXEMIA AND CLINICAL SEPSIS. J Kotani*, E Lin*, .N Avallone*, M Goshima*, S Calvano, S Lowry. Dept of Surgery, UMDNJ-Robert Wood Johnson Medical School. New Brunswick, NJ 08901. Introduction: Decreased neutrophil (PMN) apoptosis (Ao) is implicated in persistent inflammation. It was hypothesized that modulation of Bcl-2 protein family members and Ao-transducing receptors regulate spontaneous and Fas-mediated Ao in PMNs during human endotoxemia and clinical sepsis. Methods: Blood from 10 subjects who received IV endotoxin (LPS) was collected over 24 hrs. Blood from 8 patients with clinical sepsis was obtained on the day of confirmed infection. Purified PMNs were cultured for 6 hrs with or without the Fas agonist (500 ng/mL). Ao was measured by propidium iodide staining using flow cytometry (FC). Cell surface Fas and TNFR, cytoplasmic Mcl-1, Bcl-2 and Bax expression in

Parameter changes in human endotoxemia and sepsis

Time after LPS i.v. (hrs)	*0*	*2*	*24*	*SEPSIS (Day 1)*
PMN Ap (%) Spontaneous	10 ± 2	3 ± 0	8 ± 3	4 ± 0.5
Fas-mediated	24 ± 2	10 ± 3 *	18 ± 4	14 ± 3 #
PMN protein expression (times of vs 12.5µg BJAB)				
Mcl-1	1.5 ± 1.1	0.2 ± 0.2 *	4.7 ± 2.4 *	1.1 ± 0.6
Bax	0.2 ± 0.1	1.2 ± 0.5 *	1.6 ± 0.9 *	3.3 ± 0.6 *
Receptor expression (MCF)				
Fas	147 ± 15 ♀	122 ± 9 ♀	242 ± 30 *	178 ± 23
TNF receptor	29 ± 0	24 ± 0 *	30 ± 0	29 ± 3

*The values of Mcl-1 and Bax expression were normalized against that in 12.5µg BJAB cells. N.D., not detected; MCF = mean channel fluorescence ± SEM; ANOVA and Newman-Keuls' test, *p<0.05 with respect to 0 hr in human endotoxemia: ♀r=0.59, p=0.02 with respect to changes in Fas-mediated apoptosis in human endotoxemia; #p=0.02, response to CH11 between normal PMN and septic PMN.*

freshly isolated PMNs were assessed with FC or western blotting. Results: Compared to baseline, spontaneous and Fas-induced Ao were reduced at 2 hrs after LPS as was cell-surface Fas and TNFR expression. Paradoxically, at this same time point, anti-apoptotic Mcl-1 protein was decreased while pro-apoptotic Bax protein was increased. By 24 hrs, PMN Ao, Mcl-1, Bax, Fas, and TNFR, had returned to normal or supra-normal levels. Compared to controls (0 hr LPS subjects), septic patients also exhibited inhibition of Ao despite normal Mcl-1 and receptors, and increased Bax expression. Conclusions: Decreased expression of Ao-transducing receptors may contribute to reduced PMN Ao during experimental endotoxemia, but not during sepsis. While changes in Mcl-1 and Bax expression are detected in endotoxemia and sepsis, PMN Ao is more likely to be modulated by other factors.

P122

LEUKOCYTE DYNAMICS MEASURED BY ORTHOGONAL POLARIZATION SPECTRAL IMAGING CORRELATES WITH VIDEO MICROSCOPY

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Objective: Venular leukocyte rolling and adhesion is a pivotal inciting event in inflammation. Intravital video microscopy (IVM) has been utilized to study dynamic interactions between leukocytes and the endothelium in animal models, but is of limited use in the clinical setting. Therefore, we aimed to validate the novel non-invasive method of orthogonal polarization spectral (OPS) imaging against the well established technique of IVM for investigation of leukocyte dynamics.
Method: Nine C57B1/6 mice (20-25g) underwent carotid cannulation, pretreatment with cromolyn sodium (5 mg/kg) to prevent mast cell degranulation, and cremaster dissection for *in vivo* microscopy. Bright field microscopy was used to visualize leukocytes. The preparation was placed on an upright microscope stage and the image was displayed via CCD. OPS images were acquired by laying a 10x probe directly over the muscle at the same location so as to image the same vessel. Single, unbranched post-capillary venules were selected for study. The number of rolling leukocytes were determined off-line and expressed as the number of cells moving past a designated point in five-minute intervals.
Results: Mean values of leukocytes per five minute interval as visualized by video microscopy and OPS were 167 $\pm$ 136 and 112 $\pm$ 70, respectively. Their correlation was significant ($r = 0.83$, $p = 0.006$).
Conclusion: OPS imaging can be done sublingually providing a non-invasive method of studying leukocyte dynamics that correlates well with IVM. Up until now, microcirculatory alterations in inflammatory conditions such as sepsis have been difficult to analyze in humans. OPS imaging makes it possible to investigate human microvascular beds in the context of research, diagnosis, and therapy. Its use at the patient's bedside to visualize and quantify leukocytes in post-capillary venules, may provide important diagnostic and prognostic information in inflammatory conditions such as sepsis.

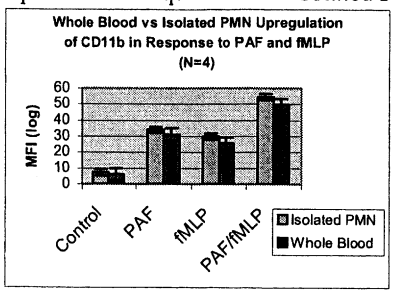
P123

CD11B AND CD62L EXPRESSION IN ISOLATED NEUTROPHILS IS COMPARABLE TO MEASUREMENT IN WHOLE BLOOD.

P. Eckels*, E. Moore, J. Johnson. UCHSC/Denver Health, Denver, CO 80262.

Background: PMNs stimulated with fMLP and/or PAF increase surface expression of CD11b while concomitantly shedding L-selectin. However, changes in surface markers due to isolation methods have been a pitfall in determining these markers of priming and activation of human PMNs in the clinical setting.

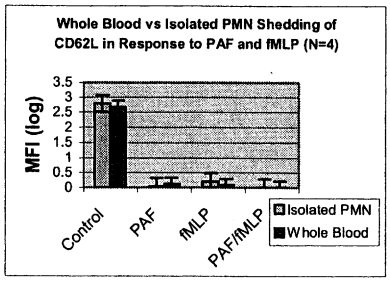
Hypothesis: Responses in CD11b and CD62L surface expression are equivalent in isolated PMNs and whole blood.



Methods: Blood collected from 8 healthy donors was divided into isolated PMNs or whole-blood for analysis. PMNs were isolated by Ficoll gradient and hypotonic lysis. Cells were either incubated with PAF or fMLP both at 1μM concentrations. Cells were stained for surface markers and fixed after 10 minutes of stimulation. Isotypes were measured and subtracted from the analysis via flow cytometry.

Results: Isolated CD11b expression was found to highly mimic the behavior of whole-blood polymorphonuclear leukocytes and their values ($p<0.05$). L-selectin appeared to be shed in both techniques to nearly the same magnitude.

Conclusion: PMN isolation techniques compare favorably to whole-blood methods in the case of surface markers CD11b and CD62L. This finding may also extend to other functional markers.



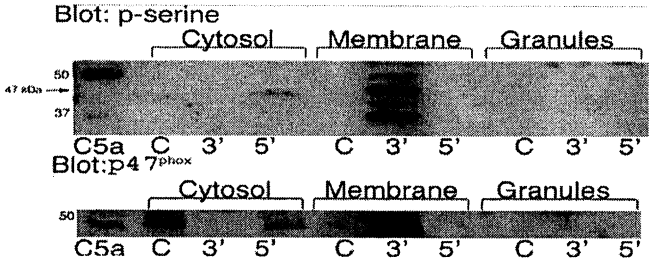
P124

COMPLEMENT PRIMING TRANSLOCATES P47 PHOX TO THE NEUTROPHIL PLASMA MEMBRANE.

L. Gries*, E. Moore, M. Kelher*, N. McLaughlin*, C. Silliman, Denver Health Medical Center and the University of Colorado, Denver, CO 80262

Background: Neutrophil (PMN) priming augments release of superoxide anion upon activation of the NADPH oxidase. An evolving concept is that different primers uniquely phosphorylate and/or translocate cytosolic components of the oxidase. LPS induces phosphorylation and translocation of $p47^{phox}$; conversely, TNF-α induces phosphorylation of both $p47^{phox}$ and $p67^{phox}$. C5a, an important mediator of the early inflammatory response, primes the oxidase at physiologic concentrations; however its effects on $p47^{phox}$ and $p67^{phox}$ remain

unclear. We hypothesized that priming with C5a will induce phosphorylation and translocation of $p47^{phox}$ to the plasma membrane. Methods: Isolated human PMNs were stimulated with C5a (10^{-10} M) for 3 minutes. Cells were then sonicated and separated into cytosol, membrane, and granule fractions. Immunoblots probed for $p47^{phox}$ and phosphoserine were performed on each subcellular fraction. Results: Priming of PMNs with C5a for 3 minutes resulted in phosphorylation and translocation of $p47^{phox}$ from the cytosol to the plasma membrane.

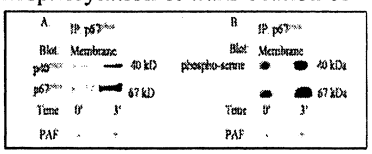


Conclusion: Priming with C5a provokes both phosphorylation and translocation of the $p47^{phox}$ subunit of the NADPH oxidase, demonstrating an effect similar to LPS priming of the oxidase. Determining the variations in NADPH oxidase assembly for different primers may facilitate selective downregulation of PMN functional responses.

P125

PAF PRIMING OF THE PMN OXIDASE INDUCES PHOSPHORYLATION & $p40^{phox}$-DEPENDANT MEMBRANE TRANSLOCATION OF $p67^{phox}$. F. Sheppard, E. Moore, N. McLaughlin*, C.Silliman

Priming of the PMN NADPH oxidase augments superoxide generation in response to a subsequent, activating, stimulus. Priming agents, such as endotoxin and interleukin-18, translocate the cytosolic oxidase subunit $p47^{phox}$ to the PMN plasma membrane, therefore we hypothesized that the rapid priming agent PAF elicits similar translocation of cytosolic oxidase subunit(s). **Methods:** Isolated, human PMNs were stimulated with buffer or 2 μM PAF for 3 minutes at 37°C and then fixed or separated into sub-cellular fractions. Sub-cellular fractions underwent immuno-precipitation, protein separation & immunoblot for *phox* proteins. Digital microscopy of intact PMNs utilizing acceptor-photobleaching fluorescent resonant energy transfer (FRET) analysis was performed to determine *phox* translocation & colocalization. A liposomal delivery system for introduction of antibodies into the cell was used for neutralization of the oxidase components. A $p67^{phox}$ deficient cell-free oxidase assay was utilized in membrane add back experiments to further determine membrane $p67^{phox}$ content. **Results:** PAF induced rapid phosphorylation & translocation of $p67^{phox}$ and $p40^{phox}$ to & co-localization at the membrane (*Fig a&b*). The intracellular neutralization of $p40^{phox}$, but not $p47^{phox}$, inhibited PAF-mediated $p67^{phox}$ translocation. *(FRET data not shown)* Add back of PAF primed membrane in the cell-free oxidase assay generated significantly more superoxide than add back of resting membrane ($p<0.05$). **Conclusion:** PAF PMN priming phosphorylates, translocates and co-localizes $p67^{phox}$ & $p40^{phox}$ to the membrane position without concomitant $p47^{phox}$ translocation. This is distinct from that previously reported for endotoxin & IL-18, and depends on functional $p40^{phox}$. These results indicate mechanistic differences between priming agents with respect to effect on oxidase subunits.



P126

MITOCHONDRIAL CYTOCHROME C IS RELEASED IN THE SEPTIC HEART LEADING TO MYOCARDIAL APOPTOSIS.

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Cardiac dysfunction occurs in septic patients. Cardiomyocyte apoptosis can lead to myocardial depression. Here we test the hypothesis that apoptosis occurs in the septic heart via the mitochondrial pathway. Under anesthesia, C57Bl6 mice underwent sham operation (S), single puncture cecal ligation and puncture (CLP, mild survivable sepsis), or double puncture CLP (2CLP, severe lethal sepsis). Mice were sacrificed at 0, 6, 16, 24, or 48 hours. Ventricular mitochondria and cytosol were isolated by differential centrifugation. Protein immunoblotting for cytochrome c was performed. DNA nicking was detected by TUNEL procedure. N=3 per group per time point. Statistical significance was determined with ANOVA and post hoc Tukey's test. Steady state mitochondrial cytochrome c levels decreased 24 and 48 hours post 2CLP and S and 48 hours post CLP ($p<0.05$). Levels of cytosolic cytochrome c decreased 16 and 24 hours post 2CLP and CLP and 16 hours following S. Levels returned to baseline 24 hours post S. At 48 hours, when mitochondrial cytochrome c levels were maximally decreased post 2CLP and persistently decreased post CLP, cytosolic levels increased dramatically following 2CLP and less so post CLP ($p<0.05$). TUNEL-positive cells increased by 5 fold 48 hours post 2CLP. Decreases in mitochondrial cytochrome c following S and CLP probably reflect temporally earlier decreases in cytosolic levels. Although cytosolic levels return to baseline following S, decreased levels persist post CLP. Decreases in mitochondrial cytochrome c with concomitant increases in the cytosol 48 hours following 2CLP indicate release into the cytosol. This is supported by the presence of TUNEL-positive cells 48 hours post 2CLP. Thus, in severe sepsis, mitochondrial release of cytochrome c occurs and is associated with myocardial apoptosis.

P127

INCREASED PERCENTAGE OF CIRCULATING CD133+ PROGENITOR CELLS IN MULTIPLE TRAUMA. D.Henrich*, Z. Shiaian*, S. Thomas*, K. Wilhelm*, I.Marzi. Dept. of trauma surgery, J.W. Goethe-University, 60590 Frankfurt, Germany

Objective: Ischemia in various tissues takes place as a direct result of multiple trauma (MT). Bone marrow-derived

endothelial progenitor cells (EPCs) may provide a therapeutic option for revascularisation of injured tissues. In recent investigations we found that sera obtained from patients with MT exert a strong effect on the EPC differentiation *in vitro*. But it is still an open question whether or not the number of EPCs in the circulation of patients after multiple trauma is also affected. Thus, here we investigated the percentage of EPCs in the circulation of patients after MT using flowcytometry.

Methods: EDTA blood was obtained from MT patients on the second day after admission, from healthy volunteers, and for determination of the influence of anaesthesia and surgical trauma, from patients undergoing elective surgery. Usually, EPC express the progenitor cell antigenes CD133 and CD34 simultanously. The percentage of CD133+ and CD34+/CD133+ cells was assessed by flowcytometry. The results were presented as mean +/- SEM. A p value < 0.05 was significant (Wilcoxon-test). The study was approved by the local ethics commitee.

Results, Table: Progenitor cell phenotypes in circulation [%].

groups	CD133+	CD34+/CD133+
multiple trauma (n=23)	0.45 +/- 0.03 *	0.07 +/- 0.01 $
elective surgery (n=9)	0.12 +/- 0.01	0.03 +/- 0.01
healthy volunteers (n=10)	0.13 +/- 0.01	0.06 +/- 0.01 $

*=p<0.05 vs surgery and volunteers; $=p<0.05 vs surgery.

Conclusion: The present study shows that the percentage of peripheral CD133+ cells is depressed through surgical trauma and is increased by multiple trauma. During differentiation EPC lose stem cell markers (CD34), thus, CD133+ cells might represent EPC in a more advanced state of differentiation. The percentage of EPC in multiple trauma could be increased by either an enhanced release of cells from the bone marrow to the circulation or an enhanced proliferation of EPC which is mediated by distinct humoral mediators.

P128

CD163, A MACROPHAGE SCAVENGER RECEPTOR AND CD95, THE APOPTOSIS RECEPTOR ARE EARLY TRAUMA RESPONSIVE SURFACE MARKERS

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Trauma-induced activation of innate immunity was expected to induce immediate early events in surface marker profiles of circulating leukocytes. Methods: Blood samples of 50 patients were taken before and 24h after prostectomy and subjected to flow cytometric analysis. Trauma-related changes in surface antigen expression in lymphocyte, monocyte, and granulocyte gates were evaluated by t-test. Differential blood counts and C-reactive protein (CRP) were also followed. Results: Medians of leukocyte counts almost doubled after trauma due to increasing granulocytes, but lymphocyte counts declined in total numbers as well as in relative amounts. Erythrocyte counts and hemoglobin (Hb) levels decreased and CRP increased to a median of 68µg/Ml. Unexpectedly, the surface activation antigens, HLA-DR, CD25 and CD69 on monocytes and lymphocytes, respectively were not found to be upregulated, 24h after trauma. The same was true for the Fc receptors, CD16, and CD64 on all cells. Also B7 molecules, CD80, CD86, the dendritic cell activation antigen, CD83, and monocytic CD14 were similarly expressed before and after trauma. However, CD163, the Hb-scavenger receptor which is an acute phase-regulated and signal-inducing endocytosis receptor was dramatically increased on monocytes (median: 40% before and 72 % post trauma). Similarly, the death receptor CD95 was upregulated in all leukocyte populations and subpopulations. Unexpectedly, neither TLR2 nor TLR4 significantly changed after trauma. B cells and C2/CD3/TCRa,ß-positive T cells remained unaltered but NK cells declined to almost undetectable levels after trauma. Therefore, CD163, a scavenger receptor, and the apoptosis receptor CD95 constitute novel markers of the early inflammatory immune response against trauma.

P129

SEPSIS INDUCES DIFFERENTIAL EXPRESSION OF ITIM RECEPTORS ON CD4+ T CELLS.

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Typically septic patients/animals develop immune dysfunction, characterized, at least in part, by impaired CD4+ T lymphocytes (Th1) response, associated with poor outcome. Thus, discerning the mechanisms of CD4+ T lymphocytes impairment in sepsis remains an important goal in understanding the pathology of this morbid condition. To this end, we proposed to study the role of leukocyte receptors containing 'immunoreceptor tyrosine-based inhibitory motifs (ITIM)' in the development of CD4+ T cell dysfunction. Therefore, we initially determined the mRNA expression of 8 ITIM receptors, i.e., CD5, CD31, ICOS, KLRG1, CTLA-4, PD1, Ly49A1 and NKG2A, during the development of sepsis in the mouse. 24 h following the induction of sepsis by cecal ligation and puncture (CLP) CD4+ T cells were isolated from the spleen using L3T4 labeled magnetic beads and TCR stimulation was mimicked by culturing cells with anti-CD3ε antibody for 4 h. In the absence of TCR stimulation the expression of CD5 and ICOS were down-regulated by CLP. TCR activation up-regulated their expression in both groups regardless of status. The expression of CD31 and KLRG1 were also down-regulated after CLP. However, while TCR activation decreased KLRG1 expression further, CD31 expression in CLP group was not affected by TCR stimulation. CTLA-4 and PD-1 are both receptors recognizing B7 family molecules and their expression were similarly up-regulated after CLP. Both receptor's expression was also further potentiated by TCR activation independent of sham-CLP or CLP. The expression of Ly49A1 was decreased in septic mouse cells, but this could not be altered by TCR stimulation. Finally, NKG2A, an MHC class I molecule recognizing receptor, was down-regulated by sepsis, but TCR activation up-regulated its expression almost 10X in CD4+ T cells from the septic mice. Our results implicate marked changes in baseline and activation induced septic mouse CD4+ T cell ITIM receptor expression, which is associated with the development of T-cell immune dysfunction. (NIH GM 46254).

P130

CECAL LIGATION AND DOUBLE PUNCTURE (2CLP) DOES NOT ALTER HEPATIC ABUNDANCE OF THE RETINOBLASTOMA PROTEIN. J Gutsche*, N Raj, C Deutschman. University of Pennsylvania School, Philadelphia, Pennsylvania 19104.

Sepsis associated complications are the leading cause of death in intensive care units. Hepatic dysfunction is an important but poorly understood component of the sepsis syndrome. In normal liver, hepatocyte destruction provokes an intense regenerative response. In previous studies we used cecal ligation and single puncture (CLP) and 2CLP in rats and mice to examine hepatic regeneration. These investigations demonstrated normal regeneration after CLP but failed hepatic regeneration despite significant levels of hepatocellular necrosis following 2CLP.[1]. This indicates that severe sepsis induces a block in the regenerative process. We also have demonstrated that 2CLP impairs tyrosine kinase activity. The progression to the DNA synthesis phase of mitosis in the cell cycle is dependent on the phosphorylation of the intranuclear retinoblastoma protein (Rb). We hypothesized that 2CLP alters hepatic regeneration by interfering with the phosphorylation of Rb. As a first step to testing this hypothesis, we examined the abundance of Rb in the hepatic cell nucleus following CLP and 2CLP. Following sham operation (SO), CLP or 2CLP, nuclear protein was obtained from nuclear lysate. These were subjected to immunoblotting. Despite failed regeneration, levels of retinoblastoma protein were unchanged by SO, CLP or 2CLP. Further, levels did not differ between the three. We concluded that reductions in levels of retinoblastoma protein are not the cause of failed liver regeneration in severe sepsis. These studies set the stage for examination of altered phosphorylation as a cause of failed hepatic regeneration following 2CLP.

1. Weiss YG. Bellin L. Kim PK. et al. Compensatory hepatic regeneration after mild, but not fulminant, intraperitoneal sepsis in rats. *American Journal of Physiology - Gastrointestinal & Liver Physiology. 280:G968-73, 2001.*

P131

DHEA TREATMENT OF ENDOTHELIAL CELLS IN VITRO RESULTS IN A REDUCTION OF THE ADHESION MOLECULE VCAM-1 IN THE EARLY SEPTIC PHASE.

T. Barkhausen* and M. van Griensven. Experimental Trauma Surgery, Hannover Medical School, D-30625 Hannover, Germany.

The recruitment of leukocytes into tissue after trauma and sepsis is mediated by adhesion molecules. This results often in tissue damage. Previous studies revealed an improved outcome associated with reduced neutrophil recruitment upon treatment with DHEA in a murine trauma model. However, the molecular mechanisms underlying these effects are not determined yet. Concerning to this, we investigated the in vitro effect of DHEA administration on the expression of the endothelial adhesion molecule VCAM-1. HUVECs of the passages 2 to 7 were seeded in 6-well plates and grown to a subconfluent state. Cells were incubated with LPS (10ng/mL) and/or DHEA (10^{-5} M; 10^{-8} M) for 2,4,6,8 and 24 hours (n=7). Untreated cells were used as controls. Afterwards, the expression level of VCAM-1 was determined by FACS analysis. After 2 hours of stimulation, the expression of VCAM-1 was significantly reduced by 40% in samples coincubated with LPS and DHEA in both concentrations (10^{-5} M; 10^{-8} M) compared to the untreated controls and to samples incubated with LPS only. In samples stimulated with DHEA alone the expression was only slightly reduced. In contrast, at the later time points the expression was increased in LPS stimulated samples with a constant incline over the time course. Simultaneously, this increase was also seen in samples stimulated with both agents. We conclude that the beneficial effect of DHEA is at least partly transmitted by the reduction of endothelial cell surface VCAM-1 in the early inflammatory phase. The rapid shedding of this adhesion molecule may reduce the attachment and diapedesis of leukocytes with additional changes in signal transduction events. This might be either a direct or an indirect effect of DHEA. To further investigate the relevance of DHEA dependent reduction of VCAM-1 expression, future studies should be performed in VCAM-1 knock-out mice and in WT mice treated with VCAM-1 antagonists.

P132

TRAUMA/HEMORRHAGIC SHOCK (T/HS) CAUSES INCREASED RBC ADHESION TO ENDOTHELIAL CELLS.

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It has been shown that T/HS causes RBC damage, and that this leads to decreased microcirculatory flow. The goal of this study was to investigate whether T/HS RBC have increased adhesion to endothelial cells. Methods: In these experiments RBC adhesion measurements were performed by optical microscopy. Rats were subjected to T/HS or Trauma/sham shock (T/SS). Animals were sacrificed at 3 hours post shock, and RBC adhesion was determined using whole blood. After hemoglobin correction T/HS RBC or T/SS RBC were added to plates with confluent Human Umbilical Vein Endothelial Cells (HUVEC) and incubated for 5 minutes at 37°C, and washed three times. The number of adherent RBC was counted microscopically in 3 randomly selected fields for each study condition. Statistical analysis was done by ANOVA.

Results:

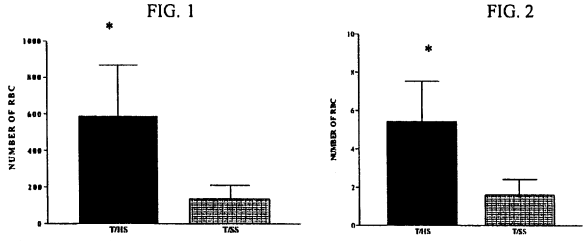


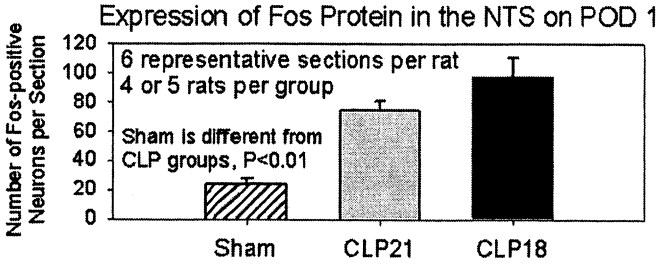
Figure 1 shows comparison of the number of the adherent T/HS and T/SS RBC. Figure 2 shows comparison of the number of adherent RBCs per HUVEC cell. * $p < 0.0001$

Conclusion: In this model, we have found that T/HS RBC have increased adhesion to HUVEC cells and this identifies an additional mechanism by which T/HS-induced RBC damage can adversely affect blood flow.

P133

ALLOSTATIC DRIVE TO THE BRAINSTEM IN THE CECAL LIGATION AND PUNCTURE MODEL OF SEPSIS. D. Carlson, G. Hoffman*, S. Amyot*, W. Chiu, and T. Scalea. R Adams Cowley Shock Trauma Center and University of Maryland School of Medicine, Baltimore, MD 21201.

Although systemic administration of endotoxin activates central neural pathways in the brainstem acutely, the activity of these pathways in the cecal ligation and puncture model of sepsis (CLP) is not known. We studied rats after sham surgery or CLP done with either a 21- (CLP21) or an 18- gauge needle (CLP18). On postoperative day (POD) 1 (21-29 h after surgery) and on POD 3 PM and POD 4 AM, anesthetized rats were perfused through the left ventricle with fixative, and brains were sectioned from the caudal medulla through the rostral pons. Neuronal activation was assessed by immunocytochemical staining for the immediate early gene product, Fos. On POD 1, Fos increased significantly after CLP in the nucleus of the solitary tract (NTS), the primary visceral sensory area in the medulla. Other areas that were activated on POD1 included the ventrolateral medulla



(VLM) and the parabrachial region of the pons. Double staining revealed that the activated population included noradrenergic and adrenergic neurons in the NTS and the VLM. On PODs 3 and 4, Fos expression in 7 of 10 CLP rats persisted in neurons in the parabrachial region including a subpopulation in the terminal field containing the peptide, CRH. Thus, CLP elicits chronic activation of central areas that have been implicated in the control of autonomic, neuroendocrine, and immune function. This persistent allostatic drive is likely to be an important component in the physiologic and behavioral derangements seen in sepsis. Supported by NIH grant GM63050.

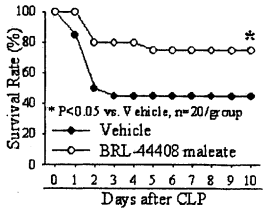
P134

A NOVEL APPROACH TO INHIBIT INFLAMMATORY RESPONSES IN SEPSIS: MODULATION OF THE SYMPATHETIC NERVOUS SYSTEM. P. Das*, R. Wu, M. Zhou, W. Dong*, D. Yang*, M. Miksa*, H.H. Simms, P. Wang. North Shore-Long Island Jewish Medical Center, Manhasset, NY 11030.

The nervous system reflexively regulates the inflammatory response in real time. Our previous studies have shown that the sympathetic neurotransmitter, norepinephrine (NE), increases TNF-α release via activation of α_{2A}-adrenoceptor (α_{2A}-AR) on Kupffer cells (KCs). In cultured KCs, NE increases the release of TNF-α by 3.4-fold and a specific antagonist for α_{2A}-AR, BRL-44408 malaete, reduces TNF-α secretion by 51%. We, therefore, hypothesize that administration of BRL-44408 malaete inhibits inflammatory responses and prevents organ injury in sepsis. To study this, sepsis was induced in male rats by cecal ligation and puncture (CLP). BRL-44408 maleate (2.5 mg/kg BW) was administered intravenously at the time of CLP. Twenty hours after CLP, the rats were sacrificed and blood and liver samples were collected. Serum levels of TNF-α, liver enzymes (i.e., AST and ALT), lactate and creatinine were measured. Gene.expression of TNF-α in the liver was analyzed. In additional groups of animals, the necrotic cecum was excised at 20 h post-CLP and the 10-day survival was recorded. The results (means±SE) are as follows:

	Sham Control	CLP Vehicle	CLP BRL-44408 malaete
TNF-α (pg/ml)	65.8±8.2	160±25.2*	91.8±8.1#
TNF-α/G3PDH (mRNA)	0.2±0.01	1.4±0.3*	0.5±0.1#
ALT (IU/L)	8.5±3.1	40.9±4.4*	20.5±4.5#
AST (IU/L)	13.6±2.1	55.7±7.7*	20.9±5.3#
Lactate (mg/dl)	10.8±0.9	32.6±2.1*	20.6±1.9#
Creatinine (mg/dl)	0.8±0.2	2.3±0.4*	1.1±0.2*#

(One-way ANOVA: *P<.05 vs. Sham; # P<.05 vs. Vehicle, n=6/group)

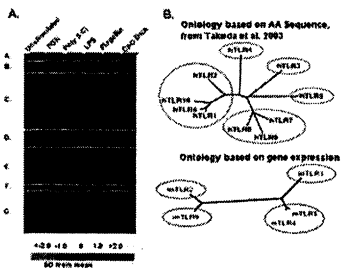


Our results indicate that BRL-44408 maleate administration decreased the expression of TNF-α, attenuated tissue injury, and reduced mortality in septic animals. Thus, modulation of the sympathetic nervous system by blocking α_{2A}-AR appears to be a novel treatment for inflammatory conditions such as sepsis (NIH R01 GM053008).

P135

ONTOLOGY OF TLR SIGNALING BASED ON DENDRITIC CELL (DC) MATURATION AND GENOME-WIDE EXPRESSION. H Tsujimoto*, S Tschoeke*, P Efron*, T Uchida*, P Scumpia*, H Baker*, W Xiao*, M Mindrinos*, R Davis*, L Moldawer, Univ. of Florida, Gainesville, FL 32610,

Introduction: Recognition of microbial infection by DCs is mediated by TLR signaling. DCs discriminate among different pathogens by common and distinct signaling pathways through different TLRs. The phenotypic response and the patterns of gene expression in response to specific TLR agonists were examined, and an ontologic tree was generated based on gene expression, and compared to their amino acid (AA) ontology. **Methods:** Murine bone-marrow DCs were generated with GM-CSF, and stimulated with specific TLR agonists: flagellin (TLR5), LPS (TLR4), CpG (TLR9), polyI:C (TLR3), or PGN (TLR2). Class II and CD86 expression, IL-12p70, TNFα, IL-10 and genome-wide expression were evaluated 24 hours after TLR stimulation. **Results:** With the exception of polyI:C (TLR3), activation of DCs with TLR2, 4, 5 and 9 agonists resulted in a comparable induction of MHCII and CD86 expression, and increased IL-12p70 and TNFα production ($p<0.05$). Using a false discovery rate of 0.001 (SAM analysis), the expression of 2,022 probe sets (out of 45,101) discriminated among the different TLR agonists. Building an ontologic tree based on the genome-wide patterns of expression (A.) revealed that TLR4 and TLR5 responses were more similar to each other than predicted by their AA sequence or their known signaling pathways (B.). TLR2 and TLR9 agonists also produced very similar gene expression patterns, despite markedly different AA sequences.



Conclusions: Despite similarities in the phenotypic response, the variation in gene expression by DCs to different TLR agonists reveals ontologies that are considerably different than predicted by either their AA sequence or known signaling pathways.

P136

ANALYSIS OF HEPATIC TRANSCRIPTIONAL ACTIVITY AFTER ENDOTOXIN EXPOSURE. D Morgan*, K Denson*, M Lerner, Y Gusev*, E Varughesekutty*, J Hanas*, R Postier*, B Smith, D Brackett. University of Oklahoma Health Sciences Center and VA Medical Center, Oklahoma City, OK 73190

Objective: To determine differential gene expression and transcriptional profiles after endotoxin and to apply bioinformatic methods to identify molecular pathway patterns involved in the innate immune response. Methods: Male Sprague-Dawley rats were given 20mg/kg of endotoxin (n=25) or saline (n=25) i.v. Five animals from each group were euthanized at each of the following time points: 10, 30, 60, 120, and 240 minutes. Hepatic expression of 1200 genes was assessed utilizing Clontech arrays. After digitization (*ArrayVision*), *GeneSpring* software was used to normalize the data and to select genes that exhibited differences in regulation at each time point. Patterns of expression were determined using 3 different methodologies: quality threshold clustering algorithms, principal components analysis, and functional classification by Gene Ontology. Statistical significance was determined using ANOVA post hoc and Student's *t*-test. *PathwayAssist* software was used to combine results with data derived from computer-driven, comprehensive literature searches for the construction of molecular pathways. These pathways permit evaluation of up & down stream interactions for potential sites of intervention. Results: Using criteria of 5-fold changes in expression (p?0.01), 52-118 genes were up-regulated over the five time points and 15-22 were down-regulated. 4 principal components were found within this group of genes. Additionally, the up-regulated genes were categorized into 5-11 clusters. The molecular pathways constructed revealed unique pathway patterns associated with each time point following the administration of endotoxin. Conclusion: New bioinformatic methodologies provide important insight into relationships in gene expression following the initiation of the immune response. By understanding these relationships we can identify key targets for potential diagnostic, prognostic, and therapeutic applications in the management of inflammatory diseases.

P137

THE ROLE OF NITRIC OXIDE AND MITOCHONDRIAL DYSFUNCTION IN THE DEVELOPMENT OF THE POST-INJURY Th-2 IMMUNE PHENOTYPE. T. Daniel*, W.J. Hubbard*, I.H. Chaudry, M.A. Choudhry, M.G. Schwacha. Department of Surgery, University of Alabama at Birmingham, Birmingham, AL 35294.

Previous studies have implicated both nitric oxide (NO) and the Th-2 type immune response as causative factors in the development post-burn immune dysfunction. Recent findings suggest that cellular redox status, (which can be modulated by NO), influences the T-cell Th-type phenotype. Nonetheless, the role of NO in the development of the Th-2 type response post-burn remains to be clearly elucidated. To study this, C57BL/6 mice were subjected to major burn injury (3rd degree, 25% total body surface area). Splenocytes were isolated 7 days later and stimulated with αCD3 or ConA. Th-1 (IL-2, IFN-γ) and Th-2 (IL-4, IL-10) cytokine production was assessed by ELISA. Mitochondrial function was assessed by MTT (oxidative metabolism) and JC-1 Mitoprobe™ (mitochondrial depolarization) assays. Addition of the NO donor, S-nitroso-N-acetyl-penicillamine (SNAP) to the splenocyte cultures at concentrations >50 μM suppressed Th-1 type but not Th-2 type cytokine production. Delaying the addition of SNAP to the cultures by 24 hr prevented the suppression of IFN-γ production. The Th-2 type shift in immune phenotype was independent of changes in cell viability, cGMP, MAP kinases, and PPAR-γ activation. Addition of SNAP to cell cultures also attenuated mitochondrial oxidative metabolism and induced mitochondrial membrane depolarization, an early marker of apoptosis. These detrimental effects of NO on mitochondrial function, however, were observed only at supra-physiologic concentrations (>250 μM). Thus, while NO suppresses T-cell Th-1 cytokine production inducing an immunosuppressive Th-2 type phenotype, it is independent of NO-induced alterations in mitochondrial function. *(funded by NIH grants GM58242 and AI049960).*

P138

ROLE OF ENDOGENOUS LIGANDS FOR THE PEROXISOME PROLIFERATORS ACTIVATED RECEPTORS ALPHA IN THE SECONDARY DAMAGE IN EXPERIMENTAL SPINAL CORD TRAUMA.

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The peroxisome proliferator-activated receptor-α (PPAR-α) is a member of the nuclear receptor superfamily of ligand-dependent transcription factors related to retinoid, steroid and thyroid hormone receptors. The aim of the present study was to examine the effects of endogenous PPAR-α ligand in an experimental model of spinal cord trauma. Spinal cord injury was induced in PPAR-α wild-type (WT) mice and PPAR-α knock out mice (PPAR-α KO) mice by the application of vascular clips (force of 24 g) to the dura via a four-level T5-T8 laminectomy. Spinal cord injury in mice resulted in severe trauma characterized by edema, neutrophil infiltration (measured as an increase in myeloperoxidase activity) and apoptosis (measured by Annexin 5 staining).An increase of immunoreactivity to TNF-α was observed in the spinal cord of spinal cord-injured PPAR-α WT mice. Absence of a functional PPAR-α gene in PPAR-αKO mice resulted in a significant augmentation of all the above described parameters.

In a separate set of experiments we have also demonstrated that the absence of PPAR-α gene in PPAR-αKO mice significantly worsened the recovery of limb function (evaluated by motor

recovery score).Thus, endogenous PPAR-α ligands reduce the degree of development of inflammation and tissue injury events associated with spinal cord trauma in the mice.

P139

THE TSUNAMI-DISASTER 2004: INJURY PATTERN AND MICROBIOLOGICAL ASPECTS. S. Gregor*, M. Maegele*, E. Steinhausen*, B. Bouillon*, M. M. Heiss*, D. Rixen*, F. Wappler*, J. Geisen*, B. Berger-Schreck*, R. Schwarz*. (Spon: D. Rixen) Merheim Medical Center, Univ. Witten-Herdecke, Cologne (Germany)

On December 26, 2004, a giant earthquake shocked south-east Asia triggering deadly flood waves (tsunami) that fanned out across the Indian Ocean. More than 200,000 people have been reported dead and millions left destitute. Shortly thereafter, European governments organized airbourne home transfer of most severely injured tourists using "MedEvac"-aircraft (Medical Evacuation). Upon arrival patients were distributed to various medical centers. One cohort of severely injured was admitted to the Cologne-Merheim Medical Center (Germany) for further surgical and ICU treatment. Materials and Methods: Seventeen severely injured tsunami victims were screened upon arrival for characteristic injury patterns. In parallel, multilocal microbiological assessment was performed to identify pathogens responsible for high level wound contamination. Results: The predominant pattern of injury comprised multiple large-scale soft-tissue wounds (range: 2 x 3cm – 60 x 60cm) located at lower (88%) and upper extremities (29%), but also head (18%). Additional injuries included thoracic trauma with hemopneumothorax and serial rib fractures (41%) and peripheral bone fractures (47%). A major problem associated with wound management was significant contamination. Microbiological assessment identified a variety of common (Pseudomonas 54%, Enterobacteriae 36%, Aeromonas hydrophilia/ veronii 27%) but also uncommon isolates with high resistencies (multi-resistent Acinetobacter and ESBL-positive E. coli 18% each). Upper respiratory tract specimens contained an unusual high rate of multi-resistent Acinetobacter species, but also MRSA, Aeromonas hydrophilia, Pseudomonas and Candida albicans. Conclusion: Apparently, common local hygiene standards could not be preserved under conditions given. These isolates need to be considered when treating Tsunami patients, especially on ICU wards.

P140

ALVEOLAR MACROPHAGES INDUCE APOPTOSIS IN ALVEOLAR TYPE 2 EPITHELIAL CELLS AFTER BLUNT CHEST TRAUMA D.H. Seitz*, M. Perl, M.G. Bachem#*, S. Braumüller*, M.S. Huber-Lang*, M.W. Knöferl. Dept. of Trauma Surgery and Dept. of Clinical Chemistry#, University of Ulm, Germany.

Alveolar type 2 epithelial (AT2) cells play an important role in both, maintenance of the alveolar epithelium and in defense of the host. Apoptosis of AT2 cells is an essential mechanism regulating these processes. Blunt chest trauma is known to activate alveolar macrophages (AM) resulting in a proinflammatory response. The present study was performed to elucidate whether blunt chest trauma induces apoptotic processes in AT2 cells and its dependency on AM. Male CD rats were subjected to either sham procedure or blunt chest trauma induced by a single blast wave. Various time points after injury (0.5h-7d) lungs were analyzed by immuno-histochemistry, stained with an anti-cytokeratin-antibody (clone MNF-116) as AT2 cell marker or anti-caspase 3. Furthermore, cultures of AT2 cells isolated from healthy rats were incubated with supernatants of AM, obtained from either trauma or sham operated animals in the presence or absence of H_2O_2. Both, Annexin 5 staining of AT2 cells and Western-blot analysis of AT2 cell lysates for active caspase 3 were used to detect apoptotic events. MNF-116 staining of lung tissue revealed a significant decrease in AT2 cell number 48h after trauma. In addition, an increased count of caspase 3 positive AT2 cells versus sham operated littermates was detected. In AT2 cell culture, increased numbers of apoptotic cells were detected after addition of AM supernatants obtained 24h after trauma compared to AT2 cell cultures incubated with AM supernatants from sham animals. Concentration of active caspase 3 was elevated in cultured AT2 cells stimulated with supernatants of AM isolated after chest trauma versus sham animals. Additional oxidative stress by exposure with H_2O_2 evoked even more distinguished differences among experimental groups. These results indicate that blunt chest trauma induces apoptosis in AT2 cells which seems to be dependent on AM most likely by release of pro-apoptotic factors, which should be subject of further investigations. (DFG KN 475/3-1)

P141

BLUNT CHEST TRAUMA INDUCES AN INFLAMMATORY RESPONSE AND INCREASES THE LETHALITY FROM SEPTIC COMPLIACTONS

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Blunt chest trauma causes an increase in circulating inflammatory mediators. Possible sources are injured lung tissue and activated immune cells. The present study was designed to characterize immunological effects of blunt chest trauma (TX) in different compartments. To evaluate the influence of altered immune responses on infectious complications, we determined if TX changes the lethality from subsequent sepsis. Male C3H/HeN mice were subjected to TX (blast wave) or sham procedure. Lungs and livers were harvested 0.5, 2 or 24h later. Blood, bronchoalveolar lavage fluids (BAL) and Kupffer cells (KU, LPS-stimulated) were obtained in a separate set of animals. IL-6 levels in plasma, BAL, KU supernatants and homogenized tissues were determined by ELISA. Additional animals were subjected to sepsis by CLP 24h after TX or sham procedure and mortality was observed for 10 days.

IL-6 [pg/ml]	Sham	0.5h TX	2h TX	24h TX
Plasma	10±3	15±3	146±32*#†	14±2
KU	9.5±1.8	20.8±3.7*#	11.3±2.3†	6.6±3.6
BAL	10±2	15±2	377±45*#†	149±72
Lung	126±12	104±9	218±19*#†	141±26
Liver	457±40	377±29	427±53#	648±115*†

(n=10, mean±SEM, one-way ANOVA, SNK, $p<0.05$ vs. *Sham, #TX 24h, †TX 0.5h)

IL-6 levels in plasma, BAL and lung tissue were markedly increased 2h after TX. As early as 0.5h after TX, KU were activated to release IL-6. Lethality from subsequent sepsis was significantly higher in the group subjected to TX 24h before CLP than in septic sham animals. These findings indicate that TX caused marked inflammatory changes in different compartments. Since TX increases the lethality from subsequent septic complications, the immune response induced by this injury should be further characterized. (DFG KN 475/2)

P142

WOUND EXCISION AND GRAFTING (WE) AFTER BURN INJURY ATTENUATES MYOCARDIAL INFLAMMATION. B. Sanders*, D.L. Maass, D.J. White, J.W. Horton. UT Southwestern, Dallas, TX 75390-9160.

INTRODUCTION: We have reported previously that excision of the burned eschar after burn over 20% TBSA improved myocardial contractile performance compared to that measured with burn over 20% TBSA in the absence of WE. Numerous studies describe that early burn WE modulates muscle catabolism, decreases both operative blood loss and incidence of wound infection. This study hypothesized that WE within 30 min after burn in adult rats attenuates cardiac inflammation, improving postburn cardiac function. **METHODS**: Group 1, sham burn; Group 2, sham burn + excision of unburned skin; Group 3, burn over 20% TBSA, no WE; Group 4, burn over 30% TBSA + no WE; Group 5, burn over 20% TBSA + WE; Group 6, burn over 30% TBSA + WE; control rats provided donor skin for wound grafting. Myocytes were prepared 24 hrs postburn (collagenase digestion) and cytokines measured, pg/ml, ELISA. **RESULTS**: Burn over either 20 or 30% TBSA promoted myocyte cytokine secretion that was attenuated by early WE; in addition, WE improved postburn cardiac function. **CONCLUSION**: Early removal of burned eschar modulates post burn inflammatory responses, providing a mechanism for WE-related cardioprotection. **NIH Grant P50 GM21681-39.**

	TNFα	IL-1β	IL-6	IL-10
Sham	88±4	13±1	74±10	18±1
Sham+E	84±7	14±2	62±9	12±1
20% Burn	438±10*	30±1*	348±11*	44±2*
30% Burn	760±36*	32±3*	354±6*	64±2*
20%Burn +WE	125±2†	19±1†	123±2†	17±.3†
30% Burn+WE	155±2†	22±1†	136±1†	19±.1†

Sham+E, sham+excision of unburned skin; *difference from sham+E at p<0.05, †difference from burn in absence of WE at p<0.05, ANOVA, Student Neuman Keuls.

P143

THE EFFECTS OF BURN INJURY ON Na, K-ATPase ACTIVITY IN THE HEART. J. Tan*, D.L. Maass, J.W. Horton. UT Southwestern, Dallas, TX 75390-9160

INTRODUCTION: Using NMR techniques, we have linked cardiomyocyte sodium accumulation to myocardial contractile dysfunction. Sodium accumulation was not related to an increase in intracellular acidosis, increased Na-H exchange or decreased ATP. This study examined the effects of burn injury on NA, K-ATPase activity in adult rat hearts after major burn injury. **METHODS**: A 3° burn injury (or sham burn for controls) was given over 40% TBSA, and rats received lactated Ringer's solution, 4 ml/kg/% burn. Subgroups of rats were sacrificed either 2, 4, or 24 hrs postburn (N=5 rats/time period). Hearts were homogenized in 10 mM histidine/0.7 mM/NaCl and centrifuged (30 min at 10,000 rpm; resulting supernatant was centrifuged (30 min at 20,000 rpm). Na, K-ATPase activity was determined from ouabain-sensitive phosphate generation from ATP by cardiac sarcolemmal (SL) vesicles. **RESULTS**: Burn injury caused a progressive rise in cardiomyocyte Na^+ (measured by fluorescent indicator SBFI). Na, K-ATPase activity (μmol/min/g, measured in the presence of 3mM ATP to ensure that ATP concentration was not a limiting factor) was progressively decreased after burn. An asterisk in the Table indicates a significant difference from controls at $p<0.05$ (ANOVA, Student Neuman Keuls). **CONCLUSIONS**: Burn-related inhibition of Na, K-ATPase likely contributes to the cardiomyocyte accumulation of intracellular sodium. Since intracellular Na^+ is one determinate of electrical-mechanical recovery after insults such as burn injury, burn-related inhibition of the Na, K-ATPase may be critical in postburn recovery of myocardial contractile function. **NIH Grant R01 GM57054.**

	SHAM	POSTBURN		
		2 Hrs	4 Hrs	24 Hrs
Na, K-ATPase	1.10±0.2	0.82±.01*	0.79±.01*	0.39±.02*
Na^+, mM	9.7±1.0	12.7±.09	16.0±.9*	39±2*

P144

CYTOKINE EXPRESSION CHANGES WITH TIME IN SEVERELY BURNED CHILDREN. CC Finnerty*, MG Jeschke*, R Przkora*, HM Oliveira*, RE Barrow, DN Herndon. Shriners Hospital for Children and Dept. of Surgery, Univ. of TX Medical Branch, Galveston, TX 77550

Objective: The release of massive quantities of inflammatory mediators (e.g. IL-1β, IL-2, IL-8, IFN-γ, TNF-α, and IL-6) is characteristic of a severe thermal injury. After thermal injury, to better understand these changes in the inflammatory response, simultaneous expression of 17 cytokines was measured over a 5-week recovery period. Serum cytokine profiles from burned children were compared to those from aged-matched non-burned children. Methods: Fifteen patients with ? 40% TBSA burned and 15 non-burned children were studied. The mean age was 7.4±1 years and time from burn to admission was 7±2 days. Patients with an inhalation injury or identified sepsis were excluded. Blood was collected at admission and weekly for five weeks after burn. Cytokine

expression profiles were determined using Luminex technology. Results: The expression of pro- and anti-inflammatory cytokines and chemokines was maximal immediately after admission. Significant increases were measured for 11 mediators: IL-6, IL-8, IL-10, IL-17, TNF-a, IL-13, IL-1b, IFN-g, MCP-1, MIP-1b, and G-CSF, $p<0.04$. These are involved in angiogenesis, cell proliferation and apoptosis, and activation as well as recruitment of immune cells to the site of inflammation and injury. Within 5 weeks, the levels of many serum cytokines decreased and approached normal levels. In some patients, however, several cytokines remained elevated at the end of 5 weeks. Conclusion: The significant increases in IL-6, IL-8, IL-10, IL-17, TNF-α, IL-13, IL-1β, IFN-γ, MCP-1, MIP-1β, and G-CSF presented in this study will help in identifying why cell signaling and immune function are compromised after a severe thermal injury.

P145

TISSUE-SPECIFIC CHANGES IN EXPRESSION PROFILES OF ENDOGENOUS RETROVIRUSES AFTER BURN INJURY. K. Cho, H. Phan*, A. Chew*, and D. Greenhalgh. Shriners Hospitals for Children Northern California and Department of Surgery, University of California at Davis, Sacramento, CA 95817

Endogenous retroviruses (ERVs) make up a significant portion of the human and mouse genome (approximately 10% in humans). Several diseases, such as insulin-dependent diabetes mellitus, have been linked to ERVs. Recent studies from our laboratory suggest that signaling events elicited from burn injury alter the expression of mouse ERVs (MuERVs). In this study, we examined the expression profiles of MuERVs in different tissues and the effects of burn injury on MuERV profiles. Nineteen different tissues collected from female C57BL/6J mice following cervical dislocation were subjected to RT-PCR analysis using a primer set flanking the nonecotropic MuERV U3 sequences which harbor the polymorphic promoter as well as envelope sequences. In addition, four different tissues (liver, lung, kidney, and spleen) collected at 3 hours and 1 day after 18% TBSA burn were examined for injury-mediated changes in MuERV expression profiles by RT-PCR amplification of U3 sequences. Electrophoretic analyses of amplified U3 sequences revealed that there are unique U3 expression profiles among 19 tissues examined. Sequencing analyses of these U3 sequences demonstrated that some sequences were unique for individual tissues and the others were overlapping sequences. Furthermore, the U3 expression profiles were substantially altered after burn injury in four tissues examined. Interestingly, the results suggested that the anesthesia alone affects the U3 expression profile. The transcriptional potentials of each U3 sequence and the genome-wide map of putative MuERVs harboring these U3 sequences as well as their neighboring cellular genes are being determined. The results from these studies may help better understand the genome-wide gene regulation, especially ERVs, in response to systemic stress signals following burn injury.

P146

EXPLORING THE HOST'S RESPONSE TO TRAUMA AND HEMORRHAGIC SHOCK USING A COMBINATION OF MATHEMATICAL SIMULATIONS AND GENOMICS

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Introduction: Trauma and hemorrhagic shock (T-HS) elicits an acute inflammatory response that predisposes the victims to subsequent organ dysfunction and death. The lack of effective therapeutic options is mainly due to the complex interplay of interacting inflammatory and physiologic elements working at multiple levels. "Systems Biology" has emerged as a new paradigm that allows the study of large portions of physiological networks simultaneously. Seeking a better understanding of the interplay among the plethora of known biological pathways, we used mathematical simulations and genomics to explore the host's response to increasing severity of stresses. ***Methods:*** We constructed a mathematical model encompassing the dynamics of the acute inflammatory response as well as global tissue damage. We asked the model whether different types of insults, i.e., sham operation, bilateral femoral fracture (T), HS alone, T-HS and T-HS+resuscitation, would induce different outcomes in C57Bl/6 mice. Subsequently, we sought to validate the results through a detailed DNA microarray analysis of liver gene expression using Affymetrix microarray (GeneChip® Mouse Expression Set 430). The microarray dataset was subjected to hierarchical clustering and then loaded into biological knowledge base software for pathway analysis (Ingenuity Pathways™). ***Results:*** Our mathematical model predicted, non-intuitively, that only the magnitude of the host's response would change with increased severity of stresses. In agreement with this finding, our microarray analysis also demonstrated that sham operation alone resulted in the majority of the observed hepatic gene/pathways changes when compared to control animals; additional T±HS±resuscitation further increased the magnitude of gene expression, but relatively few additional genes were recruited. ***Conclusions:*** Insults of different magnitude seem to evoke similar genetic & molecular pathways by the host. The use of multiple systems biology approaches may provide invaluable insight into the complex global physiological interactions that occur in response to trauma and hemorrhagic shock.

P147

CONTRIBUTIONS OF Na^+, Cl^-, AND ALBUMIN TO BASE EXCESS IN VENTILATED TRAUMA PATIENTS.
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Introduction: Since IV fluid intervention is influenced by base excess, determining the influence of Na^+, Cl^-, and albumin (alb) on base excess (BE_{total}) as an indicator of patient perfusion status could be important. **Methods:** Review of ICU ventilated trauma patients' (1/1/00 – 4/20/04) synchronous lab values for: base excess (BE_{total}), Na^+, Cl^-, and alb. The contributions to BE_{total} were then calculated as follows: $BE_{Na}=0.3(Na-140)$, $BE_{Cl}=102-(Cl \times 140/Na)$, $BE_{albumin}=0.34(45-albumin\ g/L)$ and subtracted

from BE_{total} to calculate the $BE_{unmeasured}$: $BE_{unmeasured} = BE_{total} - (BE_{Na} + BE_{Cl} + BE_{albumin})$. **Results:** 259 patients with 1,169 sets of lab values. The mean absolute difference between BE_{total} and $BE_{unmeasured}$ with alb was 6.5 ± 4.9 (SD) mEq/L, $p<0.001$ (without alb was 4.6 ± 3.1 mEq/L, $p<0.001$).

# of data sets	BE difference >2mEq/L	BE difference >4mEq/L	BE difference >6mEq/L	BE difference >8mEq/L
with alb n=160	86% (79% more acid)	66% (65% more acid)	50% (49% more acid)	31% (30% more acid)
no alb n=1009	77% (5% more acid)	54% (3% more acid)	28% (1% more acid)	14% (0% more acid)

When contributions of Na^+, Cl^-, and albumin were accounted for, the BE from unmeasured ions was an average of 6.5mEq/L different (most frequently more acidic) than what was reported with the blood gas (BE_{total}). **Conclusions:** In ventilated trauma patients, the incidence of substantial differences in BE_{total} versus $BE_{unmeasured}$ is very high. Clearly, if the contributions of these 3 strong ions (Na^+, Cl^-, albumin) are not considered, the metabolic status indicated by the BE_{total} will frequently be quite different than that indicated by the $BE_{unmeasured}$. Whether knowing the $BE_{unmeasured}$ would lead to changes in patient management (i.e. different rates and types of IV fluids) and, more importantly, whether these changes would improve patient outcome needs to be determined.

P148

IL-1 RECEPTOR ANTAGONIST LEVELS CORRELATE WITH HEMODYNAMICS AFTER SEVERE INJURY

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Introduction: Arrest of hemorrhage and resuscitation are the cornerstones of modern trauma management. We have previously demonstrated improved survival using resuscitation schemes based upon left ventricular power (LVP). Similarly, patients with lower ratios of aortic input impedance (Ea) to ventricular contractility (Ees) have improved survival. The systemic inflammatory response (SIR) also is an important determinant of outcome. Elevated post-traumatic interleukin–1 receptor antagonist (IL-1ra) levels are associated with organ dysfunction/failure and death. Lacking are studies investigating the relationship between SIR and hemodynamic (HD) performance. This study examined the relationship between IL-1ra and post-traumatic resuscitation. **Methods:** Severely injured patients admitted to a Level 1 trauma center requiring a pulmonary artery catheter (PAC) were evaluated. HD variables were recorded at four hour intervals while the PAC was in place. Blood was obtained daily for the first five days the PAC was in place and analyzed for IL-1ra levels by ELISA. The relationship between HD parameters and cytokine levels was investigated. **Results:** 16 patients met study criteria. Survivors had significantly higher mean arterial pressure (MAP) and LVP, with lower IL-1ra levels (See Table). Decreased IL-1ra levels correlated with improved RATIO (Ea/Ees), stroke work index (SWI), and LVP, indicating improved ventricular-arterial coupling ($p<0.05$). Oxygen delivery (DO_2I), consumption (VO_2I), MAP, and LVP over time were associated with decreases in IL-1ra levels as well ($p<0.05$). **Conclusion:** Lower levels of IL-1ra were associated with improved HD parameters and survival. Changes in cardiovascular performance over time inversely correlated with levels of IL-1ra. The functional relationship of these interesting findings remains unknown and requires further investigation.

Variable	Surv	Nonsurv	p
DO_2I(mL/min/m^2)	728	545	0.066
MAP(mmHg)	85.3	79.5	0.044
RATIO(Ea/Ees)	1.7	2.3	0.043
LVP(mmHg*L/min/m^2)	369	254	0.006
IL-1ra (pg/mL)	10142	22519	0.042

P149

INCIDENCE, MORBIDITY, AND MORTALITY RELATED TO DEEP VENOUS THROMBOSIS IN A PEDIATRIC BURN POPULATION

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Background: The true incidence of Deep Venus Thrombosis is also not well described for pediatric burn patients. From 1994 to 1999 routine care in our hospital included heparinized saline administration in central venous catheter flushes, which ceased in September 1999. Since September 1999 we only use saline flushes. We have hence chosen to evaluate the incidence, morbidity and mortality related to DVT during these two time periods. Method: Clinical records of the incidence and treatment of DVT were reviewed for the time period January 1994 to June 2004. No prophylactic measures were taken to prevent DVT, other than early mobilization. Patients were clinically diagnosed with DVT. Confirmatory studies were performed with venous duplex imaging done by a radiologist or registered vascular technologist. Treatment of DVT was with intravenous heparinization to an aPTT of 60-80 seconds, followed by oral coumadin therapy to an INR of 1.5-2.0 for three to six months. Autopsy records were also reviewed to elucidate any deaths related to DVT. Results: From 1994 to 2004 we admitted 2592 patients and observed a total of 16 patients with DVT (incidence 0.006%). From 1994 to 1999 1209 patients were admitted, with two patients (14±3 years, mean TBSA 81±15%, 3rd 81±15%) having DVT, however they presented with DVT at admission. There were no patients developing DVT during our treatment. From 1999 to 2004 we admitted 1383 patients, with 14 patients (7±1 years, mean TBSA 54±7%, 3rd 49±7%) developing DVT under our care (incidence 0.01%), $p<0.05$. There were no fatal pulmonary emboli, or deaths directly related to DVT. Discussion: Based on our findings, we suggest that DVT prophylaxis is indicated in pediatric burn patients. Prospective studies are still needed to determine the sub-clinical incidence of this disease process.

P150

MANAGEMENT OF GASTRIC INJURY AND SHOCK

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Methods: This is a study of 544 consecutive patients with gastric trauma admitted to a level one urban trauma center from

1980 to 2003. After IRB approval, clinical data was gathered prospectively and retrospectively through the trauma database and chart reviews. **Results:** The overall mortality for full thickness gastric injuries was 20% (109/544). The factors associated with the highest mortality were initial OR systolic blood pressure < 60 mmHg (89%), final OR pH < 7.00 (85%), initial systolic blood pressure in emergency department < 60 mmHg (80%), final OR temperature < 34.0 °C (79%), arterial (a) to end tidal (ET) PCO_2 gradient > 13 (52%), and a transfusion requirement > 10 units of blood (50%). An increased PCO_2 (a-ET) gradient indicates increased dead space, often due to inadequate resuscitation. Of the patients in severe shock on arrival to the emergency department who survived, 75% developed infectious complications and had a length of stay of 33 ± 28 days. **Conclusions:** Patients with gastric injuries and a presenting emergency department systolic blood pressure < 60 mmHg had a poor prognosis. The ability to quickly resuscitate the patient, control hemorrhage, limit blood transfusions to 9 units, and maintain OR temperature > 34.0 °C was associated with improved outcome. PCO_2 (a-ET) gradient and arterial pH can be used intra-operatively as predictors of decreased resuscitation and mortality. For those patients who presented in severe shock, infection was a major contributor to morbidity, and should be watched for closely.

PCO_2 (a-ET) Gradient	Mortality
1.0-8.9	2%
9.0-12.9	6%
13.0-16.9	30%
17.0-18.9	50%
19.0+	63%

Worst pH	Mortality
< 7.00	85%
7.00-7.09	55%
7.10-7.19	26%
7.20-7.29	13%
7.30-7.39	3%
>7.40	13%

P151

EFFICACY AND UTILITY OF WOUND SURVEILLANCE CULTURES AND VENTILATOR ASSOCIATED PNEUMONIA IN THE SEVERELY BURNED. C. White*, J. Ward*, S. Wolf, L. Cancio*, J. Holcomb*. US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, TX 78234.

Prompt and accurate administration of effective antibiotics for ventilator-associated pneumonia (VAP) impacts morbidity and mortality as well as hospital costs in burned patients. Our objective was to predict the causative organisms for VAP from surveillance wound cultures and thus determine if these cultures could be used to predict appropriate initial therapy in ventilated burned patients. We compared the results of surveillance wound and respiratory cultures from all ventilated burned patients with the diagnosis of VAP from 1 January 1999 to 31 March 2004. The most common organisms in surveillance wound cultures were *Staphylococcus* species and *Streptococcus*. The most common isolates for VAP were *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The overall incidence of the causative pneumonia organism in surveillance wound cultures was 22 of 56 (correlation 39.3%, CI 26.5 to 52.1). However, this number increased significantly when *Klebsiella* (100%), *Proteus* (75%) and *Pseudomonas* (62.5%) species were found in the surveillance wound cultures. Moreover, antibiotic susceptibilities patterns matched in 72% of these paired isolates. This correlation did not improve in the presence of tracheostomy or inhalation injury. Our results suggest that surveillance wound cultures with these gram negative isolates have utility in selecting effective antibiotics for VAP in this population.

Key Words: respiratory cultures, *Klebsiella*, *Pseudomonas*, *Proteus*, antibiotics

P152

GI TONOMETRY IS A MONITOR OF ONSET OF ACS

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Gastric tonometry measures CO_2 accumulation that occurs with gut hypoperfusion, and gastric mucosal PCO_2 ($PgCO_2$; mmHg) was promoted to be an endpoint of shock resuscitation. Increased $PgCO_2$ despite resuscitation consistently predicted adverse outcomes, but clinicians became frustrated because it could not be corrected with more aggressive interventions. Concurrently, abdominal compartment syndrome (ACS) emerged to be a frequent complication in this patient population. We, therefore, hypothesized that increased $PgCO_2$ occurs as a result of resuscitation induced ACS. We analyzed data from 152 severely injured patients admitted over 36 mo. 23 developed ACS, which was decompressed 8±1 hr after ICU admit. The data include $PgCO_2$ q1hr (continuous monitor) and urinary bladder pressure (UBP; mmHg), base deficit (BD; mEq/L) and lactate concentration ([lactate]; mM) q4hr. Responses in the 1st 8 hr of ICU resuscitation are compared in patients who developed ACS and those who did not (nonACS: ISS 27±1, age 39±1, 76% male, 85% blunt; ACS: ISS 28±2, age 41±2, 78% male, 87% blunt). Data were analyzed with t tests and ANOVA (mean±SEM; *p<0.05 nonACS v ACS; ^p<0.05 re t=0). **Results:** The table compares responses of the two groups to early resuscitation.

	nonACS (n=129)				ACS (n=23)			
t (hr)	UBP	$PgCO_2$	BD	[lactate]	UBP	$PgCO_2$	BD	[lactate]
0	15±1	46±2	6±0.4	5.6±0.2	19±3	41±3	9±1	8.2±1.0
4	16±1	49±1	^4±0.4	^4.9±0.3	*^27±4	*^60±4	9±1	8.8±1.2
8	15±1	50±2	^2±0.3	^4.2±0.2	*^28±3	*^62±6	8±1	9.3±1.1

Both groups started resuscitation with surprisingly high UBP, similar normal $PgCO_2$, and BD and [lactate] consistent with severe shock. The nonACS patients responded well (BD and [lactate] decreased), and UBP and $PgCO_2$ did not increase. ACS patients did not respond well. UBP and $PgCO_2$ increased rapidly. After decompression, UBP decreased to 21±3 mmHg and $PgCO_2$ decreased to 58±6 mmHg. **Conclusion:** Gastric tonometry is a convenient, continuous, early indicator of gut hypoperfusion that occurs with aggressive resuscitation.

P153

AN AUTOMATED FLUID BALANCE MONITOR FOR RESUSCITATION OF BURN SHOCK.

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Objective: Accurate and timely measurement of urinary output (UO) and net fluid balance (fluid in-urine out) are required for therapeutic decisions on infusion rate and, the need for inotropes in shocked patients. Unfortunately, this is subject to errors of volume, time and calculation. We have designed a Fluid Balance Monitor (FBM), which measures and continuously displays fluid infusion rates, urinary output, and calculates cumulated volumes and net fluid balance.
Methods: We tested the robustness of the FBM in 12 adult burn patients admitted within 48 hrs of injury. Our measurements did not influence patient care in any way as the displays were not available to the clinical staff. The FBM is a standard windows XP laptop programmed in Lab VIEW to display a detailed time course of urinary output, infusion rate and net fluid balance. Fluid infusion rates were measured with a noninvasive flow probe clamped on standard IV tubing (Model 402 Flow Meter Transonic, Ithaca, NY) and interfaced with a National Instruments PCMCIA DAQ card. Urinary volume was collected with a digital Bard Criticore Urimeter (Model 02N Bard, Medical Covington, GA) via a RS-232 serial port.
Results: 12 Patients were monitored continuously for 51±32 SD hrs (12-110 hrs), average TBSA was: 53% ± 26, type of injury was flame (n=8), scald (n=3) and electric (n=1). Inotropics were used in only 1 patient. The FBM displayed UO averaged over 10-min increments providing greater resolution than standard hourly UO collections.
Conclusion: The Fluid Balance Monitor machine provided useful data with great time resolution for urinary output, infused volumes and net fluid balance in patients with burn injuries. Detailed displays and the use of electronic fluid balance monitors may reduce the occurrences of under and over resuscitation that can occur even in the best burn centers.

P154

ROLE OF NADPH OXIDASE AND iNOS IN POST-TRAUMA MYOCARDIAL APOPTOSIS. T. Christopher, L. Tao*, H. Liu*, X. Ma*. Thomas Jefferson University, Philadelphia, PA. We recently demonstrated that overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) play causative roles in cardiomyocyte apoptosis induced by nonfatal mechanical trauma. The present study was designed to determine the molecular sources responsible for increased ROS and RNS production in the traumatized heart. Adult male mice were subjected to nonfatal Noble-Collip drum trauma. Nitric oxide (NO) production and iNOS expression were determined at different time points after trauma. ROS production was determined in the presence and absence of DPI, a selective NADPH oxidase inhibitor. NO production was not increased until 3 hours after trauma and reached its maximal level 6 hours after trauma (6.5-fold over sham trauma). No iNOS expression was detected in heart isolated immediately after trauma. However, clear iNOS expression was detected 3 hours after trauma, and the intensity of the iNOS band further increased thereafter. ROS production (determined by lucigenin-enhanced chemiluminescence) was also significantly increased (2.6 fold over sham trauma) 6 hours after trauma, which was abolished by pre-incubation of traumatized cardiac tissue with DPI. This result suggests that NADPH oxide is a major source of ROS production in the traumatized heart. To further establish a causative link between iNOS-derived NO/NADPH oxidase-derived ROS and post-trauma cardiomyocyte apoptosis, mice were treated with 1400W (a selective iNOS inhibitor) or DPI immediately after trauma. These treatments significantly reduced nitrotyrosine formation in the traumatized heart and markedly decreased trauma-induced cardiomyocyte apoptosis. Taken together, the present study provides direct evidence that iNOS and NADPH oxidase are the major sources for NO and superoxide production, and interventions that inhibit iNOS/NADPH oxidase expression/activity may have therapeutic value in nonfatal trauma patients.

P155

NITRIC OXIDE (NO) DONORS PRODUCE CARDIAC MYOCYTE INFLAMMATORY CYTOKINE RESPONSES THAT ARE cGMP-DEPENDANT. D.L. Maass and J.W. Horton. UT Southwestern, Dallas, TX 75390-9160.

INTRODUCTION: While NO promotes cardiac myocyte secretion of cytokines, accumulation of Na^+/Ca^{2+}, and impairs cardiac function, the mechanisms for these effects remain unclear. In this study, we propose that NO donors SNAP (S-nitroso-N-acetyl-penicillamine) or PAPA (PAPA NONOate) promote cardiac myocyte secretion of inflammatory cytokines by increasing intracellular cGMP levels. Strategies that specifically inhibit cGMP production would be expected to prevent NO donor related cardiac myocyte inflammatory responses. **METHODS**: Cardiac myocytes were prepared (collagenase digestion), cells plated ($5x10^4$ cells/microtiter well), challenged with vehicle or NO donor in the presence/absence of methylene blue (MB, 10μM/l to inhibit cGMP). In parallel studies, 8-bromo-cGMP was added to cardiomyocytes (1.0 mM)/$5x10^4$ cells; TNFα, IL-1β, IL-6 (pg/ml) and cGMP (pmol/ml) levels were measured in the supernatant. Other myocyte aliquots were treated with BPI prior to NO donor challenge to determine if cytokine responses were related to LPS contamination. **RESULTS**: NO donors increased myocyte secretion of TNFα, IL-1β, IL-6, cGMP while MB ablated these effects. BPI studies confirmed that cytokine responses to NO donors were not related to LPS contamination. 8-bromo-cGMP recapitulated the effects of NO donors on myocyte cytokine secretion. (*difference from SNAP alone, $p<0.05$). **CONCLUSION**: The effects of SNAP or PAPA are in part cGMP-dependent; cGMP alters cardiac function by

modulating cardiac myocyte secretion of cytokines. **NIH GM2P50-21681-39.**

	TNFα	IL-1β	IL-6	c-GMP
PAPA, 1.0mM	355±24	155±15	236±44	391±3
PAPA+MB	27±2*	87±4*	65±4*	166±2*
PAPA+BPI	336±18	160±16	241±12	404±3
8bromo-cGMP	300±13	104±9	264±8	425±10

P156

OXYGEN SUBSTRATE LIMITATION REGULATES NITRIC OXIDE PRODUCTION BY CYTOKINE-STIMULATED MACROPHAGES

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Introduction: Excessive production of nitric oxide (NO) by cytokine-stimulated macrophages via inducible nitric oxide synthase (iNOS) contributes to the pathogenesis of septic shock by disrupting both circulatory and pulmonary function. The resulting tissue hypoxia is intriguing because molecular oxygen is a substrate for NO synthesis. Our data and others show that subacute (18 hours) hypoxia concurrent with cytokine stimulation of macrophages decreases NO production. The most obvious mechanism for this decrease is oxygen substrate limitation. However, subacute hypoxia also decreases iNOS dimerization and iNOS protein concentration. All previous studies have been performed with subacute oxygen exposures due to the limitations of standard cell culture. Our laboratory has overcome those limitations by developing a forced convection cell culture system. Using this system, we evaluated the hypothesis that oxygen substrate limitation regulates NO production. **Methods:** Lipopolysaccharide- and interferonγ-stimulated RAW 264.7 macrophages were exposed in random order to one of 8 oxygen tensions for 3 min each. An electrochemical probe continuously measured real time NO concentration. **Results:** Data follow Michaelis-Menton kinetics. Preliminary results suggest a cellular iNOS Km for oxygen of 6 Torr. **Conclusions:** Tissue oxygen tensions are normally between 5 and 70 Torr. During hypoxia-associated diseases, such as septic shock, tissues will rapidly become hypoxic. Our results show that NO production will be immediately reduced by decreased oxygen availability. Therefore, overproduction of NO as a cause of tissue damage may not apply to hypoxic tissues. The mechanisms by which oxygen regulates NO production must be elucidated to fully understand the role NO may play during the pathogenesis of septic shock and other hypoxia-associated diseases.

Funding Source: NIH GM64486

P157

BURN SERUM INDUCED ANTIOXIDANT ACTIVITY DEFECTS AND MITOCHONDRIAL DAMAGE IN CARDIOMYOCYTES.

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Introduction: In vivo burn induced mitochondrial damage and decreased antioxidant defense have been described by us in heart. The present study determined whether burn serum challenge in cardiomyocytes produced similar abnormalities. **Methods**: Burn serum (BS) was collected 24 hr after 40% TBSA burn in adult SD rats. Isolated rat cardiomyocytes were exposed to BS (10% by volume) or control medium for 18 hours. After subcellular fractionation, antioxidant activities (glutathione peroxidase, GPx; superoxide dismutase, SOD; and catalase) were measured in both cytosol and mitochondria. Mitochondrial integrity was examined by measuring mitochondrial outer membrane damage and cytochrome C release from mitochondria to cytosol. **Results:** BS challenge decreased the activity of cytosolic GPx, SOD and catalase 53%, 19% and 12% respectively, and decreased the activity of mitochondrial GPx and SOD 78% and 13% but caused no change in mitochondrial catalase. BS challenge damaged mitochondrial outer membrane and promoted release of cytochrome C from mitochondria to cytosol. **Conclusion**: Burn serum challenge impairs antioxidant activities and alters mitochondrial integrity in cardiomyocytes. **Supported by NIH R0157054.**

		Control	BS 24 hr
Antioxidant Activities			
Cytosol	GxP (unit/g)	1796	840
	SOD (unit/g)	870	702
	Catalase (unit/mg)	71	62
Mitochondria	GxP (unit/g)	405	91
	SOD (unit/g)	667	583
	Catalase (unit/mg)	65	67
Mitochondrial Outer Membrane Damage(%)		9	19
Cytosol Cytochrome C (Fold)		1	2

P158

USE OF ANTIBODY ARRAYS TO IDENTIFY SIGNALING PATHWAYS FOR TRANSFUSION RELATED NEUTROPHIL (PMN) CYTOTOXICITY.

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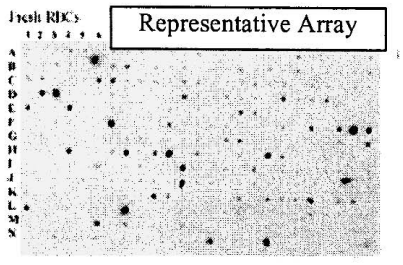
Transfusion of aged stored red blood cells (RBCs) is associated with hyperinflammatory acute lung injury and multi-system organ failure. Plasma from aged stored RBCs primes PMNs and delays apoptosis, but the precise mechanisms and signaling pathways are unknown. The purpose of this study was to employ antibody array technology to identify PMN signaling proteins whose phosphorylation is significantly altered by exposure to aged stored RBCs. **METHODS:** PMNs were isolated from healthy donors and incubated with the plasma fraction of fresh or aged (42 days) RBCs. Antibodies to 350 common cell-signaling proteins were spotted on nitrocellulose membranes and incubated with cell lysate of treated PMNs. Membranes were then probed with anti-phos-



photyrosine antibodies and visualized with ECL. Densitometry was performed and proteins with consistent and marked changes were selected for Western blotting.

Relative change in expression of protein, as related to hours of incubation with aged RBC plasma:

	1	5	24
Bad	↓	0	0
TRAF-6 0	0	0	
PIAS-1	0	0	↑
PIAS-3	↓	↑	0

RESULTS: The array identified changes in phosphorylation of several signaling proteins involved in apoptosis. Changes in protein expression were dependent upon the duration of PMN incubation with RBC plasma (Table). **CONCLUSION:** Arrays allow simultaneous investigation of many potentially relevant cell-signaling proteins. By targeting proteins that are differentially phosphorylated, the signaling pathways involved in transfusion-related immunomodulation may be elucidated.

P159

CANDIDA INFECTION MAY UNRAVEL RELEVANCE OF TLR2 SIGNALING AND SINGLE NUCLEOTIDE POLYMORHPHISM (SNP) ARG753GLN IN SEPSIS

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Eleven human TLRs recognize a wide variety of pathogen associated molecular patterns. TLR2 is a member of the recognition complex for antigenes of Gram-positive bacteria, of candida, and also of CMV. Signal transduction involves activation of the cytoplasmic MyD88 signaling pathway and leads to induction of cytokines. Two cytoplasmatic SNPs of TLR2 have been reported to affect cellular activation: Arg677Trp and Arg753Gln. We asked whether these SNPs might play a role in the manifestation of septic shock, cytokine response to pathogens, and concomitant reactivation of CMV. Thirty-eight ICU patients were studied for their clinical course, infectious complications, inflammatory cytokines and TLR2 genotype.

Methods: Genomic DNA was extracted from whole blood. TLR2 sequence was determined by pyrosequencing. Cytokines where measured by a multiplexing approach for 22 cytokines and Luminex technology.

Results: Two patients with heterozygous SNP Arg753Gln of TLR2 were identified and thirty-six patients were homozygously wildtype. Arg677Trp SNP was not found. During staphylococcal sepsis, patients heterozygous for TLR2 SNP753 elicited a cytokine response similar to the pattern of wildtype patients. In addition, septic shock associated CMV reactivation incidence was identical to wild type patients. However, SNP Arg753Gln heterozygosity was found to be associated with profoundly lower levels of inflammatory cytokine release during the course of severe infectious complications by candidial sepsis manifesting in various episodes of septic shock.

Thus, Arg753Gln SNP may modulate TLR2 signaling induced by fungal infection. It may be hypothesized that TLR2 SNP Arg753Gln contributes to immunological anergy associated with candida infection. Patients with multiple episodes of sepsis and septic shock followed by severe candidiasis should be studied for TLR2 specific signalling in greater detail.

P160

A ROLE OF SERINE/THREONINE PHOSPHATASES IN MLN T CELL SUPPRESSION IN ALCOHOL AND BURN INJURY.

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Serine/threonine-specific protein phosphatases (PP) are involved in signaling pathways controlling T cell functions. We observed that a combined insult of EtOH intoxication and burn injury suppresses mesenteric lymph node (MLN) T cell IL-2 and IFN-? production by inhibiting p38 and ERK-1/2 activation. In this study, we tested the hypothesis that a combined insult of EtOH intoxication and burn injury up-regulates serine/threonine phosphatases which result in decreased T cell function. To test this hypothesis, male rats (250g) were gavaged with EtOH to achieve a blood EtOH level of 100 mg/dL prior to burn or sham injury (25% TBSA). Twenty-four hours after injury, rats were sacrificed and MLN T cells were isolated. T cell IL-2 and IFN-??production after their stimulation with ConA in the presence or absence of PP inhibitors [Calyculin A (CA) and Okadaic acid (OA)] was determined. In addition effects of PP inhibitors was also determined on T cell p38 and ERK-1/2 activation. The suppression in IL-2 and IFN-? production was attenuated in T cell cultured in presence of PP inhibitors (Fig.1). Similarly, treatment of T cell with PP inhibitors also prevented the decrease in p-38 and ERK-1/2 phosphorylation. In summary, these findings suggest that a combined insult of EtOH intoxication and burn injury suppresses MLN T cell IL-2 and IFN-? production by up-regulating serine/threonine protein phosphatases. (supported by NIH AA12901).

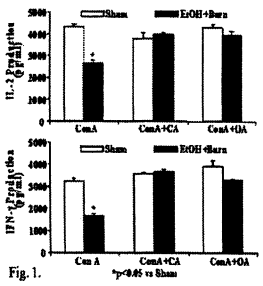


Fig. 1.

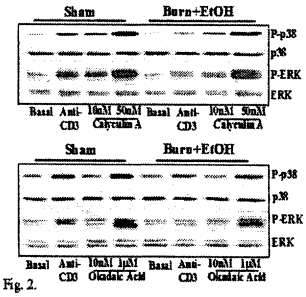


Fig. 2.

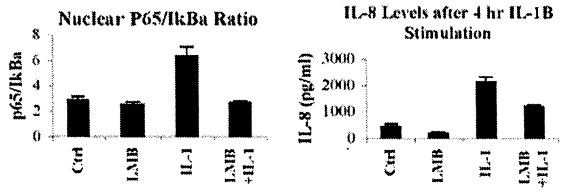
P161

INHIBITION OF NUCLEAR EXPORT ATTENUATES NF-κB SIGNALING.

M .Walsh*, C. Hamiel*, A. Banerjee, A. Cheng, R. McIntyre. University of Colorado, Denver, CO 80262

Activation of NF-κB is mediated by signal induced phosphorylation of IκBα, enabling p65 NF-κB binding to DNA. Termination of gene expression occurs when free IκBα enters the nucleus and binds p65. Leptomycin B (LMB) specifically inhibits Exportin-1, the transport protein that mediates nuclear export of free IκBα as well as the inactive IκBα/p65 complex. We hypothesized that inhibition of IκBα export would increase nuclear IκBα and attenuate NF-κB signaling. **Methods:** Human microvascular endothelial cells (HMVECs) were treated with LMB (20 nM) or vehicle for 1 hr. Cells were then stimulated with IL-1β (100 ng/ml) for 10, 60 or 240 minutes. Supernatants

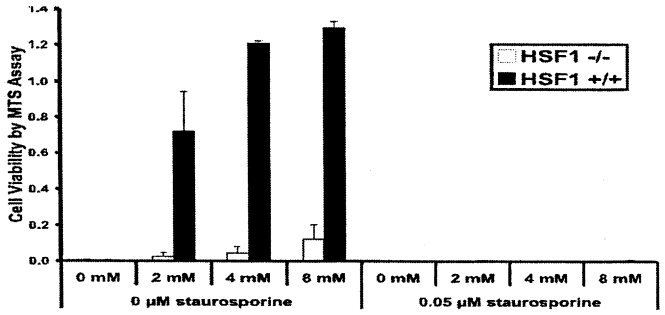
were collected for IL-8 and MCP-1 ELISA analysis, and cell lysates were used for western blotting. Additionally, HMVECs were analyzed by immunofluorescence microscopy for ICAM-1 expression and cellular P65/IκBα localization. **Results:** LMB treatment induced sequestration of IκBα in the nucleus (p=.0012). Thus while IL-1β stimulation increased active NF-κB (assessed as nuclear p65/IκBα ratio) 225% over control levels (p<0.0001), IL-1β+LMB treated cells demonstrated no significant change. Western blotting demonstrated that Il-1β+LMB treated cells demonstrated 38% less IκBα phosphorylation compared with IL-1β treated cells (p=0.02) after 10 minutes of stimulation. Accordingly, IL-1β +LMB treated cells demonstrated a 44% decrease in IL-8 (pg/ml; 2132±197 vs 1192±76; p<0.0001) production, a 33% decrease in MCP-1 (pg/ml; 9285±442 vs 6226±320; p< 0.0001) and a 90% (p<0.0004) decrease in ICAM-1 expression compared to IL-1β only. **Conclusion:** Inhibition of IκBα export effectively attenuates p65 DNA binding and diminishes signal induced NF-κB activation by sequestration of IκBα/p65 complexes in the nucleus.



P162

INHIBITION OF PROTEIN KINASE C ACTIVITY PREVENTS GLUTAMINE MEDIATED HSP-70 INDUCTION AND CELLULAR PROTECTION A. Morrison,* P. Wischmeyer. University of Colorado Health Sciences Center, Denver, CO, 80262.

Glutamine (GLN) has been shown to prevent cell death via a heat stress protein (HSP) dependent pathway. The mechanism leading to this induction of HSPs is currently unknown. Protein Kinase C (PKC) activation has been shown to be involved in the regulation of HSP pathway activation. Thus, we hypothesized that GLN may be acting to upregulate HSP expression and provide cellular protection via activation of PKC. To evaluate this, fibroblasts from Heat Shock Factor-1 (HSF-1) knock-out (HSF-/-) and wild type mice (HSF+/+) were treated with 0.05 μM staurosporine (STP) for 15 min., then treated with either 0 or 8 mM GLN and immediately exposed to a sub-lethal heat stress (43° C for 45 min). These cells were then assayed via western blot for HSP-70 expression. In a separate experiment, cellular protection against a lethal heat stress was assayed via the MTS assay. Cells were treated with +/- STP and GLN doses ranging from 0-8 mM immediately prior to lethal heat stress (44° C for 50 min). STP treatment led to significant inhibition of GLN mediated HSP-70 expression in HSF +/+ cells. The cell viability results are shown in figure. No toxicity from STP treatment alone was observed. Inhibition of PKC activity with STP led to inhibition of GLN-mediated HSP-70 induction and blocked GLN-mediated cellular protection. GLN may activate the HSP pathway via enhanced PKC activity.



P163

CHANGES IN INTRACELLULAR CALCIUM REGULATE HEPATOCYTE NITRIC OXIDE SYNTHESIS.

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A number of cell surface receptors can alter signalling pathways that induce changes in intracellular calcium (Ca2+) concentrations and Ca2+ can regulate many areas of hepatocyte metabolism. Ca2+ can also alter the expression of genes through the activation of kinases and phosphatases that are stimulated by increased intracellular Ca2+. The role of Ca2+ in regulating hepatocyte iNOS expression and activity is unknown. We tested the hypothesis that Ca2+ regulates hepatocyte iNOS expression by culturing rat hepatocytes in vitro with proinflammatory cytokines to induce iNOS in the presence of the ionophore A23187 and the calcium channel blocker nifedipine and measured the effects on iNOS expression. Total RNA was collected at 3 hours and iNOS mRNA expression measured by Northern blot. iNOS protein expression was measured by Western blot from cytosol collected at 24 hours and iNOS enzyme activity was assessed by measuring supernatant nitrite in 24 hour cultures using the Griess reaction. Proinflammatory cytokines stimulated a significant increase in iNOS mRNA levels, cytosolic iNOS protein levels, and supernatant nitrite (Table). The Ca2+ ionophore A23187 significantly decreased cytokine-stimulated iNOS mRNA, iNOS protein, and supernatant nitrite while nifedipine increased supernatant nitrite compared to cytokines alone. These data demonstrate that increased intracellular Ca2+ inhibits cytokine-stimulated iNOS expression. They suggest that Ca2+-mediated signalling downregulates iNOS expression and support the hypothesis that Ca2+-stimulated kinases may mediate this effect.

	Cyt	A23187 10^{-6}	A23187 10^{-5}	Nifed 10^{-5}
NO_2,% control	100± 0	38 ± 0*	7 ± 1*	154 ± 11*
INOS mRNA	++++	+++	+	N/A
INOS protein	++++	++++	+	N/A

P164

DIFFERENTIAL REGULATION OF MAPK ACTIVATION BY TNF IN ADHERENT AND NON-ADHERENT NEUTROPHILS: A ROLE FOR δ-PKC L.E. Kilpatrick, S. Sun*, C. Ho* and H.M. Korchak*. Univ. of Pennsylvania and Children's Hospital of Phila., Philadelphia, PA 19104

TNF is a unique cytokine whose signaling is linked to both anti-apoptotic and pro-apoptotic pathways. In neutrophils (PMN), TNF activates members of the mitogen-activated protein kinase (MAPK) cascade which are important effectors of PMN apoptosis. TNF signaling is influenced by adherence and the ligation of β-integrins. Previously, we identified δ-PKC as a critical regulator of TNF signaling in adherent PMN but the role of δ-PKC in MAPK activation is not known. In this study, we examined the role of crosstalk between β-integrins and TNF signaling on activation of the MAP kinases p44/42 MAPK (ERK1/2) and p38 MAPK. TNF triggered activation of p44/42 and p38 MAPK in both non-adherent and fibronectin (FN)-adherent PMN. However, TNF mediated activation of p44/42 and p38 MAPK was 2-fold greater in non-adherent PMN as compared to FN-adherent PMN suggesting β-integrins downregulate MAPK activation. In non-adherent PMN, inhibition of δ-PKC with the specific antagonist δ-V1.1 PKC Tat peptide significantly inhibited TNF mediated activation of both p44/42 MAPK (68% of TNF+Tat carrier peptide alone, $p<0.02$) and p38 MAPK (85% of TNF+Tat, $p<0.03$). Thus, in non-adherent PMN, δ-PKC is a positive regulator of p44/42 and p38 MAPK activation by TNF. Conversely, in FN-adherent PMN, δ-PKC inhibition enhanced p44/42 MAPK (158% of TNF+Tat, $p<0.01$) activation but had no effect on p38 MAPK suggesting differential regulation of these MAPK by TNF in FN-adherent cells. These results indicate that in FN-adherent PMN, δ-PKC is a negative regulator of p44/42 MAPK activation but has no regulatory role in p38 MAPK activation. Thus, β-integrin signaling converts δ-PKC from a positive regulator of TNF mediated activation of p44/42 and p38 MAPK to a negative regulator of p44/42 activation. (Supported by NIH GM64552 and AI24840).

P165

Title: CpG ODN STIMULATION OF HUMAN BRONCHIAL EPITHELIAL CELLS. N. Parilla, V. Hughes*, M. Haas*, K. Page* Cincinnati Children's Hospital, Cincinnati, OH

OBJECTIVE: Bacterial DNA does not exist alone, but in the presence of organisms and inflammatory mediators such as interleukin (IL)-1β or tumor necrosis factor (TNF)α. Unmethylated bacterial CpG motifs have been shown to increase cytokine expression in immune cells. However, the role of CpG motifs on human airway epithelium is currently unknown. Therefore, we investigated the effect of CpG oligodeoxynucleotides (ODN) in the presence or absence of either IL-1β or TNFα.
METHODS: SV40-transformed human bronchial epithelial cells (16HBE14o-) were treated with synthetic CpG ODN or control ODN in the presence or absence of IL-1β or TNFα. Toll like receptor (TLR)9 expression was determined by RT-PCR and western blot. Expression of IL-8 was determined by quantitative real time PCR and ELISA. Cells were pretreated for 1 hr with chloroquine, a TLR9 signaling inhibitor or PD98059, an extracellular regulated kinase (ERK), inhibitor prior to treatment with CpG ODN and IL-1β. ERK and IκBα were detected by western blot.
RESULTS: 16HBE14o- cells express TLR9, the receptor for CpG ODN. Treatment with CpG ODN in the presence of either IL-1β or TNFα synergistically increased IL-8 mRNA and protein levels. CpG ODN alone did not regulate IL-8 expression, nor did the control ODN. Chloroquine attenuated CpG ODN's effect, suggesting the importance of TLR9 activation. Since IL-8 is primarily regulated by nuclear factor (NF)-κB, we tested whether CpG ODN regulated NF-κB activation. CpG ODN had no effect on IκBα degradation, a marker for NF-κB translocation to the nucleus. However, we found that CpG ODN increased ERK phosphorylation, and pretreatment of cells with PD98059 attenuated CpG ODN's effect.
CONCLUSIONS: This study shows that in human bronchial epithelial cells CpG ODN in the presence of inflammatory cytokines (IL-1β or TNFα) synergistically increased IL-8 expression by ERK activation.

P166

GENDER DEPENDENT INHIBITION OF TNF SIGNALING IN PRIMARY HUMAN SMOOTH MUSCLE CELLS C. Hamiel*, F. Gamboni-Robertson*, G.Escobar*, A.Banerjee. University of Colorado Health Sciences Center, CO 80262

Cholesterol enriched microdomains in the plasma membrane (rafts and caveolae) provide platforms for assembly of receptors with signaling complexes. Because of the prominent differences in inflammation affecting vasculature (e.g. atherosclerosis, trauma) between males and females we investigated the expression of ICAM 1 stimulated with TNFa in primary vascular smooth muscle cells (hVSMC). We hypothesized that cholesterol dependent scaffolding would affect TNF signaling in males.
Methods. Primary human VSMC were isolated from arterial vessels of liver donors and grown in culture. Cells between passages 2-7 were treated with TNFα (10ng/ml) for 24 hours. ICAM expression was assessed by quantitative fluorescent microscopy and Western blots. Cholesterol was depleted by 3.5mM β-methylcyclodextrin (Cyclo). Inhibition was assessed as a percent of the ICAM inducible in the donor (means ±SEM).
Results. TNFα increased ICAM expression >100 fold irrespective of gender, n=4 each. Cyclo reduced it by (59±3%, $p<0.01$) in males but hardly in females (18±12%, NS)). However chemokine synthesis (e.g. IL-6) and cell division were unaffected. In contrast, blocking Clathrin mediated endocytosis (Chloroquine 50uM) inhibited both males and females similarly (76% and 69% respectively, $p<0.01$ vs TNFα stimulated ICAM expression).

Conclusion: Male hVSMC maybe more dependent on cholesterol dependent rafts and caveolae, for TNFα signaling. ICAM induction efficiency by TNFα did not appear statistically different in the limited age ranges investigated, but inhibiting clathrin scaffolds affected both genders. This suggests that vascular inflammation in males and females could be treated differently and that the specific TNFα signaling pathway used by females needs exploration.

P167

DIMINSHING POSITIVE INOTROPIC EFFECTS OF BigET-1 AND p38-MAPK UPREGULATION FOLLOWING SEPSIS.

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In the present study we hypothesized that bigET-1 in adult rat ventricular myocytes (ARVM) would lead to a progressive unresponsiveness to contractility during sepsis. Sepsis was induced in male-SD rats (350-450g) using cecal inoculum (200mg/kg i.p.). The heart was harvested from each anesthetized rat and single ARVM isolated by retrograde perfusion with collagenase. The mechanical properties were assessed using a video-based edge detection system (IonOptix Co-op). Peak shortening (PS), maximal velocity of myocyte shortening (+dL/dt) and maximal velocity of relengthning (-dL/dt) were measured at 0, 3, 6 and 24h following bigET-1 (100nM) in sham and septic ARVM. Septic ARVM exhibited a depressed contractile function (PS & ±dL/dt) at 0h as compared to sham group. BigET-1 at 3h could increase ±dL/dt and PS as compared to 0h sepsis but at 6h there was no significant difference in the ±dL/dt and PS values, as compared to the respective vehicle treated sham and septic groups. At 24h post treatment septic ARVM remained unresponsive to varying doses of bigET-1, as opposed to sham, however they displayed an increased contractile response as compared to the respective sham groups. Immunoblot analysis at 0, 5, 15 and 30 minute intervals showed a progressive increase in phosphorylation of p38-MAPK in septic ARVM. The data suggest that bigET-1 exerts its positive inotropic effect only upto 3h in septic ARVM. However its effect on the phosphorylation of p38-MAPK remains sustained until 24h after ARVM are in culture, with more pronounced effects at the early stages of sepsis. We concluded that sepsis caused a diminished contractile response to bigET-1 in ARVM possibly via p38-MAPK phosphorylation. *(Supported by NHLBI, 66016).*

P168

PHOSPHORYLATED IκB LEVELS DO NOT PREDICT THE INFLAMMATORY READOUTS IL-8, ICAM-1 AND MCP-1 IN PULMONARY ENDOTHELIAL CELLS

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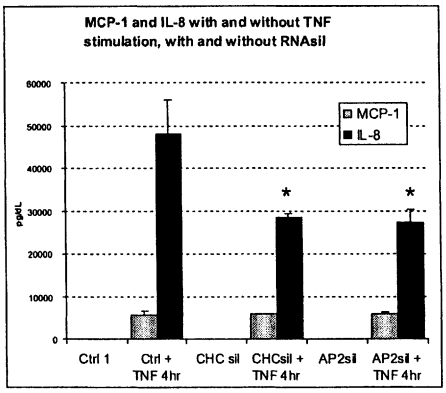
The endothelial gene products IL-8, ICAM-1 and MCP-1 are widely cited as inflammatory response markers, classically thought to be modulated by NF-kB. Conversely, production of these markers has often been equated to NF-kB activation. We investigated the dependence of NF-kB activation in IL-8, ICAM-1 and MCP-1 expression by disrupting TNFα signaling scaffolds with small interfering RNA (siRNA) directed at endocytosis. **Methods**: Human microvascular endothelial cells (HMVEC) were exposed to a mock transfection, or transfection with clathrin heavy chain (CHC) siRNA or AP2 siRNA. HMVEC were treated with TNFα 10ng/ml for 10 min or 4 hr. NF-kB activation was indirectly determined by measuring Phospho-IkB (pIKB) by western blot (WB). ICAM-1 was detected by WB and quantitated by fluorescent; IL-8 and MCP-1, by ELISA. **Results**: TNFα increased pIkB, ICAM-1, IL-8, and MCP-1 expression ($p<0.05$). However, mock transfection alone differentially augmented these markers despite no increase in pIkB. Interfering with CHC and AP2 decreased pIκB, ICAM-1 and IL-8 ($p<0.05$), but not MCP1. **Conclusion**: ICAM-1, MCP-1 and IL-8 expression induced by TNFα may not be exclusively determined by the NF-kB pathway in HMVEC.



P169

HYPERTONIC SALINE REGULATES T CELL FUNCTION THROUGH OPPOSING EFFECTS OF EXTRACELLULAR ATP AND ADENOSINE

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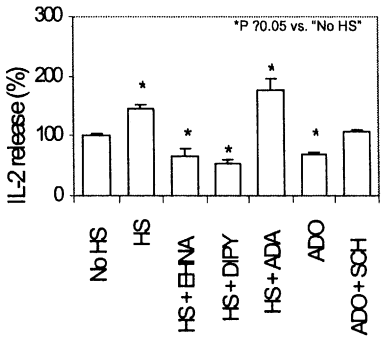
Hypertonic saline (HS) enhances IL-2 release and the proliferation of T cells. We previously showed that ATP release is involved in these enhancing effects of HS. ATP can be converted to adenosine (Ado) by a number of endogenous enzymes. Here we examined the role of Ado in the response of isolated human mononuclear cells to HS. HS (40 mM) enhanced IL-2 release of T cells. This enhancement was sensitive to drugs that alter extracellular Ado levels. Agents that increase extracellular Ado, such as the adenosine deaminase (ADA) inhibitor EHNA and the nucleoside transporter inhibitor dipyridamole (DIPY) abolished HS-induced enhancements of IL-2 release, while

the Ado-metabolizing enzyme ADA further enhanced IL-2 release (Fig). Ado suppressed IL-2 release, an effect abolished by the A2A adenosine receptor antagonist SCH58261, suggesting that this receptor subtype mediates the immunosuppressive actions of Ado. These data suggest that HS may exert its immunomodulatory effects on T cells through opposing effects of ATP and Ado. Thus, the balance of these opposing effects represents potential pharmacological targets to increase the beneficial immunomodulatory effects of HS resuscitation in trauma patients. Support: NIH Grant GM51477.



P170

CHOLINERGIC AGONISTS INHIBIT HMGB1 RELEASE AND IMPROVE SURVIVAL IN SEPSIS.

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Physiological anti-inflammatory mechanisms represent efficient systems that can be exploited for the treatment of inflammatory disorders. Here we report that acetylcholine, the principal neurotransmitter of the vagus nerve, inhibits HMGB1 release from human macrophages by signaling through a nicotinic acetylcholine receptor. Nicotine, a more selective cholinergic agonist, is more efficient than acetylcholine (IC_{50}=2.3±0.5μM vs Nicotine IC_{50}=1.2±0.4μM), and inhibits HMGB1 release induced by either endotoxin or TNF. Nicotinic stimulation preserves nuclear location of HMGB1, and inhibits the NF–κB activation through a specific "nicotinic anti-inflammatory pathway" that requires the α7 nicotinic acetylcholine receptor (α7nAChR). *In vivo*, nicotine administration attenuates serum HMGB1 levels, prevents lethal endotoxemia, and improves survival in established polymicrobial peritonitis (survival in nicotine-treated group = 84% (32/38); survival in vehicle-treated group= 51% (19/37); P<0.05), even when treatment is started after the appearance of the clinical signs of sepsis. These results reveal acetylcholine as the first known physiological inhibitor of HMGB1 release from human macrophages, and suggest that selective nicotinic agonists for the α7nAChR might provide a therapeutic potential for the treatment of "established" sepsis in a clinically relevant time frame.

This research was supported by the Faculty Awards Program of the North Shore Research Institute, NIGMS, the North Shore-LIJ GCRC, and DARPA.

P171

PREOPERATIVE IMMUNE-ENHANCING NUTRITIONAL SUPPLEMENTATION CORRECTS TH1/TH2 IMBALANCE UNDERGOING ELECTIVE SURGERY FOR COLORECTAL CANCER.

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Aim: Recent studies showed that Th1/Th2 balance shifts toward Th2 dominance on cancer bearing state or by surgical stress. Preoperative immunonutrition is reported to improve outcome in patients with gastrointestinal cancer. This study is designed to evaluate whether preoperative immunonutrition modulate Th1/Th2 balance following colorectal surgery.

Methods: Thirty-six patients with colorectal cancer were divided into two groups as follows: oral intake supplementation for 5 days before surgery with a formula enriched with arginine, ω-3 fatty acids and ribonucleic acid (supplemented group; n=19); and no supplementation before and after surgery (control group; n=17). Blood sampling was performed before supplementation (POD -5), just before the operation (POD 0), postoperative day 3 7 and 14 (POD 3, 7 and 14). The proportions of $CD4^+$ T-cells producing intracellular cytokines (interferon (IFN)-γ and interleukin (IL)-4) were measured by flow cytometry.

Results: On POD-0, the proportions of $CD4^+$ T-cells producing IL-4 significantly decreased and Th1/Th2 ratio significantly increased, compared with those on POD -5. On postoperative periods, the proportions of $CD4^+$ T-cells producing IFN-γ in both groups maintained preoperative levels. The proportions of $CD4^+$ T-cells producing IL-4 in control group showed a gradual increase from the preoperative level. In contrast, supplemented group maintained the preoperative level. The Th1/Th2 balance gradually shifted toward Th2 dominance in control group, however, supplemented group maintained the preoperative level.

Conclusion: Preoperative immunonutrition corrects impaired Th1/Th2 balance both on cancer bearing state and on postoperative periods. This correction may be one of the important determinants of clinical benefits of immunonutrition.

P172

ADENOSINE A2A RECEPTOR ACTIVATION PROMOTES T HELPER 2 LYMPHOCYTE DIFFERENTIATION.

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Objective: Sepsis induces a shift in lymphocyte development from a T helper (Th)1 to a Th2 profile, which

may compromise the ability of the host to combat the invading microorganisms. Extracellular levels of the endogenous purine mediator adenosine are increased in patients with sepsis. Adenosine has potent immunoregulatory effects, most of which are mediated by Gs-coupled A2a receptors on immune cells, including T lymphocytes. Since activation of Gs-coupled pathways has been reported to augment Th2 versus Th1 differentiation, we hypothesized that A2a receptor stimulation would increase the Th2/Th1 ratio. **Methods:** CD4+ T cells were purified from spleens of CD-1 mice using antibody-coated magnetic beads. Cells were treated with plate-bound anti-CD3 antibody and anti-CD28 antibody in the presence of IL-12 to induce Th1 development, or with anti-CD3/CD28 in the presence of IL-4 to achieve Th2 cell development. The cells were also treated with increasing concentrations of the A2a receptor agonist CGS-21680 in the presence or absence of the A2a antagonist ZM241385. After 5 days, the cells were washed and restimulated with soluble anti-CD3 antibody for 24 hours, following which supernatants were collected and analyzed for Th1 (IFN-γ) and Th2 (IL-4 and IL-5) cytokine content using ELISA. **Results:** CGS-21680 increased IL-4 and IL-5 production by Th2 cells but failed to affect IFN-γ production by Th1 cells. The potentiating effect of CGS-21680 on IL-4 and IL-5 production was prevented by ZM241385. **Conclusion:** A2a receptor stimulation produces an increased Th2/Th1 ratio by enhancing Th2 cell development while not affecting Th1 cell differentiation. Endogenous adenosine may contribute to the phenotypic shift from predominance of Th1 cell function to that of Th2 cells observed in sepsis.

P173

A PIVOTAL ROLE FOR NK-CELLS AND DHEA IN A MURINE TRAUMA/SEPSIS MODEL

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NK-cells are part of the innate immune response. During trauma and sepsis, the number of NK-cells are increased and are associated with better outcome. The outcome can be improved by the administration of the steroid hormone DHEA. Administration of DHEA to mice subjected to femur fracture/hemorrhage and subsequent CLP results in improved survival. Furthermore, the percentage of circulating NK-cells is increased. Therefore, the positive effects of DHEA may be mediated via NK-cell function. This hypothesis was tested using the murine trauma model with subsequent sepsis in combination with NK-cell depletion. Sepsis was induced by CLP, which was preceded 48 hours by a femur fracture, produced using a blunt guillotine device. This fracture was combined with fluid substitution after 1h. 1h after CLP, an NK-cell depleting antibody was administered intravenously. Additionally, animals were daily treated with DHEA 25 mg/kg. Animals were sacrificed 96h after CLP. NK-cell depletion resulted in 100% survival regardless of DHEA treatment. The serum concentrations of IL-6, TNF-α and IL-10 were significantly reduced in the NK-cell depleted animals. DHEA partly further diminished these levels. Moreover, CD4+ T-cells showed a marked reduction in the blood compartment. Lung injury, evaluated by protein extravasation and histologically, was significantly less pathologic in DHEA treated animals and in all NK-cell depleted animals. Especially, granulocyte infiltration was significantly reduced. Thus, the depletion of NK-cells has a positive effect in a multi-hit model of trauma with subsequent sepsis. The effect of DHEA may be partly mediated by NK-cells, as it was still able to reduce cytokine serum levels in NK-cell depleted animals. However, as NK-cell depletion per se was advantageous, the effect of DHEA is hard to determine. Therefore, it is important to further elucidate the functional activity of NK-cells during trauma and sepsis together with DHEA treatment.

P174

ENHANCEMENT OF DENDRITIC CELL PRODUCTION AND FUNCTION BY FMS-LIKE TYROSINE KINASE-3 LIGAND DECREASES SUSCEPTIBILITY OF MICE TO BURN WOUND INFECTION.

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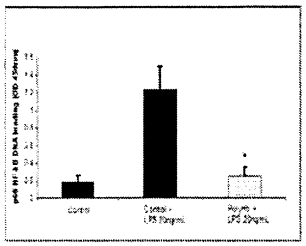
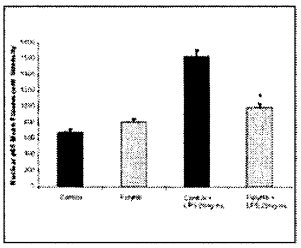
Burn injury causes susceptibility to infections with opportunistic organisms such as *Pseudomonas aeruginosa*. We have examined the effects of Fms-like tyrosine kinase-3 ligand (Flt3L), a hemopoietic cytokine and dendritic cell (DC) growth factor, on resistance to *P. aeruginosa* wound infection in burned mice. Survival after burn wound infection was significantly greater in mice treated with Flt3L compared to control-treated mice, and was associated with increased numbers of splenic DCs and enhanced bacterial clearance within burn wounds. Resistance to burn wound infection was also conferred by adoptive transfer of DCs that had been harvested from spleens of Flt3L-treated, but not control-treated, mice. To determine differences in properties of DCs harvested from Flt3L- and control-treated mice, *in vitro* antigen uptake and surface expression of MHC II, CD11c, and costimulatory molecules were examined in freshly isolated splenic DCs by flow cytometry. In the absence of antigenic stimulation, surface expression of the costimulatory molecules CD80, CD86, and CD40 was low and not different between treatment groups. Although the percentages of DCs expressing high levels of surface MHC II and CD11c were greater after Flt3L treatments, uptake of FITC-ovalbumin was also greater. Therefore, while increased surface expression of CD11c and the antigen presentation molecule MHC II suggests that Flt3L-treated mice may have more mature DCs, high antigen uptake and low costimulatory molecule expression in DCs from Flt3L-treated mice are suggestive of an immature phenotype. In conclusion, these data suggest that Flt3L increases resistance of mice to burn wound infection through modulation of DC production and properties that may prime for enhanced bacterial clearance.

P175

A POLYMERIZED HEMOGLOBIN BASED OXYGEN CARRIER INHIBITS NF-kB ACTIVATION IN HUMAN PULMONARY ENDOTHELIAL CELLS. A Cheng, E Moore, M Walsh,* C Hamiel,* J Johnson, A Banerjee. Denver Health/UCHSC, Denver, CO 80262

Previous clinical trials and in vivo studies using polymerized hemoglobin (PolyHb) suggested that this hemoglobin-based oxygen carrier (HBOC) has anti-inflammatory properties. In this study we hypothesize that the HBOC attenuates TLR_4-dependent NF-kB activation. **Methods:** Human pulmonary endothelial cell cultures were pre-incubated with a 50% (v/v) PolyHb/cell media mixture for 5 hrs. After pre-incubation, cells were stimulated with LPS in fresh media for 30 min. Nuclear extracts of p65 NF-kB DNA binding was determined, and cellular p65 intensity was quantitated by immunofluorescent microscopy. **Results:** The PolyHb-LPS group decreased p65 DNA binding in nuclear lysates by 79% compared to the Control-LPS group, (1.232 ± 0.273 vs. 0.255 ± 0.05, $p < 0.05$). By microscopy, PolyHb-LPS group had decreased intensity of p65 in the nucleus compared to the Control-LPS group (MFI/Cell: 176.0 vs. 234.9, $p<0.05$). Additionally, the nuclear: cytoplasmic ratio of p65 in the PolyHb-LPS group was decreased compared to the Control-LPS, 21% vs 26% ($p<0.05$).





Conclusions: Polymerized hemoglobin attenuates LPS-stimulated NF-kB activation of human pulmonary endothelium, partly through inhibiting p65 translocation to the nucleus. This suggests that the anti-inflammatory properties observed clinically using this HBOC is through modulating the NF-kB pathway. Further studies are needed to elucidate the molecular mechanism(s) of this potential beneficial down-regulation of the innate immune response to inflammatory stress.

P176

EXPRESSION OF TLR-2, TLR-4, CD14 AND CD11b/c ON NEUTROPHILS AND MONOCYTES IN WHOLE BLOOD FROM SEPTIC PATIENTS. M. Brunialti*, P. Martins*, M. Fernandes*, L. Martos*, L. Martins*, F. Machado*, M. Assuncao*, R. Salomao. Federal University of S. Paulo, Brazil.

Cellular response is modulated during sepsis, with down regulation of monocytes derived inflammatory cytokines, and increased ROS production by neutrophils. The effects of bacterial products are mediated through surface receptors, such as TLRs and CD14, and possible CD11. In this study we evaluated the expression of these cell surface receptors in monocytes and neutrophils in whole blood from patients with different stages of sepsis by flow cytometry. Neutrophils and monocytes were identified based on cell size and granularity and the expression of CD66b and CD14 respectively. Results of the receptors expression are shown as mean ±SD of the geometric mean fluorescence intensity (GMFI) in tables 1 and 2.

Table1. Surface expression of receptors on monocytes.

	sepsis (n=11)	s. sepsis (n=9)	shock (n=15)	h. donnors (n=15)	p
CD14	121±52	110±54	122±37	211±64	NS
CD11b	438±352	332±365	251±175	207±169	NS
CD11c	231±142	158±75	160±104	179±104	NS
TLR-2	57±29	48±24	51±22	59±25	NS
TLR-4	10±6	12±10	10±6	8±6	NS

Table2. Surface expression of receptors on neutrophils.

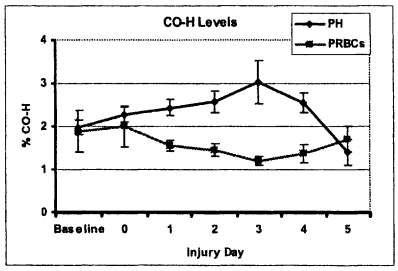
	sepsis	s. sepsis	shock	h. donnors	p
CD11b	471±466	316±287	176±123	239±272	NS
CD11c	123±67	106±57	88±43	77±55	NS
TLR-2	15±19	16±9	9±6	11±5	NS
TLR-4	5±4	7±5	5±5	4±2	NS

In conclusion, TLR-2 and TLR-4 were similarly expressed in patients and h. donnors, while a trend to enhanced expression of CD11b/c was found in the early stages of sepsis. Although not significantly CD14 was decreased on monocytes from septic patients.

P177

EVIDENCE FOR THERAPEUTIC INDUCTION OF HO-1 IN PATIENTS TRANSFUSED WITH POLYMERIZED HEMOGLOBIN. J. Johnson, E. Moore, J. Long* and A. Cheng*, Denver Health Medical Center and the University of Colorado Health Sciences Center, Denver, CO, 80204

Background: Transfusion of human polymerized hemoglobin in lieu of banked red blood cells appears to ameliorate postinjury leukocyte priming, cytokinemia and organ dysfunction. While these effects may be due to the avoidance of PRBCs, it may be that polymerized hemoglobin has a protective effect. Heme compounds induce Heme Oxygenase-1 (HO-1), which is anti-inflammatory, in part due to enzymatic production of carbon monoxide (CO). Preliminary in vitro studies show induction of HO-1 by PH. We hypothesized that patients transfused with PH would have evidence of higher CO production than a similar group transfused with PRBCs. Methods: A retrospective analysis of carboxyhemoglobin (CO-H) levels in critically injured patients resuscitated with PH or PRBCs. CO-H levels are measured on routine arterial blood gasses. A worst values (highest) approach was used for the serial measurements. Demographic data, ISS, blood pressure and heart rate were collected to compare baseline characteristics. Values were compared by Student's t-test. Results (See Figure): CO-H levels



were higher in the group transfused with PH, peaking on the third postinjury day (3.03 ± 0.49 vs 1.20 ± .09, $p<.05$). There were no statistically significant differences in baseline characteristics. Conclusions: Patients transfused with PH have an increased CO-H compared to patients resuscitated with PRBCs. This suggests increased CO production from induction of HO-1 and corroborate our in vitro observations. Increased CO production may be a mechanism by which PH has an anti-inflammatory effect.

P178

AN UNANTICIPATED EFFECT OF TLR2 STIMULATION ON IMMUNE FUNCTION AFTER INJURY

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The biological activities of bacterial lipopeptide, (BLP), a Toll-like receptor 2 (TLR2) agonist that promotes innate immune cell activation and interferon-γ (IFN-γ) production suggested to us that it may act as an adjuvant in vivo to help boost immune functions in normal or burn-injured mice. To test this hypothesis, we established an effective in vivo dose for BLP treatment in mice by measuring the influence of BLP on antibody isotype formation in immunized groups of sham- and burn-injured male C57BL/6J mice. Using the optimal dose (500 ng/mouse) of BLP, we then tested whether BLP treatment might protect burn mice from developing suppressed resistance to sepsis induced by the cecum ligation and puncture (CLP) method at 7 days after sham or burn injury. Surprisingly, we found that BLP treatment did not improve immune function. Instead, we observed a tendency for BLP treated mice to display lowered resistance to CLP challenge (% survival shown in Table, n=18 mice per group). Though a

Sham	Sham + BLP	Burn	Burn + BLP
68%	56%	28%	11%

negative outcome, we next examined several potential mechanisms that might account for this finding. First, we found that spleen cells prepared from BLP-treated sham or burn injured mice produced less IFN-γ when stimulated with polyclonal T-cell stimuli. Second, BLP treatment reduced cellular responses to bacterial lipopolysaccharide (LPS), BLP, and bacterial DNA (CpG ODN) as judged by TNFα, IL-6, IL-10, and MCP-1 production levels. Thus, it appears that BLP treatment promotes a more immune suppressive type response in injured mice than anticipated. Our findings also suggest the possibility that an early activation of the TLR2 pathway may play a role in initiating suppressed immune function after severe injury.

P179

INDUCTION OF TOLERIZING DENDRITIC CELLS MAY CONTRIBUTE TO T CELL DYSFUNCTIONS IN TRAUMA PATIENTS.

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This laboratory has previously shown that trauma patients' dendritic cells (DC) are dysfunctional. One aspect of DC dysfunction is the development of tolerizing DC (Tol DC) when partially differentiated immature DCs are exposed to IL-10. We hypothesize that some patients' dysfunctional DC might have additional defects beyond their failure to act as antigen presenting cells. We have made tol DC by culture of normal control monocytes (n=10) with IL-4 + GM-CSF for 3 days followed by treatment with IL-10 for 2 days parallel to treating their monocytes with IL-4+GM-CSF (classic DC). Normals' tol DCs significantly ($p<0.05$) inhibited (27% ± 6 as compared to their classic DC) naïve autologous T cell proliferation in response to immobilized anti-CD3+CD28. It has been suggested that tol DC can also further suppress responses of autologous anergic T cells rendering them even more unresponsive. Anergic T cells were generated in normal controls by treatment of their T cells with immobilized anti-CD3+ soluble CD47 for 4 days (negative signaling anergy induction system). Addition of controls' tol DCs to their autologous anergic T cells further intensified the defects in those anergic T cells (42% ± 4 depression of proliferation as compared to addition of their classic DC). In preliminary experiments, we investigated the effects of adding trauma patients' dysfunctional DCs to their still proliferation responsive T cells. The patients' dysfunctional DCs suppressed the anti-CD3+CD28 induced proliferative responses of autologous T cells by 45%. These data strongly suggest that some trauma patients' dysfunctional DCs may also be tolerogenic.

P180

COMPLEMENT C3 IS NECESSARY FOR EARLY SUPPRESSION OF INTRAPULMONARY BACTERIAL GROWTH.

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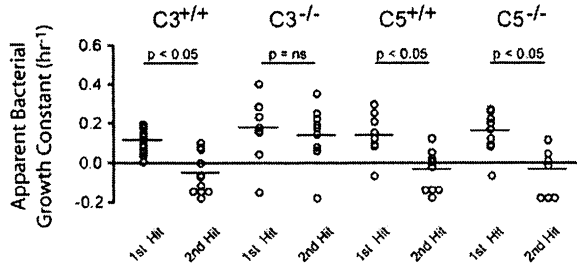
A central task for host defense early in pneumonia is suppression of bacterial growth, which may be accomplished in part by complement activation. To study C3 and C5 in early lung defense we devised a 2-hit model whereby bacterial growth rates throughout the course of pneumonia could be monitored. A galactose-nonfermenting (*Gal*$^-$), but equally virulent, strain of *Klebsiella pneumoniae* was established that could be differentiated from *Gal*$^+$ WT. Separated by 4 hours, the 2 strains were introduced by aspiration into mice. At 24 hours, lung culture on gal indicator agar allowed the two inocula to be independently quantified such that 1st order kinetic growth constants (as hr^{-1}) could be calculated for each 'hit.' Differences in growth constants between the 1st and 2nd hit were a reflection of changes in the fate of

bacteria entering a progressively defended lung. C3- and C5-deficient mice and their controls were studied. After initial infection, $C3^{+/+}$, $C5^{+/+}$, and $C5^{-/-}$ mice suppressed the growth of subsequent inocula (growth constant < 0 for each for the 2nd hit), while $C3^{-/-}$ animals failed to control the growth of the 2nd bacterial hit. Our data demonstrate a novel means of characterizing C3's contribution to early suppression of bacterial growth in acute pneumonia.



P181

EVIDENCE OF MYOCARDIAL HIBERNATION IN THE SEPTIC HEART.

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Cellular hibernation has been proposed as a potential mechanism of sepsis induced organ dysfunction. Sepsis-associated cardiac dysfunction may be due to myocardial hibernation. Described as an adaptive response to ischemia and hypoxia, hibernating myocardium is hypocontractile and demonstrates characteristic changes including a switch in substrate from fatty acids to glucose, upregulation of glucose transporters (GLUT1 and GLUT4), and glycogen deposition. Here we evaluate septic myocardium for these specific changes. Under anesthesia, male C57Bl6 mice underwent cecal ligation and double puncture (CLP). Mice were studied at 48 hours post procedure and compared with sham operation (SO) and non-operative controls (T0) (N=3). Cardiac performance was measured with ECG-gated MRI. We performed cardiac PET imaging following 18-FDG injection, immunoblotting for GLUT1 and GLUT4 using chemiluminescence, and PAS staining to determine cardiomyocyte glycogen content. Blood glucose and p_aO_2 were measured and myocardial perfusion was assessed with SPECT scanning following (99m)Tc sestamibi injection. Statistical significance was determined with student's t-test. Cardiac output and stroke volume were significantly decreased in septic mice. CLP increased cardiac 18-FDG density > 1.5 fold ($p<0.001$), significantly increased steady state GLUT4 levels ($p<0.02$), and enhanced myocardial glycogen content. Increased myocardial glucose uptake occurred in septic mice despite significantly decreased blood glucose ($p<0.05$). P_aO_2 and myocardial sestamibi uptake were unchanged following CLP. These findings indicate the presence of myocardial hibernation in the septic heart in the absence of hypoxemia or hypoperfusion. Thus, myocardial hibernation may underlie sepsis-associated cardiac dysfunction.

P182

EFFECT OF C5A-BLOCKADE ON CARDIAC PERFORMANCE DURING SEPSIS

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The pathogenesis of sepsis and multi-organ dysfunction syndrome is not yet completely understood. We hypothesized that C5a, which is generated in excess during sepsis, plays an important role in sepsis-induced cardiac dysfunction. We sought to evaluate the effect of C5a-blockade during sepsis on cardiac performance in vivo and at the sarcomere level in vitro.
Methods: Sepsis was induced in rats by cecal ligation and puncture (CLP). Goat anti-rat C5a or an irrelevant goat anti-rat IgG was given i.v. at the time of CLP. 24h after CLP, the left ventricle was catheterized and peak left ventricular pressure (LVP) was recorded. C5a receptor presence was assessed and verified by Western Blot analysis of myocyte suspension lysates (CLP and sham animal derived), RTD-PCR and confocal microscopy using a specific fluorescent antibody.
Results: After 24h, CLP and CLP+IgG control animals showed a decreased in maximum LVP values, while sham animals maintained a normal peak LVP. CLP+anti-C5a treated rats demonstrated increased LVP compared to controls ($p<0.001$, ANOVA). Cardiomyocytes were then isolated and sarcomere contraction assessed using a CCD variable rate video camera system with sarcomere length detection software. Peak relative sarcomere shortening was decreased 24h post-CLP compared to shams ($p<0.01$). In CLP+anti-C5a treated animals, measured peak sarcomere shortening was significantly improved vs. cardiomyocytes from CLP alone animals ($p<0.01$).
Conclusion: These results support the hypothesis that C5a plays an integral role in sepsis-induced cardiac dysfunction. Strategies involving blockade of C5a may help to minimize organ dysfunction and improve the survival of patients with sepsis.

* These authors contributed equally to the body of the work.

P183

DUAL EFFECTS OF MESENTERIC LYMPH ISOLATED FROM RATS WITH BURN INJURY ON CONTRACTILE FUNCTION IN RAT VENTRICULAR MYOYCTES

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Burn injury initiates a series of pathophysiological changes: a progressive fall in left ventricular contractile function, despite aggressive fluid resuscitation has been reported in both clinical and experimental studies. Although gut-derived mesenteric lymph from animals with 40% burn injury (burn lymph) appears to trigger myocardial contractile dysfunction, the underlying cellular mechanisms remain unclear. We examined the physiologically relevant concentrations of burn lymph on excitation-contraction coupling in rat ventricular myocytes. Burn lymph (0.5-4%), but not control mesenteric lymph from sham-burn animals, induced dual positive and negative inotropic

effects, depending upon the concentration. At lower concentrations (0.5-1 %), burn lymph initially increased the amplitude of myocyte contraction (1.8 ± 0.3-fold, n=12), which was followed by a decrease in contraction (by 50 %). At higher concentrations (≥ 2 %), burn lymph completely blocked contraction. These effects were reversible upon washout. The initial positive inotropic effect was associated with a prolongation of action potential duration (APD_{90}, 2.1 ± 0.4-fold, n=10), leading to significant increases in the net Ca^{2+} influx (1.7 ± 0.1-fold, n=8). The negative inotropic effect was accompanied by a shortening and triangulation of the action potential plateau (overshoot decrease by 68 ±12 %, n=4). There were no significant changes in the resting membrane potential. Voltage-clamp experiments revealed that the stimulatory effects of burn lymph were due to an inhibition of the transient outward K^+ currents (I_{to}) that prolongs action potential duration, and the inhibitory effects were due to a concentration-dependent inhibition of Ca^{2+} currents (I_{Ca}) that lead to a reduction of action potential plateau. These burn lymph-induced changes in cardiac myocyte Ca^{2+} handling can contribute to burn-induced contractile dysfunction and ultimately to heart failure.

P184

POSTBURN ADMINISTRATION OF ORAL ANTIBIOTICS.
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INTRODUCTION: While pre-injury oral antibiotics attenuate burn-related myocardial inflammation and improve cardiac contractile performance, there is little clinical relevance of pre-injury therapies. In this study, oral antibiotics were initiated 4 hrs postburn and repeated 8 hrs postburn. We hypothesized that decontamination of the digestive tract in the early postburn period would reduce myocardial inflammatory responses. **METHODS**: In Group 1, adult rats were given sham burn plus vehicle (water) by oral gavage 4 and 8 hrs after sham burn. In Group 2, polymixin E, 15 mg; tobramycin 6 mg, 5-flucytosin, 100 mg were given by oral gavage 4 and 8 hrs after sham burn; Group 3 rats were given burn (40% TBSA) + conventional fluid resuscitation + vehicle as described for Group 1. In Group 4, burned rats received fluid resuscitation + antibiotics (as described for Group 2). Cardiac myocytes (collagenase digestion) were prepared 24 hrs postburn. **RESULTS**: In the absence of antibiotics, burn promoted myocyte cytokine secretion and impaired LVP (mmHg). Oral antibiotics initiated 4 hrs postburn attenuated myocyte cytokine secretion (pg/ml, ELISA) and improved LVP. **CONCLUSION**: Our data confirm that antibiotic therapy (initiated in a clinically relevant manner after burn injury) reduced myocardial inflammation and provided a measure of cardioprotection.

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	Sham+ Vehicle	Sham+ Vitamin	Burn+ Vehicle	Burn+ Vitamin
LVP	96±4	100±3	55±3*	68±6*†
TNF-α	96±1	83±2	507±6*	231±7*†
IL-1β	7±1	3±1	46±2	11±1†
IL-6	56±2	15±1*	143±18*	78±1*†

All values are mean±SEM, *difference from sham+vehicle at $p<0.05$, †difference from burn+vehicle at $p<0.05$ ANOVA, student Neuman Keuls

P185

EFFECT OF VARIATION IN BODY TEMPERATURE ON CARDIAC FUNCTION IN MICE: ECHOCARDIGRAPHIC ASSESSMENT.
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Introduction: Anesthesia impairs thermoregulatory homeostasis, with dramatic body temperature changes occurring rapidly in small animals. Cardiac output would be expected to parallel variations in metabolic demand due to changes in body temperature, but the impact of hypothermia and hyperthermia on cardiac performance in anesthetized mice has not been fully characterized.
Materials and Methods: 10 C57-Bl/6 mice were studied under light levels of isoflurane anesthesia. Cardiac function was measured at baseline (Bas1), hypothermia (Hypo), and after animals had recovered (Bas2), and with hyperthermia (Hyper). Temperature was measured with a rectal probe.
A high-resolution ultrasound system was used to assess stroke volume (SV, μL) by Doppler in the aortic outflow tract, and fractional shortening (FS, %) by M-mode in the short axis view. Cardiac output (CO, mL/min) was calculated as SV*HR.
Results: No clinically relevant differences were observed in hemodynamic parameters when body temperature was changed. See table.

	Bas1	Hypo	Bas2	Hyper
Temp	37.3±0.7⌠*	30±0.2*	37.3±0.3⌠*	40±4⌠
HR	466±50*	412±41*	434±36*	561±52
FS	32±3⌠	53±7	31±6⌠	36±4⌠
SV	55±5	59±10	50±9	48±8
CO	26±4	24±4	22±3*	27±3

⌠ $P<0.05$ vs Hypothermia; * $P<0.05$ vs Iperthermia.

Conclusion: Contractility changes compensated for changes in heart rate. Cardiac performance is conserved in healthy mice over a broad range of body temperature.

P186

THE EFFECT OF ANTIRETROVIRAL THERAPY ON THE MYOCARDIAL RESPONSE TO ENDOTOXIN.
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Human immunodeficiency virus infection (HIV-1) has long term effects not only on the immune system but also on the

cardiovascular system. Cardiac dilation has been reported in HIV infected individuals, especially in those who have progressed to AIDS. In order to determine if antiretroviral therapy for HIV infection compromises cardiovascular reserve, we studied the cardiac response to an inflammatory stimulus. A model of HIV infection was studied using mice in which the HIV protein Tat was expressed and then animals were treated with zidovudine (AZT) or water for 3-4 weeks. Endotoxin was used as an acute stressor on the cardiovascular system. Whereas treatment of control mice with AZT did not affect their ventricular performance, AZT did cause a small decrease in myocardial performance of the Tat mice. Additional stress with endotoxin resulted in cardiac depression that was greatest in the Tat mice given either water or AZT in their drinking water. The AZT did not exacerbate the negative effect of Tat expression. These differences in myocardial response to endotoxin were not matched by comparable changes in mRNA for cytokines in hearts from these groups. Although endotoxin did increase mRNA in all groups at 2 hr, by 4 hr when heart function was studied, the mRNA for tumor necrosis factor and interleukin-6, two cytokines that have been implicated in myocardial injury, was not different in the Tat vs. control mouse hearts. The myocardial dysfunction induced by endotoxin was exacerbated by the presence of the HIV protein Tat but not by the consumption of the antiretroviral agent.

P187

EFFECT OF FR901533 *IN VIVO* AND *IN VITRO* ON LV FUNCTION DURING SEPSIS

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We tested the hypothesis that modulation of endothelin (ET) converting enzyme (ECE) during sepsis would affect myocardial function. The study was performed in male Sprague-Dawley rats (350-400g). Animals were randomized into sham-sepsis and sepsis groups. The animals were further sub-divided into saline and FR901533 (FR)-treated. Experiments were performed *in vivo* in conscious animals for 24-h and *in vitro* in isolated perfused hearts at 24-h, after induction of sham-sepsis and sepsis. Sepsis and sham-sepsis were induced using 200 mg/kg cecal inoculum in 5% dextrose water (D_5W) and D_5W injected i.p., respectively. For *in vivo* studies, animals were anesthetized and aseptic surgery was performed for left ventricular (LV) cannulation. After animals regained complete consciousness, sham-sepsis or sepsis was induced and FR (1mg/kg.hr) or saline was infused for 6 hours. Myocardial and plasma ET-1 did not change at 24-h either in saline or FR treated groups. Saline-treated septic animals showed a significant elevation of LV isovolumic relaxation rate constant, *tau*, with elevated LV end diastolic pressure (LVEDP) as compared to saline group at 12- and 24-h. FR-treated septic animals showed no significant change in *tau* or LVEDP at 24-h. *In vitro*, sepsis produced a significant prolongation of *tau* as compared to sham. FR-treatment (0.1, 0.5 and 1 μM) in septic hearts depressed *tau*, significantly at 0.5 μM as compared to no treatment. FR significantly improved left ventricular function (LV systolic pressure, LV developed pressure and rates of LV contraction and relaxation) in septic hearts at all doses *in vitro* as compared to no treatment. We concluded that blockade of ECE-1, both *in vivo* and *in vitro*, ameliorated sepsis-induced myocardial dysfunction. *This work was supported by NHLBI (#066016) and a predoctoral fellowship from AHA (#0510075Z) awarded to SB.*

P188

EFFECT OF P38-MAPK INHIBITION ON SEPSIS-INDUCED MYOCARDIAL DYSFUNCTION

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We tested the hypothesis that endothelin-3 via p38-mitogen activated protein kinase isoforms (MAPK) would affect myocardial function during sepsis. Sepsis was induced using cecal inoculum (200 mg/kg suspended in 5 ml D5W, i.p.) in male Sprague-Dawley rats (350-400 g). Twenty-four hours post-sepsis induction, the heart was harvested, subjected to isolated heart preparation, and paced at 300 beats/min. Left ventricular pressure (LVP) was recorded using a fluid-filled latex balloon connected to the Biopac data acquisition system. Septic rats exhibited a significantly depressed LVSP (85.85 ± 8.18 mmHg) as compared to sham (106.75 ± 4.98 mmHg) group. ET-3 (1 nM) produced 10% increase in LVSP and a significant increase in LVDP in septic animals. SB203580 (1μM), per se, increased LVSP by 6 mmHg in sham rats while in septic rats it increased by 11 mmHg. In septic rats, a maximal increase of 6 mmHG in LVSP was seen with 1 nM ET-3. SB203580, per se, had no effect on LVDP in sham rats, but ET-3 (1 nM) + SB203580 (1 μM) produced an increase in LVDP. Septic rats showed a significantly depressed +dP/dt as compared to sham. In septic rats, SB203580 ameliorated sepsis-induced depressed rates of LV rates of contraction and relaxation, +dP/dt and –dP/dt, respectively. The effect was further increased with ET-3 (0.1, 1, 10 nM). Septic rats showed a significantly elevated LV isovolumic relaxation rate constant, *tau*. In both sham and septic rats SB203580 (1μM) caused a decrease in *tau*. When given with ET-3, septic rats showed a maximal increase in *tau* at 1 nM dose. We concluded that p38-MAPK inhibition ameliorated the deleterious effect of ET-3 on sepsis-induced myocardial dysfunction. *Supported by NHLBI # 066016 and AHA # 0510075Z.*

P189

A SELECTIVE ESTROGEN RECEPTOR-α AGONIST REDUCES HEPATIC INJURY FOLLOWING TRAUMA-HEMORRHAGE.

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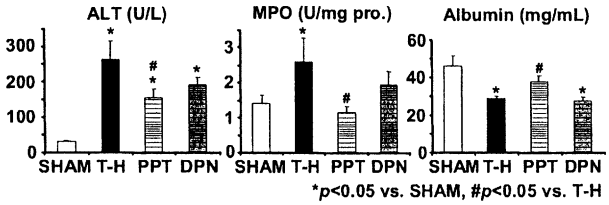
Although estrogen (E2) produces salutary effects on hepatic function following trauma-hemorrhage (T-H), it remains unknown whether E2 receptor (ER)-α or ER-β is responsible for

producing the salutary effects of E2. To examine this, we used a selective ER-α (propyl pyrazole triol; PPT) and ER-β (diarylpropiolnitrile; DPN) agonist following T-H. Male Sprague-Dawley rats (275~325g BW) underwent a laparotomy, hemorrhagic shock (mean BP 40mmHg for 90min) and resuscitation (4x shed blood volume with Ringer's lactate). PPT (5μg/kg), DPN (5μg/kg), or vehicle (10% DMSO) was administrated subcutaneously during resuscitation. Twenty-four hrs thereafter, plasma levels of hepatic injury markers (ALT and AST) and albumin were measured. Myeloperoxidase (MPO) activity in the liver was also determined. One-way ANOVA and Tukey's test were employed for group comparison and differences were considered significant at $p<0.05$. Our results (n=6/group) show that T-H significantly increased plasma levels of ALT, AST and hepatic MPO activity compared to sham. In contrast, plasma albumin levels decreased significantly following T-H. Treatment of rats with PPT (ER-α agonist) after T-H significantly attenuated the increase in ALT, AST and MPO activity as well as the decrease in serum albumin levels. However, treatment of animals with DPN (ER-β agonists) did not influence the above parameters following T-H. Thus, ER-α plays a key role in E2-mediated salutary effects on hepatic function following T-H. (NIH grant R37 GM 39519).



P190

ESTRADIOL INDUCES HEAT SHOCK PROTEIN EXPRESSION AND PROTECTS CARDIAC AND HEPATIC FUNCTIONS FOLLOWING TRAUMA-HEMORRHAGE. L Szalay*, T Shimizu*, T Suzuki*, MA Choudhry, MG Schwacha, KI Bland and IH Chaudry. University of Alabama at Birmingham, Birmingham, AL, 35294.

Although studies indicate that 17β-estradiol (E2) administration following trauma-hemorrhage (T-H) improves cardiac and hepatic functions, the underlying mechanisms remain unclear. Since the induction of heat shock proteins (HSP) can protect cardiac and hepatic functions, we hypothesized that these proteins contribute to the salutary effects of E2 following T-H. Male Sprague-Dawley rats (~275g) underwent laparotomy, hemorrhagic shock (~40 mmHg for ~90min) and resuscitation (4x the shed blood volume as Ringer's lactate with or without E2 administration (1mg/kg BW). Five hrs thereafter, heart performance was determined by cardiac output and $dp/dt_{(max)}$ measurement, and liver function was measured by indocyanine-green (ICG) uptake and bile production. HSP expressions were determined by real-time PCR. As shown below, T-H impaired cardiac and hepatic functions, increased HSP32, but reduced HSP60 expressions in the heart. In the liver, both HSP32 and HSP70 were elevated. E2 treatment attenuated the T-H-induced alterations in organ functions, and enhanced expression all of the observed HSPs. The E2-amplified HSP expressions along with the improvement of organ functions suggest a role for HSPs in the mediation of salutary effects of E2 on organ functions following T-H. (NIH R37 GM 39519).

	Sham	T-H	T-H+E2
Cardiac output (mL/min/100g)	40 ± 1	24 ± 2*	35 ± 2$^{?}$
+dp/dt(max) (mmHg/sec)	12922±270	8191±551*	10262±459*$^{?}$
Heart HSP32 fold	1 ± 0.4	7.1 ± 2.2*	16.9 ± 4.2*$^{?}$
Heart HSP60 induction	1 ± 0.2	0.5 ± 0.1*	1.3 ± 0.3$^{?}$
Heart HSP70 of the Sham	1 ± 0.1	1.2 ± 0.3	3.9 ± 1.5*$^{?}$
Liver ICG uptake (μg/g/min)	71 ± 3	45 ± 6*	57 ± 3*$^{?}$
Bile production (mg/min/kg)	26 ± 3	15 ± 2*	32 ± 7$^{?}$
Liver HSP32 fold	1 ± 0.1	30 ± 3.3*	67 ± 5.9*$^{?}$
Liver HSP60 induction	1 ± 0.08	1.1 ± 0.08	1.8 ± 0.15*$^{?}$
Liver HSP70 of the Sham	1 ± 0.07	1.5 ± 0.12*	2.3 ± 0.2*$^{?}$

Values are mean ± SE (n=5-6/group). *$p<0.05$ vs. Sham; $^{?}p<0.05$ vs. T-H. One-way ANOVA and Tukey's test.

P191

EVALUATION OF INHALED INDOMETHACIN AS TREATMENT FOR SEVERE PULMONARY CONTUSION. W Weiss *, A Batchinsky*, B Jordan*, A Delgado*, A Gerena-Alvalle*, D Cancelada*, L Cancio. U.S. Army Institute of Surgical Research, Fort Sam Houston, TX 78234

Objective: Intravenous (i.v.) indomethacin attenuated post-injury hypoxia in a porcine model of blunt chest trauma. Its side effects preclude its i.v. use in many patients. We evaluated nebulized indomethacin as a treatment in a similar model.

Methods: Swine were anesthetized and ventilated. Injury (INJ, n=10) and injury plus indomethacin groups (INDO, n=10) sustained right-chest blunt trauma by a captive-bolt apparatus, then 12 ml/kg hemorrhage 10 min after injury, and resuscitation with LR and shed blood 30 min after hemorrhage. Animals in the control group (CTRL, n=14) received sham injury. To optimize delivery, indomethacin, 5 mg/kg, was given just before injury by a novel high-output nebulizer (Aeroneb®). Plasma was analyzed for drug levels by HPLC. Bronchoalveloar lavage (BAL) for cell count and total protein was performed at 0, 1, 2, 3, 4, and 6 h. Arterial blood gases (ABGs) were obtained hourly. The primary endpoint was end-of-study PaO_2.

Results: Both INJ and INDO groups demonstrated a decrease in PaO_2 to near-ARDS levels by 1 h after injury, with partial recovery by the end of the 6-h study. There were no significant differences between INJ and INDO groups at any timepoint. Systemic levels of indomethacin were low (0 to 8.54 ng/mcL).

Conclusions: Nebulized indomethacin was ineffective treatment in this injury model. Efforts are under way to evaluate other therapies for hypoxic lung failure following trauma.

P192

MELATONIN LIMITS LUNG INJURY IN BLEOMYCIN TREATED MICE.

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Melatonin is the principal secretory product of the pineal gland and its role as an immumo-modulator is well established. Recent evidence shows that melatonin is a scavenger of oxyradicals and peroxynitrite and exerts protective effects in septic shock, hemorrhagic shock and inflammation. The aim of this study was to investigate the effect of melatonin on the lung injury caused by bleomycin (BLM) administration. Mice subjected to intratracheal administration of BLM developed significant lung injury characterized by a marked neutrophil infiltration (assessed by myeloperoxidase (MPO) activity) and by tissue edema. In addition, an increase of immunoreactivity to nitrotyrosine, Poly-ADP-ribose (PAR) was also observed in the lung of BLM-treated mice. Also, lung injury induced by BLM administration was correlated with a significant lost of body weight and with a significant mortality. Administration of melatonin (10mg/kg i.p.) daily significantly reduced the (i) lost of body weight, (ii) mortality rate, (iii) infiltration of the lung with polymorphonuclear neutrophils (myeloperoxidase activity), (iv) edema formation and (v) histological evidence of lung injury. Administration of melatonin also markedly reduced the nitrotyrosine and PAR formation. Taken together, our results demonstrate that treatment with melatonin significantly reduces lung injury induced by BLM in the mice.

P193

THE HEMODYNAMIC CONSEQUENCES OF TWO METHODS FOR ALVEOLAR RECRUITMENT RS Syring*, CM Otto, RE Spivack*, K Markstaller*, JE Baumgardner Univ of Pennsylvania, Philadelphia, PA, USA and Univ of Berne, Switzerland.

INTRODUCTION: Extrinsic PEEP (PEEPe) is used to limit tidal recruitment of atelectasis as a lung protective ventilation strategy. However, high PEEPe can cause hemodynamic depression, which can contribute to morbidity and mortality. An alternative method to limit tidal recruitment uses short exhalation times with low PEEPe. Recruitment with this method is thought to be mediated by intrinsic PEEP (PEEPi). In a surfactant-depletion model, we used a fast PaO2 probe to demonstrate recruitment of atelectasis with moderate respiratory rates (RR). We measured the effects of increased RR on PEEPi, and we compared the effects of recruitment by RR, versus recruitment by PEEPe, on cardiac output. **METHODS:** Seven NZW rabbits were saline lavaged and ventilated with pressure controlled ventilation, FIO2 of 1.0, I:E of 2:1, and Pplat of 24 to 30 cmH2O. Lung recruitment was attempted in each rabbit via 2 methods: (a) moderate RR (22-24/min) and low PEEPe (2-4 cmH2O); and (b) low RR (6-7/min) and high PEEPe (11-14 cmH2O). Recruitment was assessed with an indwelling PaO2 probe, PEEPi by volumetric flow versus time, cardiac output by arterial pulse contour, and mixed venous saturation by blood gas analysis. **RESULTS:** Equivalent lung recruitment was achieved with moderate RR, low PEEPe and low RR, high PEEPe. No intrinsic PEEP was detected at any ventilator setting. Recruitment by moderate RR and low PEEPe resulted in significantly improved cardiac output ($P=0.003$) and mixed venous saturation ($P<0.001$). **CONCLUSIONS:** In surfactant depleted rabbits, equivalent alveolar recruitment was achieved using moderately elevated RR or elevated PEEPe. Recruitment with RR occurred in the absence of intrinsic PEEP. In addition, the moderately elevated RR setting consistently resulted in improved hemodynamic stability, with both higher cardiac output and presumptively improved tissue oxygen delivery, as suggested by higher mixed venous oxygen saturations. Supported in part by NIH R01GM64486; and DFG MA2398/3-1

P194

REGIONAL AND TEMPORAL DISTRIBUTION OF iNOS ACTIVITY IN A MODEL OF ACUTE LUNG INJURY

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Introduction: Acute lung injury (ALI) is recognized as a heterogeneous condition that progresses over time. Dependent regions of the lung are prone to collapse and/or alveolar flooding; mid-regions are prone to tidal recruitment and intermittent hypoxia; and non-dependent regions of the lung are prone to overdistension and stretch. We hypothesized that inducible nitric oxide synthase (iNOS) activity would vary with spatial distribution and time in a surfactant depletion model of acute lung injury. **Methods:** Following saline lavage in NZW rabbits, we adjusted mechanical ventilation to provide tidal recruitment in approximately 25% of the lung using a recently described fast intra-arterial fluorescence quenching oxygen probe. Lungs were harvested at either 0, 1.5, 3, or 6 hours after lavage; frozen; sectioned into 8-9 slices from nondependent to dependent regions; and homogenized. Tissue homogenates were analyzed for iNOS activity by conversion of radioactive arginine to citrulline. All samples were normalized to dry lung weight. **Results:** In normal lungs and immediately following saline lavage, iNOS activity was negligible in all regions. With increasing duration of ventilation following lavage iNOS activity increased preferentially in the homogenates from dependent regions of the lung. The iNOS activity in samples from upper, overdistended lung regions was similar to baseline at all time points. **Conclusions**: In this surfactant depletion model of ALI, iNOS activity showed substantial spatial and temporal heterogeneity. Consistent with the known hypoxia-inducible nature of iNOS, the predominant localization of iNOS activity was in the dependent lung regions. Whole lung iNOS assays that do not account for this spatial heterogeneity may miss important features of ALI.

Supported by DFG Ma2398/3-1, NIH GM64486

P195

PRESSURE-VOLUME CURVE MORPHOLOGY IN THE INJURED LUNG REFLECTS A CHANGE IN ALVEOLAR NUMBER NOT SIZE.

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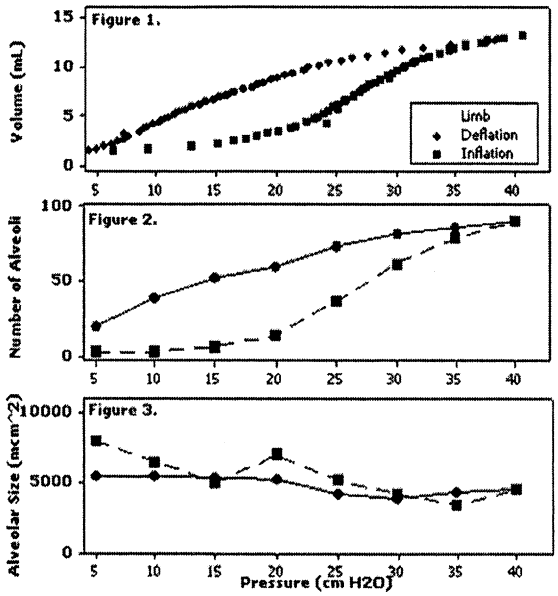
Rationale: This study tested our hypothesis that the shape of the pressure-volume curve (P-V) in the injured lung is due to a change in alveolar number rather than size. ***Methods:*** Rat lung injury was accomplished by saline lavage. Twelve regions of subpleural alveoli were recorded with *in situ* microscopy and a P-V curve was generated. The size and number of alveoli were measured by computer image analysis. ***Results:*** With incremental inflation and deflation (Figure 1) there was a corresponding change in alveolar number (Figure 2) while alveolar size remained relatively static (Figure 3). ***Conclusion:*** Alveoli in the acutely injured lung recruit and derecruit without evidence of overdistention. Thus, strategies to reduce ventilator-induced lung injury should stabilize alveoli rather than prevent alveolar overdistention.



P196

ACUTE INTESTINAL EDEMA CAUSES A DECREASE IN STIFFNESS AND RESIDUAL STRESS

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We have shown that acute edema impairs intestinal transit and wanted to know whether this could be due to changes in the physical characteristics of intestine. We hypothesized that acute edema will change the intestine's physical characteristics, measured by standardized engineering measures of stiffness and residual stress. Methods: Rats were randomized to three groups: sham, mild edema (ME, 80 cc/ kg of NS resus.), and severe edema (SE, 80 cc/kg of NS resus. & intestinal venous HTN). A duodenal catheter was placed and, 30 minutes prior to sacrifice, FITC Dextran was injected. At sacrifice, dye concentrations were measured. Segments of ileum were hung in a tissue bath and attached to a tensiometer. Ileum segments were stretched in increments of 1 mm, recording the new length and corresponding force from the tensiometer. Next, two transverse cuts were made yielding a 1-2 mm thick ring shaped segment of tissue and then cut radially to open the ring. The opening angle was measured. The engineering stress and strain are measurements used to characterize the properties of a biological material and can be expressed by the equations:

σ(stress) = F/A ε(strain)=(L - L_o)/L_o EM(Elastic Modulus)= σ/ε

Where stress is the force (F) in one axis divided by cross-sectional area (A), strain is the change in length divided by the initial length (L_o) and elastic modulus (EM) is a measure of stiffness of a material and is derived from stress divided by strain. Maintenance of the physiologic shape of the intestine requires a specific residual stress. A reliable indicator of this stress is the opening angle (OA) of the intestine.

Results	Sham	ME	SE	Units
Transit	4.82±0.77†	4.55±1.07†,‡	3.61±0.69‡	geom.center
EM	349±14†	282±13‡	250±12‡	kilo Pascals
OA	127±8.5†	91±10.5‡	55.6±9.5*	Degrees
Different symbols (†,‡,*) indicate statistical significance, p<0.05				

Conclusions: Acute intestinal edema leads to a significant loss in stiffness and residual stress and is a plausible explanation for how acute edema impairs intestinal transit.

P197

DECREASED BLOOD TRANSFUSION DURING TRAUMA RESUSCITATION IS ASSOCIATED WITH A DECREASED RISK OF POSTINJURY MULTIPLE ORGAN FAILURE

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Background: In 1997, blood transfusion was identified as an independent risk factor for the development of postinjury multiple organ failure (MOF). Since then, numerous clinical and basic science studies have characterized the immunologic consequences of blood transfusion on the postinjury systemic inflammatory response. The present study was designed to compare the use of blood transfusion during trauma resuscitation before and after the effects of blood transfusion were elucidated. We hypothesized that blood use for trauma resuscitation has decreased over time and that decreased blood transfusion during resuscitation is associated with a decreased risk of postinjury MOF. Methods: Data were prospectively collected on trauma patients at risk for postinjury MOF from 1992 through 2003. Study criteria were age >16yrs, admission to the ICU, ISS>15 and survival >48hrs. Isolated head injuries were excluded. MOF was defined as a Denver MOF score ≥4 time after 48 hours postinjury. Blood transfused within 12 hours of injury was defined as blood transfused during resuscitation. The study was divided according to injuries occurring before 1997 (time period 1) and during or after 1997 (time period 2). Results: Data were collected on 1277 patients. The proportion of patients receiving blood transfusion during trauma resuscitation was significantly lower for time period 2 (173/760, 22%) compared to time period 1 (140/517, 27%) after adjusting for patient age and injury severity ($p < .001$ by multiple logistic regression). The hemoglobin level on postinjury day 3 was also lower for patients in time period 2 vs. patients time period 1 (9.8 +/- .06 vs. 10.7 +/- .08, $p < .001$) MOF

was identified in 300 patients (23%). The incidence of MOF was significantly lower in time period 2 compared to time period 1 after adjusting for patient age and injury severity (p = .02 by multiple logistic regression). Conclusions: Our resuscitation strategy has changed since the effects of blood transfusion were described. Limiting the use of blood transfusion during resuscitation is associated with a decreased risk of postinjury MOF.

P198

IS S-NITROSYLATION OF N-ETHYLMALEMIDE-SENSITIVE FACTOR (NSF) A MECHANISM UNDERLYING INFLAMMATION-INDUCED EPITHELIAL HYPERPERMEABILITY? L. Wang*, S. Dhanisetty*, M. P. Fink, and R.L. Delude. Department of Critical Care Medicine, University of Pittsburgh School of Medicine, 3550 Terrace Street, Pittsburgh, Pennsylvania 15261.

We and others have shown that lung, liver and gut epithelial permeability is increased in endotoxemic mice via a nitric oxide (NO·)-dependent mechanism. Similarly, we and others have shown that cytomix (a mixture containing IL-1β, TNF-α and IFN-γ) increases the permeability of Caco-2 (enterocyte-like) and Calu-3 (pneumocyte-like) epithelial monolayers via an NO·-dependent mechanism. Both *in vivo* and *in vitro*, these inflammation-induced changes in permeability are associated perturbations in the expression and subcellular localization of tight junction (TJ) proteins. Therefore, we hypothesized that NO·-induced epithelial barrier dysfunction may be caused, at least in part, by S-nitrosylation of NSF, a protein that is important for the proper transport of proteins from the Golgi apparatus to the plasmalemma. We introduced TAT-NSF222, a peptide inhibitor of NSF, or a scrambled version of the peptide into Caco-2 monolayers. Whereas the scrambled peptide failed to increase permeability, TAT-NSF222 significantly increased permeability to FITC-dextran (MW_{AVG} 4 kDa; FD4) (p=0.002). Using the biotin switch assay developed by Jaffrey *et al.* (*Nat Cell Biol* **3**:193), we showed that NSF is S-nitrosylated in Caco-2 cells stimulated for 6 h with cytomix or incubated for the same period with DETA-NONOate (an exogenous NO· donor). Furthermore, we showed NSF in hepatic tissue is S-nitrosylated 6 h after inject of LPS into C57Bl/6J mice, but is not modified in control animals. These data support the view that NO·-dependent S-nitrosylation of NSF may play a role in the pathogenesis of inflammation-induced epithelial barrier dysfunction both *in vivo* and *in vitro*.

P199

DIETARY STEARIDONIC ACID INCREASES N-3 FATTY ACID CONTENT IN RAT LIVER PHOSPHOLIPIDS. J. Phipps*, B. Daley, B. Enderson, L. Jones*, J. Whelan*, M. Karlstad. Department of Surgery, University of Tennessee Medical Center, Knoxville, TN 37920.

We have shown that enteral nutrition containing eicosapentaenoic acid (20:5n-3; EPA) provided beneficial anti-inflammatory effects in critically ill patients with acute lung injury. The use of stearidonic acid (18:4n-3; SDA), derived from terrestrial sources, offers an alternative strategy for increasing EPA content in tissue phospholipids and may provide the health benefits typically associated with long-chain n-3 polyunsaturated fatty acids from fish. We examined whether diets supplemented with SDA can be used as a means to increase EPA content in liver phospholipids and to determine the relative effectiveness of SDA in increasing tissue EPA as compared with dietary EPA. Male Long-Evans rats (n=18) were randomly divided into 3 dietary groups and fed a modified US-17 diet providing 200 $kcal \cdot kg^{-1} \cdot day^{-1}$ and containing either 2% oleic acid (18:1n-9; OA) (n=7), 1% EPA /1% OA (n=8), or 1% SDA/1% OA (n=7) for 2 weeks. Liver phospholipid fatty acid composition (mol%) was determined by gas chromatography.

Fatty Acid	OA	EPA	SDA
20:4n-6 (AA)	28.4 ± 0.56^a	25.6 ± 0.39^b	27.6 ± 0.47^a
20:5n-3 (EPA)	0.21 ± 0.05^a	1.18 ± 0.17^b	0.62 ± 0.07^c
22:5n-3 (DPA)	0.51 ± 0.05^a	1.69 ± 0.10^b	1.03 ± 0.02^c
22:6n-3 (DHA)	6.84 ± 0.36^a	6.96 ± 0.25^a	7.12 ± 0.42^a

Values with diff. superscripts are significantly diff. (P ? 0.05); ANOVA

There was a small reduction in AA content with EPA, but not SDA, as compared with OA. SDA increased liver phospholipid EPA content as compared with OA with approximately 50% efficiency as compared with EPA. EPA and SDA significantly increased DPA as compared with OA; however, there was no significant increase in DHA content with either EPA or SDA. These results show that dietary SDA can increase EPA content in liver phospholipids and the relative effectiveness of SDA conversion to EPA and incorporation into tissue phospholipids was equivalent to one half of dietary EPA. Also, EPA and SDA are not appreciably desaturated to DHA.

P200

LIPOPROTEIN ABNORMALITIES IN EARLY MURINE SEPSIS. J Stewart*, CS Deutschman. University of Pennsylvania, Philadelphia, PA 19104.

Background: Lipoprotein concentrations change rapidly in patients with severe sepsis, resulting in 50-70% reduction in levels of LDL, HDL, and chylomicrons. Previous work has shown that triglyceride-rich lipoproteins bind endotoxin (LPS), forming lipoprotein-LPS complexes, and protect against endotoxic shock and death in rodent models of gram-negative sepsis. The role of HDL in this process is complex and remains poorly studied. Recent data suggests that structural changes in the HDL molecule occur in sepsis. HDL appears to be the main contributor to the decrease in lipoprotein levels seen in early sepsis. **Objective:** To determine whether cecal ligation and double puncture in murine subjects results in similar patterns of lipoprotein abnormalities seen in early sepsis in humans. **Methods:** Under general anesthesia, 6-8 wk old C57/BL6 mice either underwent cecal ligation and double puncture (2CLP) or sham operation. Animals were sacrificed after 0, 16, or 24 hrs. Serum lipid profiles were drawn and analyzed. **Results:** When compared to sham-operated subjects, 2CLP subjects exhibited significantly decreased levels of HDL at 16 hrs ($p<0.001$).

Sixteen-hour 2CLP subjects exhibited a mean HDL of 10.3-mg/dL ± 7.9 mg/dL while their sham cohorts showed a mean of 71.3 mg/dL ± 1.6gm/dL. In addition, when compared to sham-operated subjects, 2CLP subjects showed elevated levels of total cholesterol at 24 hrs post operatively ($p<0.05$). Finally, among the 2CLP groups, the 16hr subjects showed significantly lower levels of HDL and total cholesterol than 24hr subjects ($p<0.005$ and $p<0.05$ respectively). There was no mortality in any study group at 24 hrs post-op.

Conclusion: The pattern of early decline in HDL levels seen here in murine subjects appears to be consistent with that seen in humans. Murine subjects appear to exhibit more rapid derangements in lipid profiles than humans though changes in HDL levels appear to be most pronounced. Investigation into the mechanism of HDL down regulation seen in early sepsis is warranted. In addition, these findings may have implications presentation of molecules such as endotoxin to parenchymal cells.

P201

THYROID STORM IN A TRAUMA SETTING: AN ARCANE DIAGNOSIS. M. Lorenzo, P. Rodriguez*. University of Puerto Rico, San Juan, PR. 00936.

Objective: Remarkably, thyroid storm following a traumatic episode has been rarely documented in the medical literature. Despite adequate medical treatment, the corresponding mortality rate is quite high (30%-40%). A high level of suspicion is required for the appropriate diagnosis and treatment of this endocrine emergency.

Method: Review of the literature and a descriptive analysis of a case involving a patient who developed a thyroid storm after severe trauma were done.

Results: A 31 year old patient was transferred to our institution after having sustained an open book fracture of the pelvis and an extraperitoneal bladder rupture from a motor vehicle crash (MVC). His past medical history included the use of Tapazole for a few years after a diagnosis of hyperthyroidism was established four years before the trauma. The patient showed an uneventful clinical course during the first two days of supportive management. Forty-eight hours post admission, he developed excessive agitation and irritability, thrombocytopenia (40,000), a body temperature of 42 degrees F, irrepressible diarrhea and tachycardia (170-180 b/m). Work-up for infection was negative and thyroid function tests were ordered. Patient showed miniscule levels of TSH and elevated levels of T3 and T4. Treatment for thyroid storm was promptly instituted, but the patient succumbed to cardiovascular collapse in less than 24 hours.

Conclusion: While the thyroid storm syndrome is rare in and of itself, we suggest that thyrotoxicosis goes undiagnosed because its features may easily be mistaken for the usual signs and symptoms found in the trauma population. A combination of tachycardia, extreme hyperpyrexia, diarrhea, uncontrollable irritability and thrombocytopenia should be fundamental clues to include thyrotoxicosis as part of the differential diagnosis of a trauma patient. Prompt recognition and aggressive therapy for this disorder are imperative for better patient outcomes.

P202

G-CSF DURING RADIOTHERAPY REDUCES BONE MARROW RECOVERY CAPACITY

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Objective: Side effects of chemo- and radiotherapy are granulo- and thrombocytopenia. The long-term effects of in vivo G-CSF stimulation of the hematopoietic system during radiotherapy are not well known. In this study, we determined the bone marrow effect of G-CSF during radiotherapy. **Methods:** In a prospective, randomized clinical trial 10 patients were (6 ? , 4 ? , 30-64 yrs, mean 50.6 yrs) assigned to large field radiotherapy. 7 pat. with non-Hodgkin lymphoma, one with Hodgkin's disease and 2 with small-cell carcinoma of the lung were included. Patients were randomized to either radiotherapy alone (group A) or radiotherapy with simultaneous G-CSF (group B) treatment and assessed for acute and late toxicity. Blood samples were analyzed before and after G-CSF stimulation. The mobilization effectivity of G-CSF on $CD34^+$ was measured with flow cytometry and colony forming units on admission and during the complete follow-up period (1, 3 and 18 months post RTx). **Results**: 50 pat. were intended to be included. The preliminary analysis revealed a significant decrease of thrombocytes and $CD34^+$ in group B. According to the study protocol interruption criteria were reached. Peripheral leukocyte counts ranged between 2800 - 4375 /µl in 9/10 pat. In group B mean thrombocyte levels dropped below 30.000 mg/l and CD34+ to 50% ($p<0.02$, Student's t-test). Hemoglobin did not vary. Differences in granulocyte counts with more neutrophils in group B were found. Lymphocyte counts in group A were significantly decreased. In group A, 3/5 pat. developed an overshooting reaction (4,7 x increase) after G-CSF-stimulation. In arm B CD34+ dropped. In arm A, 3/5 pat. had an initial overshoot reaction compared to none in group B. CFU (>40cells) and cluster (4-39 cells) showed considerable variations. **Conclusion**: Our results demonstrate that simultaneous treatment with G-CSF during radiotherapy reduces the mobilization of $CD34^+$ progenitor cells and exhaust the bone marrow capacity while peripheral leukocyte counts remain at baseline levels.

P203

ALBUMIN ADMINISTRATION MAINTAINS POST-ADMINISTERD ANTITHROMBIN III (AT III) TROUGH ACTIVITY IN CRITICALLY ILL PATIENTS.

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There has been limited pharmacokinetic (PK) information regarding AT III agent in critical patients. In this study, we performed PK analysis to determine whether AT III agent at 500 units three times a day (divided group) or at 1500 units just once per day (combined group), is more effective for maintaining AT III trough activity in critically ill patients, Also, we defined the

effects of albumin administration on changes in serum AT III activity after AT III application in such patients. **Methods:** This study has been approved by the IRB. Twenty critical patients compatible with DIC were included in this study. The female/male ratio was 8/12. Ages ranged from 25 to 85 years. APACHE II scores were 10 to 35. Albumin of 12.5g was given twice a day for three days. AT III agent was administered by either method for three days. Data are expressed as mean ± SD. Statistical analysis was done by Mann-Whitney U-test or ANOVA. **Results:** AT III trough activity in the divided group was significantly higher than that in the combine group after the treatment. Half-life of the AT III distribution phase in the patients was remarkably shorter than previously reported values in patients with congenital AT III deficiency. This suggests an increased vascular permeability in the critical patients. Distribution volume of the agent inflated to 5.1 ± 0.3L (the previous control: 2.4 ± 0.2L). Albumin administration elevated AT III trough activity over those without albumin treatment. **Discussion:** This is the first report to demonstrate that AT III agent should be administered in divided doses to maintain the trough level in patients with increased vascular permeability and/or distribution volume. Albumin infusion sustained post-administered AT III trough level: its mechanism may be linked to certain binding capability of albumin.

P204

SERUM ALBUMIN LEVELS, BUT NOT THROMBIN-ANTITHROMBIN III COMPLEX (TAT), ANTICIPATE CHANGES IN POST-ADMINISTERD ANTITHROMBIN III (AT III) ACTIVITY IN CRITICALLY ILL PATIENTS.

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Maintaining serum AT III levels is requisite for obtaining anti-coagulatory and anti-inflammatory effects of AT III agent. We examined whether serum AT III levels after administration of an AT III agent would change depending on serum TAT, a marker of AT III consumption, and /or albumin levels in such patients, and also performed pharmacokinetic (PK) analysis for AT III. **Methods:** This study has been approved by the IRB. Twenty critical patients compatible with DIC were included in this study. The female/male was 8/12. Ages ranged from 25 to 85 years. APACHE II scores were 10 to 35. AT III agent was administered 500 units three times per day for three days. Data are expressed as means ± SD. Mann-Whitney U-test or ANOVA was used for statistics. **Results:** AT III trough levels in patients, even those with a high TAT of more than 20 μg/l (normal: < 4.1 μg/l), increased from 54 ± 9 to 104 ± 10 % after the three-day administration, but in patients with low serum albumin level below 2.0 g/dl, especially subjects who had open drainage for local infection, AT III levels did not increase. Serum albumin levels before AT III administration were well correlated with serum AT III levels (R=0.72, p=0.005), but TAT was not. The half-life of the distribution phase (α‽ 1/2) in the patients was shortened to 1/3 value of which was previously reported in patients with congenital AT III deficiency, suggesting increased vascular permeability. The distribution volume of the agent inflated to 5.1 ± 03L (previous control: 2.4 ± 0.2L). **Conclusion:** We report here for the first time that changes in AT III can be anticipated by serum albumin levels before AT III administration, but not by TAT. These findings might be explained, at least in part, by the shortened α t1/2 and increased distribution volume observed in the critical patients.

P205

DROTRECOGIN ALFA IMPROVES PHYSIOLOGIC PARAMETERS IN POST-SURGICAL PATIENTS WITH SEPSIS SYNDROME.

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Objective: To evaluate the outcome, physiologic and pharmacological response to drotrecogin alpha in post operative SICU patients with sepsis syndrome.

Materials and Methods: A retrospective review was performed on nine consecutive postoperative surgical patients receiving drotrecogin alpha in a tertiary care teaching hospital. All patients met clinical criteria for SIRS with organ dysfunction and vasopressor support. Data acquired included hemodynamic respiratory, pharmacological and laboratory parameters assessed at 0, 24, 48, and 96 hours post infusion. AP2, ICULOS and Mortality were obtained. Data are presented as mean ± sd

Results: Mean age was 66.1±9.4 years. Five of nine survived (44% mortality). The mean AP2 score was 29.2 ± 3.1 at time of infusion (Predicted Mortality 73.4±8.6). Mean ICULOS was 28.1±11.7 days. All nine patients demonstrated decreased pressor requirements at 96 hours No patient experienced complications during drotrecogin alfa infusion and all completed 96 hours of infusion.

% Change (mean±sd)	24hours	48hours	96hours
MAP	?11.7 ± 29.7 %	?14.5 ± 12.9 %	?22.4 ± 23.9 %
PaO2/FiO2	?45.8 ± 63.6 %	?50.3 ± 49.4 %	?64.1 ± 95.8 %
HR	?3.4 ± 6.0 %	? 14.0 ± 11.7 %	? 19.9 ± 17.7 %
24 hr Urine Output (cc)	?23.3 ± 41.0 %	?30.7 ± 38.2 %	?42.8 ± 45.7 %
Cardiac Index	?18.0 ± 38.7 %	?24.4 ±30.5%	?17.3± 41.9 %

Conclusion: Our data demonstrate that cardiovascular, respiratory, and renal function improved during the 96 hours of infusion with drotrecogin alfa. Pressor requirements were decreased in all patients and the observed mortality in this group was significantly better than the AP2 Predicted Mortality.

P206

SEVERITY OF EMERGENCY DEPARTMENT HYPOTENSION PREDICTS ADVERSE HOSPITAL OUTCOME

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Arterial hypotension often signifies inadequate systemic perfusion. We hypothesize that in a heterogeneous emergency

department (ED) population with clinically suspected circulatory shock, the severity of hypotension on presentation predicts in-hospital outcome. We performed a secondary analysis of patients with non-traumatic shock enrolled in a non-interventional, randomized, controlled trial. The setting was an urban, tertiary ED, census >100,000 visits per year. Patients included non-trauma ED patients, aged >17 years, with initial ED vital signs consistent with shock (systolic blood pressure <100 mmHg or shock index >1.0), and agreement of two independent observers for at least one sign and symptom of inadequate tissue perfusion. Measurements included inter-observer agreement for signs and symptoms of shock, relationship between the depth and duration of ED hypotension and adverse hospital outcome (in-hospital mortality, need for intensive care unit services, and acute organ failure) and logistic regression analysis for independent predictors of adverse hospital outcome. Of 202 patients who qualified, 190 patients were included: the in-hospital mortality rate was 15%. The sign or symptom of shock with the highest inter-observer agreement was "unresponsive" (?=0.74). The adverse hospital outcomes increased with each decile decrease in the lowest ED systolic blood pressure (SBP) from 17% if SBP >89 mmHg versus 50% if SBP <80 mmHg. Forty percent of patients with an adverse hospital outcome had sustained hypotension (all ED SBP <100 mmHg for ?60 min). Sustained hypotension was the strongest independent predictor of an adverse hospital outcome (odds ratio 3.1; 95% CI 1.5-7.1). Mortality among patients who present to the ED with undifferentiated shock is high. The depth and duration of systolic blood pressure appears to have a dose-response relationship to adverse hospital outcome.

P207

CENTRAL VENOUS PRESSURE RESPIRATORY VARIATIONS DURING MECHANICAL VENTILATION FOR THE ASSESSMENT OF VOLUME STATUS AFTER CARDIAC SURGERY. GA Westphal*, LF Poli de Figueiredo, E Silva*, M Caldeira Filho*, Mauricio Rocha e Silva. Centro Hospitalar UNIMED, Joinville, SC and Research Division, Heart Institute, InCor, LIM11, University of São Paulo Medical School, São Paulo, Brazil.

Variations in intra-thoracic pressure interferes with venous return and cardiac output. Pulse pressure variations through arterial lines (ΔPp) during mechanical ventilation have been recommended for volume replacement in hypovolemic patients. **Hypothesis:** Our hypothesis is that central venous pressure variations (ΔCVP) may be used to identify postoperative hypovolemia. **Method:** 28 patients submitted to cardiac surgery, during the initial two hours of postoperative care, requiring mechanical ventilation (tidal volume of 8-10 mL/kg, peak pressure of 25-35 cm H_2O, 1:3 I:E relationship). All patients received both arterial and central venous lines preoperatively. They were all under residual anesthetic effects, with no spontaneous efforts. Tracing were obtained by multi-parametric monitors and transferred to a software that calculated ΔPp and ΔCVP variations during the inspiratory (Pins) and expiratory (Pexp) phases of the respiratory cycles:

ΔPp or ΔCVP (%)=100x[(Pinsp-Pexp)/(Pinsp+Pexp)/2]

A ΔPp ≥ 13% was considered hypovolemia. *Kappa* coefficient was used for concordance between ΔPp and ΔCVP. **Results**: There was no difference (p=0.3) in mean CVP values in patients with ΔPp≥13% or <13% - p = 0,3. The ΔCVP related to a ΔPp=13% was -4%. Although no linear correlation between ΔCVP and ΔPp could be drawn, for all 15 patients with a ΔPp≥13%, a ΔCVP was <-4%. Among patients with a ΔPp<13%, a ΔCVP was >-4% in only two patients (*kappa*=0.85). **Conclusion:** CVP variations during mechanical ventilation may be a useful additional tool to identify hypovolemic patients after cardiac surgery.

P208

AN IMPLANTABLE MICRO-SENSOR FOR REAL-TIME TISSUE PERFUSION MONITORING. W. Xu*[1], M.N. Ericson*[2], G.L. Cote*[3], J. Baba*[2], C.L.Britton, Jr.*[2], M.A. Wilson.[1] [1]University of Pittsburgh and VA Pittsburgh Healthcare System, Pittsburgh, PA 15240; [2]Oak Ridge National Laboratory, Oak Ridge, TN 37831; [3]Texas A&M University, College Station, TX 77843

Visceral organ ischemia/reperfusion is common clinically after hemorrhagic shock, aortic revascularization, and with organ transplantation. Perfusion defects may be subtle until irreversible organ injury has occurred. Minimally invasive detection of visceral microvascular organ blood flow and blood oxygen saturation would be of benefit in such clinical situations. An advanced, non-invasive, and implantable micro-sensor was developed using photonics-based measurements coupled with integrated signal processing to provide real-time monitoring of tissue perfusion and blood oxygen saturation. LED wavelengths (660 nm, 810 nm, 940 nm) were initially selected based on the isobestic point and absorption curves of oxygenated and deoxygenated hemoglobin. Initial characterization of this system was conducted using an *in vitro* oxygenated, blood-perfused circuit with porcine liver. Peristaltic flow was produced with a cardiac cycle simulator, and flow was maintained at preset values using time-transit Doppler flowmetry. Reflected light was quantified by a photodetector and data were analyzed by fast-Fourier transforms. Results indicate that there is a near linear relationship of the sensor to measured flow between 1 and 4 ml/min (R^2 = 0.99) for each wavelength when data are reported as normalized power in the frequency domain. Arterial oxygen saturation was calculated from AC and DC values at the 660 nm and 940 nm wavelengths and confirmed by comparison to a commercial blood gas analyzer. Due to low signal to noise ratio at 660 nm, oxygen saturation could not be reproducibly calculated. In order to increase tissue penetration, the 660 nm LED was replaced with a 735 nm LED that resulted in improved calculated of arterial oxygen saturation. Flow detection at 735 nm remained highly correlated to measured flow. These preliminary results demonstrate the feasibility of this optical detection system for monitoring of tissue perfusion and blood oxygenation.

P209

SYNCHRONIZATION OF RR-INTERVAL AND ARTERIAL BLOOD PRESSURE OSCILLATIONS IN BRAIN INJURY

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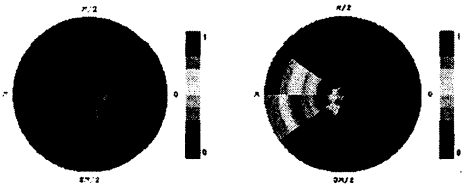
Objectives. The goal of the research was to develop a novel mathematical framework capable of capturing nonstationary coupling of RR intervals (RRI) and arterial blood pressure (ABP) time series. *Methods.* We employ complex wavelet transforms to determine the time evolution of the instantaneous phase difference between the RRI and ABP time series. We quantify phase dynamics with the help of a synchronization index γ which may vary between 0 (uniform distribution of phase differences) and 1 (phase locking of RRI and ABP). *Results.* In the low frequency part of the spectrum (0.02-0.07 Hz) the group-averaged synchronization index for healthy volunteers was 0.15, indicating weak coupling between the two time series. In patients with traumatic brain injuries (TBI) or spontaneous cerebral hemorrhage we observed pathological synchronization of RRI and ABP ($\gamma = 0.70$). *Conclusions.* The autonomic nervous system plays an important role in systemic blood pressure control, e.g., via baroreflex feedback. The observed synchronization may originate as a result of head trauma or shock.



Polar density plot of phase difference between RRI and ABP time series. Left: Healthy volunteer; Right: TBI patient.

P210

DETECTION AND PREDICTION OF SHOCK STATES IN PATIENTS WITH VENTRICULAR FAILURE

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Rationale: Early detection and prediction of shock conditions in patients with ventricular failure is complicated by shifts in the hemodynamic profile. The physiologic pattern in ventricular failure may be incomplete or overlap with other conditions. The implementation of an intelligent physiologic monitoring system with predictive capabilities can alert caregivers to potential life-threatening conditions in patients with ventricular failure earlier than traditional monitors. A system based on Bayesian networks and intelligent signal processing mechanisms can analyze physiologic measurements and their tendencies to predict emergency conditions in the context of ventricular failure. ***Methods:*** A multi-agent intelligent system was created by using multiple inferencing techniques to detect and classify problems. It incorporates physiological data from sensors indicating the patient's condition and computes the probabilities of having one or more common types of emergency conditions. These conditions include ventricular failure and all shock like states: hypovolemic, cardiogenic, obstructive, septic and other distributive. Also, based on tendencies of changing readings, the system can predict these same conditions. ***Results:*** This system has detected shock conditions and demonstrated predictive capabilities in physiologic data from postoperative cardiac patients and those with chronic ventricular failure. ***Conclusion:*** Multi-agent Bayesian network based intelligent techniques can be used to analyze physiologic measurements to support care givers decision-making. The advantage obtained is through early prediction of emergency conditions and intelligible organization of physiologic information.