

SPHINGANINE-1-PHOSPHATE AMELIORATES ACUTE LIVER FAILURE AND ACUTE KIDNEY INJURY INDUCED BY HEPATIC ISCHEMIA AND REPERFUSION IN MICE. S.W. Park*, S.W. Chen*, M. Kim*, K.M. Brown*, V.D. D'Agati* and H.T. Lee* (Sponsor: S.M. Lee) Columbia University, New York, NY 10032.

Hepatic ischemia and reperfusion (IR) injury is a major complication after liver surgery. Furthermore, acute kidney injury is frequent after hepatic IR and greatly increases the postoperative complications. Sphinganine-1-phosphate (Sg1P) is a sphingolipid with uncharacterized physiological effects. We recently determined that plasma levels of Sg1P fell significantly after liver IR in mice. In this study, we hypothesized that repletion of plasma Sg1P would protect against liver and kidney injury after liver IR. C57BL/6 mice were subjected 60 min of partial hepatic IR and treated with either vehicle or with Sg1P (0.1 mg/kg prior to reperfusion and 0.2 mg/kg 2 hr after reperfusion). Vehicle-treated mice subjected to liver IR developed acute liver and kidney injury with elevated plasma alanine aminotransferase (ALT=15076±1174 U/l, N=6, vs. Sham=62±31 U/l, N=6) and creatinine (Cr=1.08±0.07 mg/dl, N=6, vs. Sham=0.50±0.08 mg/dl, N=6) 24 hr after IR. However, liver and kidney injury were significantly attenuated with Sg1P treatment (ALT=9340±1184 U/l, N=6, P<0.01; Cr= 0.53±0.07 mg/dl, N=6, P<0.01). Sg1P markedly inhibited liver and kidney necrosis and apoptosis 24 hr after IR. Moreover, Sg1P markedly inhibited neutrophil infiltration and preserved the liver and kidney vascular integrity. Finally, hepatic IR caused severe F-actin cytoskeleton degradation of hepatocytes as well as renal tubular epithelial cells. F-actin degradations were attenuated by Sg1P. Therefore, our results show that Sg1P improves acute liver failure as well as acute kidney injury after hepatic IR via inhibition of necrosis and apoptosis and by improving vascular integrity. Harnessing the mechanisms of cytoprotection with Sg1P activation may lead to new therapies for perioperative hepatic IR and subsequent remote organ injury.

POLYMICROBIAL SEPSIS ACTIVATES TH1 AND PROTECTS AGAINST *LISTERIA* INFECTION.

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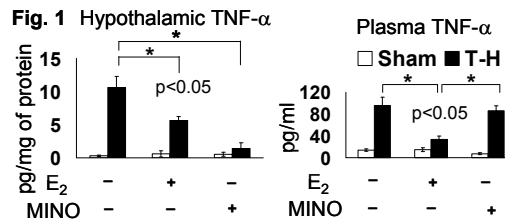
Objective: Although polymicrobial sepsis induces a Th1 to Th2 shift that contributes to immune suppression and potentiates secondary infection, the host's ability to eradicate microbes requiring a subsequent Th1 response is unknown. *L. monocytogenes* (LM), an intracellular pathogen, requires a Th1 response for total eradication. We hypothesize that the sepsis-induced Th1 to Th2 immune shift should impair subsequent Th1 immune responses and prevent successful LM eradication. **Methods:** Female 8 week old B6 mice underwent either cecal ligation and puncture (CLP) (LD₁₀) or sham procedure and at 3 days (n=7 mice/group) received LM (1x10⁵ cfu/mL, iv, LD₀). After five days LM colonization was assessed in the spleen and liver. Mice also underwent CLP or sham (n=20 mice/group) and at 3 days given LM (1x10⁶ cfu/mL, iv, LD₅₀) and followed for survival. Splenic macrophages (CD11b⁺F480⁺) were evaluated for oxidative burst.

Results: Mice undergoing LM infection after sham had 15,000 cfu/spleen and 1000 cfu/liver (means); however, no *Listeria* could be found in the liver or spleen from the CLP mice administered LM (p<0.001). Mice receiving ovalbumin-labeled LM 3 days after CLP had a 3 fold greater ovalbumin-specific IgM response compared to shams (p<0.01). At day 3 after CLP splenic macrophages demonstrated a 2-fold greater oxidative burst compared to sham mice (p<0.01). Mice that underwent CLP and LM infection (LD₅₀) incurred a 60% greater survival benefit compared to similar treated shams (p<0.01).

Conclusion: Although a Th1 to Th2 immune profile shift is associated with immune suppression, Th1 immunity is intact and protective against LM following CLP. The enhanced Th1 immunity after CLP promotes efficient macrophage clearance, antigen presentation, and specific IgM antibody production in response to LM infection.

MECHANISM OF THE ANTI-INFLAMMATORY EFFECT OF 17 β -ESTRADIOL ON BRAIN FOLLOWING TRAUMA-HEMORRHAGE. H Akabori*, F Moeinpour*, KI Bland*, IH Chaudry. Center for Surgical Research, Department of Surgery, University of Alabama at Birmingham, AL 35294

Although 17 β -estradiol (E2) is reported to improve the inflammatory response after trauma-hemorrhage (T-H), it remains unknown whether E2 plays any role in the central nervous system following T-H. Microglial (MG) cells, resident central macrophages, are thought to play a central role in exacerbating cell-mediated inflammation. We hypothesized that T-H upregulates MG cell-mediated inflammatory response in the brain, and E2 produces central anti-inflammatory effects via negative regulation of MG. Male Sprague-Dawley rats were subjected to sham operation (cannulation plus laparotomy) or T-H (midline laparotomy, mean BP of 35 \pm 5 mmHg for 90 min followed by resuscitation) and immediately sacrificed. Rats received vehicle or E2 (1 mg/kg BW i.v.) at the onset of resuscitation. Minocycline (MINO, 40 mg/kg BW i.p.), MG inhibitor, was administered 1 hr before T-H. In T-H rats, compared to shams, the plasma TNF- α levels and hypothalamic TNF- α contents increased (Fig. 1), along with the activation of MG cells. Furthermore, T-H increased MG TNF- α productive capacity *in vitro* (data not shown). E2 administration following T-H prevented these inflammatory responses. In rats pretreated with MINO, decreased MG TNF- α production and hypothalamic TNF- α levels were observed, but plasma TNF- α levels were not altered following T-H. Thus, T-H induces inflammatory responses even in the hypothalamus and E2 may be a useful adjunct for downregulating MG cell-mediated inflammatory response to T-H. (NIH RO1 GM39519)



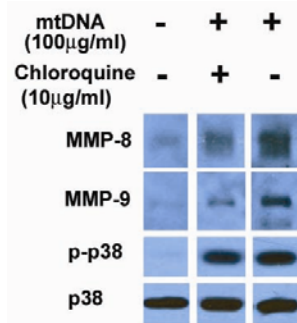
MITOCHONDRIAL DNA ACTIVATES NEUTROPHILS VIA TLR9 AND P38 MAPKINASE.

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Background: Bacterial DNA (bDNA) is unlike eukaryotic nuclear DNA (nDNA) in that it is circular, contains unmethylated CpG motifs and is not protected by histones. Thus it is perceived by the innate immune system as containing “Pathogen associated molecular patterns” (PAMPs) that activate an immunostimulatory “Danger” response via Toll-like receptor 9 (TLR9). bDNA circulates in sepsis but should not be present in sterile SIRS after trauma. Mitochondrial DNA (mtDNA) however, is similar to bDNA because mitochondria evolved from prokaryotic ancestors and retain their own genome. It is unknown whether mtDNA is released by injury or is capable of eliciting the same immune responses as bDNA. We hypothesized that if injured tissues released mtDNA it might activate PMN via TLR9, potentially contributing to SIRS. **Methods:** Human PMN were incubated with human liver-derived mtDNA or nDNA, with or without pre-treatment by the TLR9 inhibitor chloroquine (CQ). PMN activation was assessed as MMP-8 and MMP-9 release from 2’ and 3’ granules respectively. P38 and P44/42 MAPK phosphorylation was examined by Western Blot. **Results:** mtDNA (but not nDNA)

induced dose-dependent MMP-8/MMP-9 release and p38 (but not p44/42) MAPK activation. All responses were partially inhibitable by CQ. **Conclusions:** mtDNA activates PMN via mechanisms that include TLR9 activation of p38 MAPK. mtDNA is potentially an important DAMP that can be released by injured cells and stimulate innate immunity. Mitochondrial DAMPs may play a role in the genesis of SIRS after tissue trauma.



TRANSFORMING GROWTH FACTOR-ALPHA PRECONDITIONING IMPROVES MESENCHYMAL STEM CELL-MEDIATED CARDIOPROTECTION DURING ISCHEMIA. J. Herrmann*, Y. Wang*, A. Abarbanell*, B. Weil*, J. Tan*, D. Meldrum. Indiana University School of Medicine, Indianapolis, IN 46202.

Mesenchymal stem cells (MSCs) are a promising therapy for acute organ ischemia in part due to their ability to release protective growth factors in a paracrine fashion. However, these cells face a hostile, inflammatory environment that may mitigate their protective abilities during ischemia/reperfusion injury. One strategy for enhancing MSC function is exposing them to exogenous growth factors prior to infusion. We hypothesized that preconditioning MSCs with transforming growth factor- α (TGF α) prior to infusion would improve myocardial functional recovery to a greater extent than with non-preconditioned MSCs in a rat model of acute global myocardial ischemia. Methods: MSCs were harvested from the femurs and tibias of adult male wild-type mice (C57BL). MSCs (passages 3-7) were preconditioned with TGF α (250 ng/ml) *in vitro* 24 hours prior to infusion. Adult male Sprague-Dawley rat hearts were isolated and subjected to 15-min equilibration, 25-min warm global ischemia, and 40-min reperfusion. Transcoronary delivery of vehicle, wild-type MSCs, or TGF- α -preconditioned MSCs was performed immediately prior to ischemia. Myocardial function was continuously recorded. Results: Postischemic recovery of left ventricular developed pressure at end reperfusion versus baseline was significantly greater in hearts infused with TGF α -preconditioned MSCs (70.0 +/- 5.1%) than untreated MSCs (55.0 +/- 2.9 %) or vehicle (33.8 +/- 4.6%; p <0.0001). In addition, there was significantly greater recovery of end diastolic pressure after infusion of TGF α -preconditioned MSCs than vehicle or untreated MSCs. Conclusion: Preconditioning MSCs with TGF α prior to transplantation for acute myocardial ischemia may further enhance their ability to protect injured tissue and improve functional recovery.

DISCOVERY OF A NATURAL NONCOGNATE LIGAND ANTAGONIST OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF): CORRELATION IN PATIENTS WITH SEPSIS. Y. Al-Abed*, C. Metz, K. Cheng*, B. Aljabari*, M. Ochani*, X. Lin, V. Pavlov, T. Coleman*, K Tracey, E. Miller, The Feinstein Institute for Medical Research, Manhasset, NY 11030

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine that plays a critical role in the upregulation of proinflammatory mediators and the pathogenesis of sepsis. Three-dimensional X-ray crystallography of MIF shows that the molecule exists as a homotrimer and we have determined that the hydrophobic cavity formed between two adjacent subunits of the homotrimer, is required for the pro-inflammatory activity of the molecule. We have designed several small molecules that fit into the site critical for the proinflammatory action of MIF, and confirmed the interaction by the crystal structure of the MIF-complex. Binding of MIF in this way inhibits its proinflammatory activity, improves the clinical outcome in sepsis, and recapitulates immunotherapy and gene deletion. However, no natural soluble ligand of MIF has been reported previously. In this study, we have discovered a natural ligand, designated MIFn1 that binds the proinflammatory site of MIF with high affinity, and effectively modulates its activity. In addition, in plasma from patients with sepsis, we found an inverse correlation between the rise level of MIF and the decreased concentration of MIFn1. Therefore, we hypothesized that supplementation of this ligand during sepsis should compensate for its dramatic reduction and improve survival in our peritonitis model of sepsis in C57/Bl6 mice. Administration of MIFn1 improved the 14 days survival rate to 60% compared to 20% observed for the vehicle treated mice. Our data identify for the first time, the presence of a natural, noncognate ligand antagonist of MIF in plasma. A better understanding of the kinetics of MIF/ligand regulation in patients with sepsis may lead to improved outcome in this devastating disease.

REGULATORY T CELLS DEMONSTRATE AN INJURY-SPECIFIC RECALL RESPONSE

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Objective: In a previous study, we demonstrated that burn injury expands and activates FoxP3+ regulatory T cell (Treg) in mice. To test whether Tregs might develop an injury-specific recall response, we transferred CD4 T cells from sham and burn FoxP3-GFP knock-in mice into sham and burn naïve mice to track the expansion and activation of injury-experienced and inexperienced Tregs. **Methods:** FoxP3-GFP mice treated with or without rapamycin, a drug that increases Treg expansion, underwent sham or burn injury. Seven days later, CD4 T cells were purified from the lymph nodes and spleen of each group. CD4 T cells from sham or burn mice were adoptively transferred into naïve mice that underwent sham or burn injury to represent a second injury response. After 7 days, transferred FoxP3-GFP positive cells were detected by FACS to measure expansion and stained for activation marker antibodies (CD62L, ICOS, and CTLA-4) to measure activation. **Results:** We observed that rapamycin treatment caused significantly higher expansion and activation of FoxP3+ Tregs in burn as compared to sham mice at 7 days after the first burn injury. When CD4 T cells from sham or burn FoxP3-GFP mice were transferred into naïve recipient sham or burn mice, we found that the combination of burn CD4 T cells into burn recipient mice caused significantly greater FoxP3+ Treg expansion and activation (ICOS, and CTLA4, lower CD62L) than other adoptive transfer combinations.

Conclusion: These results indicate that injury activates Tregs because rapamycin caused a further increase in FoxP3 T cell expansion. The observation that injury-experienced Tregs respond more vigorously to the second injury suggests that Tregs develop an injury-specific memory-like recall response.

SEPSIS-INDUCED SUPPRESSION OF TH1 PHENOTYPE IS NOT DUE TO THE RISE IN TH2 CELLS

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Background and Objective: Sepsis alters CD4 T-cells, suppressing the Th1 phenotype and inducing apoptosis. Once stimulated, naïve CD4 T-cells exclusively commit to one of the T helper (Th) phenotypes by transcription of lineage-specific genes.

Methods: Male C57Bl/6 mice had cecal ligation and puncture (CLP, n=4) or sham (n=4) surgery. CD4 T cells were enriched from disaggregated spleens (to >90%) using positive immunomagnetic selection at 8 and 18 hrs after surgery and were analyzed by quantitative RT-PCR for mRNA expression of markers that reflect lineage commitment: T-bet (Th1), Gata3 (Th2), Ror γ t (Th17), Foxp3 (Treg), PUMA (apoptosis) and IL2 and IL6 (representing polyclonal activation). In a separate cohort of animals, intracellular PUMA and phospho-p53 (Ser46) protein abundances were measured by FACS at 18-20 hours.

Results: Remarkable downregulation of *T-bet*, and *Gata3* was found in CD4 T-cells from septic animals compared to sham. *ROR γ T* expression was not altered by sepsis. *IL2* and *Foxp3* had modest (<2-fold) increases at 8 hrs, but were normalized by 18 hrs. *IL6* and *PUMA* showed significant and sustained sepsis-induced upregulation (>6 fold, $p < 0.01$). Intracellular staining for phospho-p53 (Ser46) and PUMA showed a significant increase in fluorescence intensity in CD4 splenocytes in septic compared to sham mice.

Conclusion: Sepsis suppressed the expression of both Th1 and Th2 lineage specific transcription factors while increasing mRNA and protein expression of PUMA, a potent pro-apoptotic protein. These data suggest that sepsis does not suppress the Th1 phenotype by increasing the number of cells that commit to the Th2 phenotype. Instead, transcription of lineage-specific markers for both Th1 and Th2 cells are initially suppressed by sepsis. Furthermore, p53- and PUMA-mediated apoptosis may play a role in the sepsis-induced depletion of CD4 splenocytes.

TYPE I INTERFERON IN THE HEMATOPOIETIC SYSTEM IS NECESSARY FOR HOST DEFENSE DURING SEPSIS.

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Type I interferon (IFN) is essential for dendritic cell (DC) maturation and priming of B cells in response to toll like receptor (TLR) ligation, two key features of host defense. Mice with impairments in type I IFN signaling (IFNAR^{-/-} and SPRET/Ei mice), were shown to be endotoxin (LPS)-resistant (Karaghiosoff, M et al. Nat.Immunol 2003 and Mahieu T. et al. PNAS 2006). In addition, IFNAR^{-/-} mice were found to resist severe bacterial peritonitis induced by colon ascendens stent perforation (CASP) surgery (Weighardt H. et al. JI 2005). In this study, using IFNAR^{-/-} mice, we examined the contribution of type I IFN to the initiation of adaptive immune responses during sepsis using the mouse model of cecal ligation and puncture (CLP). We find that type I IFN is not necessary for splenic DC maturation but is required for the increased activation of B cells as assessed by CD69 expression and T cell independent antibody production to NP-Ficoll immunization. Interestingly, we found that despite IFNAR^{-/-} mice being resistant to LPS challenge, we find that they have a 50% increase in late mortality (after 48 hours) to CLP when compared to SvEv control mice (p=0.0002). Additionally, using bone marrow chimeras (SvEv→SvEv vs SvEv→IFNAR^{-/-}), we found that signaling of type I IFN in the hematopoietic system was required for protection against sepsis mortality (40% difference, p=0.006). These data indicate that type I IFN is required for optimal B cell activation during sepsis and type I IFN within the hematopoietic compartment is necessary for survival in sepsis.

SPERMINE PROTECTS MICE AGAINST LETHAL SEPSIS BY ENHANCING “EARLY” BUT ATTENUATING “LATE” CYTOKINE RESPONSES.

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The pathogenesis of sepsis is partly attributable to dys-regulated inflammatory response mediated by exogenous (e.g., bacterial endotoxin) and endogenous (e.g., HMGB1) stimuli. **Objectives:** To investigate the role of an endogenous anti-inflammatory molecule, spermine, in animal models of lethal systemic inflammatory diseases. **Results:** Here we report that intraperitoneal administration of spermine (10 mg/kg, twice daily, for three days) conferred significant protection against lethal sepsis (induced by cecal ligation and puncture), but not lethal endotoxemia. *In vivo*, spermine significantly enhanced peritoneal levels of IL-6, M-CSF, RANTES, sTNFR1 and sTNFR2 during early sepsis (i.e., 24 h post CLP), but dramatically attenuated peritoneal levels of HMGB1 and several surrogate markers of sepsis (e.g., IL-6, KC, MCP-1, TIMP-1, sTNFR1 and sTNFR2) during late sepsis (i.e., 52 h post CLP). When given at low concentrations (e.g., 20 μ M), spermine enhanced HMGB1- (but not LPS-) induced release of G-CSF, IL-6, RANTES, and sTNFR2 *in vitro*. At high concentrations (100 – 500 μ M), however, spermine dramatically attenuated HMGB1-induced release of G-CSF, KC, IL-6, MIP-2, RANTES, and sTNFR2. In light of the abundant accumulation of HMGB1 in the peritoneal cavity, as well its capacity to bind spermine *in vitro*, we propose that spermine is uniquely poised as an endogenous regulator for extracellular HMGB1 at sites of infection or injury. **Conclusions:** Spermine exerts protective effects against experimental sepsis by enhancing early inflammatory response (to fight against bacterial infection), and attenuating late inflammatory response (to facilitate its resolution). *(Supported by the NIH Grants R01GM063075 and R01GM070817 to HW).*

GENE- AND PROTEIN EXPRESSION PROFILING IN LIVER IN A SEPSIS-BABOON MODEL

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Objective: This study was designed to investigate the differences of Gram+ and Gram- induced sepsis in general and the specific genetic and protein expression patterns in liver. For a detailed understanding of the interdependence of activated pathways and their link to the septic response state, many molecular events must be monitored simultaneously. **Methods:** Male adult papio ursinus received over a period of 2 hours either a non-lethal dose of bacteria (*S. pyogenes* [Gram+ group] or *E.coli* [Gram- group]) or LPS suspended in saline or physiological saline (control group). After the animals had been monitored for 6 hours, liver samples were collected. To obtain specific gene expression (GE) patterns for liver, baboon RNA from both stimuli was hybridized on LBI-custom designed micro arrays. Pathway analysis for deregulated genes and linking of genomic and proteomic data were performed using a newly developed visualization tool called "Caleydo" (caleydo.org). Protein expression (PE) profiling for over-expressed soluble liver proteins was performed using 2-DE and mass spectrometry. **Results:** In liver, 32 genes were identified (29 for Gram+, 28 for G- and 21 for LPS), involved in e.g. the Fibrinolysis Pathway, Coagulation and Complement Cascades or Lipid Metabolism. Both bacterial stimuli provoked the identical PE profile (29 proteins) in liver. The majority of up-regulated proteins are involved in e.g. stress response, ROS response or immune response. Our data provide a deeper insight into the interdependence of pathways in liver activated at this stage of sepsis for genetic and proteomic analysis. This project is financially supported by GEN-AU.

CALCULATION OF PATHWAY GENETIC LOAD ALLOWS SIMULTANEOUS EVALUATION OF MULTIPLE LOCI.

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Objective: Despite the general success of genome-wide association studies, much heritability remains unidentified in many disease states. Some of this 'missing' heritability may lie in epistatic interactions among multiple loci, which are typically ignored. We present Pathway Genetic Load (PGL) as a novel method for simultaneous evaluation of epistatic interactions. Methods: Research was approved by the UTSW IRB. We evaluated alleles at six loci in the TLR4 signaling and response pathway (CD14, TLR4, LBP, IL-6, TNF- α , and IL10) for an effect on risk for complicated sepsis (sepsis-related organ failure or shock) and death after burn injury in 155 patients admitted to Parkland Hospital, Dallas, TX with $\geq 15\%$ TBSA burns and without significant non-burn trauma (ISS ≤ 16), traumatic or anoxic brain injury or spinal cord injury, who survived >48 hours. Clinical data were collected prospectively and candidate genotypes were determined by TaqMan assay. PGL was calculated for each individual patient as the sum of mutant alleles at all six loci, with presence of risk alleles scored as 0, 1, or 2 at each candidate locus. Data for PGL and each individual locus were analyzed by multivariate logistic regression. Results: After adjustment for percent TBSA burn size, inhalation injury, age, gender and race, PGL was associated with increased probability for complicated sepsis (aOR=1.59; 95%CI=1.11-2.29; $p=0.011$) and death (aOR=1.75; 95%CI=1.11-2.76; $p=0.017$). In analyses of individual loci, significant associations were observed only between alleles in TLR4 and TNF- α and complicated sepsis (TLR4: aOR=3.22; 95%CI=1.09-9.49; $p=0.034$, TNF- α : aOR=2.28; 95%CI=1.06-4.92; $p=0.035$) and between CD14 and death (aOR=2.87; 95%CI=1.26-6.55; $p=0.012$). Conclusion: Relative variability of aORs indicate greater power to detect genetic associations with PGL compared to the analysis of loci individually. Supported by NIH/NIGMS 5P50GM21681-43 and 5T32GM008593-13.

SMALL INTERFERING RNA (SIRNA) MEDIATED POLY (ADP-RIBOSE)
POLYMERASE (PARP) -1 INHIBITION UPREGULATES THE HEAT SHOCK
RESPONSE

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Introduction: PARP-1 is a nuclear protein that plays a pivotal role in DNA damage repair and genomic integrity. Persistent activation of PARP-1 leads to ATP depletion and cell death. PARP-1 inhibition is protective in animal models of inflammation, shock and diabetes. Our previous studies demonstrate that both pharmacological inhibition and genetic deficiency of PARP-1 up regulate the heat shock response. To further confirm that heat shock response upregulation is not a functional compensation of an absent PARP-1 gene, we hypothesize that siRNA mediated PARP-1 inhibition upregulates the heat shock response.

Methods: Immortalized mouse fibroblasts from wild-type controls were treated with PARP-1 siRNA. At 24 h after siRNA treatment, cells were subjected to heat shock at 43 °C for 45 min followed by recovery at 37 °C up to 4 h. Heat shock protein (HSP)-70 expression was assessed by Western blot assays and HSP-70 mRNA was evaluated by Real-time reverse transcriptase-PCR analysis. Electrophoretic mobility shift assays were performed to examine the DNA binding activity of HSF-1.

Results: SiRNA mediated PARP-1 gene silencing resulted in significant reduction of PARP-1 mRNA and protein expression. PARP-1 siRNA treated wild-type cells demonstrated a significant increase in HSP-70 protein and mRNA expression. This was accompanied by a significant increase of heat shock factor-1, the main transcription factor for HSP-70.

Conclusion: This study validates our previous results that decreasing PARP-1 activity upregulates the heat shock response. Hence, we propose that augmentation of the heat shock response is a possible mechanism for the salutary effects of PARP-1 inhibition in inflammation.

TRAUMA / HEMORRHAGE-INDUCED INFLAMMATION IN MICE: INSIGHTS FROM DATA-DRIVEN MODELS

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Objective: Trauma accompanied by hemorrhagic shock (T/HS) elicits a global acute inflammatory response. We have previously created computational simulations of inflammation and tissue damage/dysfunction to gain insight into this complex response in mice. In the present study, we utilized multiplexing cytokine analysis coupled with statistical modeling to gain further insights into the inflammatory response to T/HS.

Methods: Male C57/Black mice (n = 6 per group) were subjected to surgical cannulation (T) ± HS using a computerized, closed-loop system (Torres et al, *Shock* 2008, In Press). The 8 experimental groups were a) 1, 2, 3, or 4 h T alone and b) 1, 2, 3, or 4 h of T/HS (25 mmHg). Plasma was assayed for 20 cytokines (Luminex™) and NO₂⁻/NO₃⁻ (nitrate reductase). Principal component analysis (PCA) was carried out to determine the primary inflammatory drivers of inflammation in T or T/HS.

Results: ~98% of the variance in the data obtained in the T group could be described by a vector combination consisting predominantly of the chemokines MIG and IP-10, along with the cytokines GM-CSF and IL-10. T/HS was characterized by an inflammatory response dominated by MIG, IL-6, IL-10, IP-10, IL-12, TNF, and KC.

Conclusion: These results support our published modeling work that suggests a central role for underlying trauma in hemorrhage-induced inflammation (Lagoa *et al*, *Shock* 2006. 26:592), as well as supporting our previous selection of TNF, IL-6, and IL-10 for inclusion in the mathematical model of inflammation in various shock states (Chow *et al*, *Shock*, 2005. 24:74). These results highlight the capacity of PCA, both for discovering novel tendencies in the data and for defining variables to be included in mechanistic simulations.

NITROGEN METABOLISM BY THE LIVER AFTER INJURY IS INFLUENCED BY TRANSCRIPTIONAL CHANGES IN COMMON WITH THE ACUTE PHASE RESPONSE.

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Objective: The hepatic acute phase response (APR) is an organ specific response to a diverse array of insults characterized by a transient switch in liver phenotype.

This phenotype change significantly affects the profile of secreted proteins. Hepatic Nuclear Factor (HNF)-4 α plays a key role in modulating this response. HNF-4 α acute phase protein targets such as Transthyretin (TTR) change during the APR, but their adaptive role is unclear. Nitrogen disposal by the liver is an important aspect of the injury response with a unique point of transcriptional control by HNF-4 α at Ornithine Transcarbamylase (OTC). We postulate that changes in nitrogen metabolism are temporally and mechanistically related to the acute phase response through the action of HNF-4 α . Methods: We performed unilateral (uFF) or bilateral (bFF) femur fracture and observed C57/BL6 mice for periods of time up to 24 hours. Quantitative PCR and electrophoretic mobility shift assays (EMSA) to HNF-4 α promoter sequences were performed. Results: Relative quantity of OTC mRNA declined early after injury in both uFF and bFF with a greater effect in bFF at 6 and 12 hours. OTC mRNA returned to control levels at 24 hours in both groups. EMSA demonstrated HNF-4 α binding to the OTC promoter declines early post injury in uFF and bFF, which is consistent with changes in mRNA. The deficit in binding is greater and more sustained in bFF.

Similarly, binding of HNF-4 α to the promoter of the classic acute phase protein TTR declined early after injury and to a greater degree in the bFF group. Conclusions:

Changes in HNF-4 α binding during the acute phase response influence pathways of nitrogen metabolism essential to injury response. A broader view of the hepatic acute phase phenotypic shift, and its adaptive nature, may more accurately characterize its contribution to the injury response as a whole.

ER STRESS IN LIVER TISSUE OF BURNED RATS IS ATTENUATED BY INSULIN TREATMENT.

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Introduction: Autophagy is part of the unfolded protein response (UPR) caused by endoplasmic reticulum (ER) stress with the aim to degrade bulk cytoplasmatic content. The autophagy pathway is regulated by 2 main pathways, via the ER stress pathway and here especially the inositol requiring enzyme (IRE1) and via the PI3K pathway. We have recently shown that burn causes increased autophagy contributing to hepatic dysfunction. We have further shown that insulin improved hepatic structure and function and we therefore asked whether insulin has an effect on hepatic autophagy post-burn. Methods: Rats received a 60% full thickness scald burn and were euthanized at 1h, 3h, 12h, 24h, and 48h post-burn and liver tissue was harvested. Rats were randomized to receive either insulin 5 IU/kg or saline (n=4 per group per time point). Hepatic autophagy was determined by protein expression of Atg5 and Atg12, as well as ER stress marker IRE-1 and PI3K signaling pathway. Statistical analysis was evaluated by ANOVA, Student's t-test corrected with Bonferronis post-hoc test. Statistical significance is set at $p < 0.05$.

Results: Hepatic markers of autophagy Atg5 and Atg12 were markedly increased post-burn, as well as ER stress markers. PI3K and Akt signaling was significantly impaired post-burn. Insulin attenuated the expression of Atg5 and Atg12, ER stress markers and restored PI3K and Akt signaling post-burn, $p < 0.05$.

Conclusion: Burns causes ER stress and autophagy in the liver which may contribute to hepatic failure and dysfunction. Insulin attenuated autophagy and ER stress which was associated with improved PI3K/Akt signaling.

LOCAL IGF-I PREVENTS SEPSIS-INDUCED MUSCLE ATROPHY. G. Nystrom, R. Frost, C. Lang. Penn State College Medicine, Hershey, PA 17033.

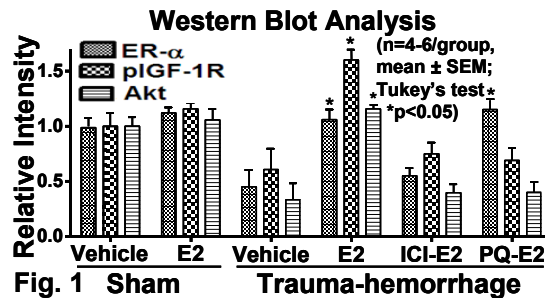
The present study tests the hypothesis that local bioavailability of IGF-I is capable of regulating muscle protein balance and that muscle-directed IGF-I can selectively maintain muscle mass during bacterial infection. Initial studies in C57BL/6 mice demonstrated that increasing or decreasing bioavailable IGF-I within muscle by local administration of either Leu²⁴ Ala³¹ IGF-I or IGF binding protein (IGFBP)-1, respectively, produced proportional changes in surrogate markers (e.g., phosphorylation of 4E-BP1 and S6K1) of protein synthesis. Next we examined the ability of a sustained local administration of IGF-I to prevent sepsis-induced muscle atrophy over a 5-day period. At the time of cecal ligation and puncture or sham surgery, mice had a time-release pellet containing IGF-I implanted next to the gastrocnemius and a placebo pellet placed in the contra-lateral limb. Data indicated IGF-I released locally only affected the adjacent muscle and was not released into the circulation. Gastrocnemius from septic mice containing the placebo pellet was atrophied and had a reduced IGF-I protein content. In contrast, locally-directed IGF-I increased IGF-I protein within adjacent muscle to basal control levels. This change was associated with a proportional increase in muscle weight and protein, as well as increased phosphorylation of 4E-BP1 and the redistribution of eIF4E from the inactive eIF4E·4EBP1 complex to the active eIF4E·eIF4G complex. Local IGF-I also prevented the sepsis-induced increase in atrogin-1 mRNA in the exposed muscle. Finally, local IGF-I prevented the sepsis-induced increase in muscle IL-6 mRNA. Thus, muscle-directed IGF-I attenuates the sepsis-induced atrophic response apparently by increasing muscle protein synthesis and potentially decreasing proteolysis. Collectively, our data suggest agents which increase the bioavailability of IGF-I within muscle per se might be effective in ameliorating the sepsis-induced loss of muscle mass without having undesirable effects on metabolic processes in distant organs. (GM 38032).

THE SEPARATE EFFECTS OF TNF- α AND REACTIVE OXYGEN SPECIES (ROS) ON ACUTE INSULIN RESISTANCE FOLLOWING TRAUMA AND HEMORRHAGE. L. Zhai*, J. Messina. Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Little is known about the mechanism of development of acute insulin resistance following acute injury. Here we studied whether trauma and hemorrhage induced acute hepatic insulin resistance in mice. Acute hepatic insulin resistance occurred as early as 30 min following hemorrhage and was most severe at 90 min (early time point). After resuscitation and 60 min of recovery, hepatic insulin signaling did not recover by 210 min (later time point). We have previously reported that TNF- α is necessary for the hepatic insulin resistance in the rat following hemorrhage (later time point). We therefore used TNF receptor 1/2 knockout (TNFR1/2KO) mice to exam the development of hepatic insulin resistance following hemorrhage at early and late time points. Insulin's ability to induce tyrosine phosphorylation of IR and IRS1, and phosphorylation of Akt, were rescued in the TNFR1/2KO mice at the later time point, but not at the early time point. This suggests that TNF- α does not cause hepatic insulin resistance following hemorrhage (the early time point), but plays a role in the continued insulin resistant state (the later time point). Reactive oxygen species are thought to play a role in the chronic development of insulin resistance and are also increased following injury. Therefore, we investigated the possible association between increased ROS and the acute development of hepatic insulin resistance. In wild type mice, ROS levels were significantly increased at early time points, but not at the later time point following hemorrhage. NAC, a nonspecific antioxidant, decreased ROS levels and rescued insulin signaling at the early time point following hemorrhage, but not at the later time point. Our results indicate that hemorrhage-induced insulin resistance has two phases: ROS play an important role in the rapid onset of acute hepatic insulin resistance, whereas TNF- α plays a critical role in the later phase.

ESTROGEN-DERIVED UPREGULATION OF INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR MEDIATES HEPATIC PROTECTION FOLLOWING TRAUMA-HEMORRHAGE (T-H). CK Hui*, H Akabori*, S Yang*, TC Hyatt*, WJ Hubbard*, R Raju*, IH Chaudry. Center for Surgical Research and Department of Surgery, University of Alabama at Birmingham, AL 35294

Although 17β -estradiol (E2) prevents organ damage following T-H, the precise mechanism responsible for its salutary effects is not fully identified. Protein kinase B (Akt) is involved in proinflammatory and chemotactic events in response to injury. Activation of insulin like growth factor-1 receptor (IGF-1R) after E2 administration has been shown to be beneficial in the recovery from brain injury following ischemia. We hypothesized that estrogen receptor (ER)- α /IGF-1R/Akt plays a role in E2-mediated attenuation of hepatic injury after T-H. Male rats underwent T-H (mean BP 40 mmHg for 90 min) and were treated with vehicle, E2, E2 plus the IGF-1R inhibitor PQ401 (PQ), or E2 plus ER antagonist ICI 182,780 (ICI), followed by fluid resuscitation. At 2 hr after resuscitation, plasma α -GST was measured as a hepatic injury marker and protein levels of hepatic Akt were determined. Results showed that T-H increased α -GST, which was markedly decreased in the E2-treated rats (data not shown). E2 treatment also increased ER- α expression, IGF-1R, IRS-1 and Akt activation compared to vehicle-treated T-H rats (Fig. 1). The above improvements were abolished by co-administration of PQ or ICI. These results demonstrate that ER- α and IGF-1R are upstream regulators of Akt. A rapid physical association between ER- α and IGF-1R upon E2 stimulation further suggests the role of ER- α on IGF-1R activation in E2-mediated attenuation of hepatic injury after T-H. (NIH R01 GM39519)

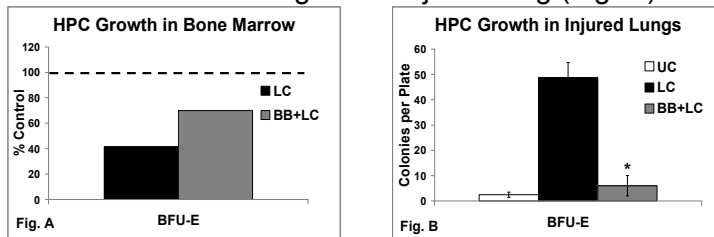


THE EFFECT OF BETA BLOCKADE ON PROGENITOR CELL GROWTH AND MOBILIZATION.

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Background: Previous work has demonstrated that hematopoietic progenitor cells (HPCs) are mobilized into the peripheral blood (PB) and subsequently sequestered in injured tissue. Norepinephrine influences both HPC growth and mobilization in a dose dependent manner. The aim of this study is to delineate the role of beta blockade (BB) in HPC growth and mobilization. **Methods:** Male Sprague-Dawley rats underwent once daily IP injections of propranolol (10 mg/kg) for 3 days to induce BB. Two groups underwent unilateral lung contusion (LC and BB+LC). HPC presence was assessed by GEMM, BFU-E and CFU-E colony growth both in injured lung and BM tissue 3h after injury. Flow cytometry, using c-kit and CD71, was used to determine mobilization into PB. * $p < 0.05$ (n=4-7/group). **Results:** BB resulted in less suppression of HPC progenitor growth in BM as compared to LC alone (Fig. A; dashed line represents control growth). BB resulted in a reduction of HPC mobilization to PB (1% vs 5%; BB+LC vs LC) as well as a decreased homing to the injured lung (Fig. B).



Conclusions: Given prior to injury, BB reduced suppression of HPC growth in BM following injury and prevented the mobilization and subsequent homing of HPCs in injured tissue. Therefore, BB may play an important role in regulating BM dysfunction and HPC mobilization following injury.

MYELOID DERIVED SUPPRESSOR CELL EXPANSION REQUIRES CXCL12
INDUCED PROGENITOR CELL DEVELOPMENT AND IS NECESSARY FOR
SURVIVAL

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Objective: Splenic myeloid derived suppressor cells (MDSCs) expand over 10 fold, and suppress CD8⁺ T cells during sepsis. Yet, the signals responsible for MDSC expansion are still unknown. Common myeloid progenitors (CMPs) generate granulocyte monocyte progenitors (GMPs) that transform into immature myeloid forms. Progenitor cells require (CXCL12) signaling for proliferation and differentiation. We hypothesize that CXCL12 regulates splenic MDSC expansion through splenic CMP and GMP proliferation and development. **Methods:** Female, 8 week, B6 mice underwent cecal ligation and puncture (CLP) (LD₂₀), sham or CLP with anti-CXCL12 antibody treatment (1 mg/day ip). At 1, 3, 5, 7, and 10 days (n=5 mice/group), splenic and bone marrow stem cells (Lin⁻Sca-1⁺c-Kit⁺) CMPs (Lin⁻Sca-1⁻Kit⁺CD34^{high}FcγR^{low}), GMPs (Lin⁻Sca-1⁻Kit⁺CD34^{high}FcγR^{high}), and MDSCs (GR-1⁺CD11b⁺) were evaluated. CLP mice receiving anti-CXCL12 (n=20 mice/group) were monitored for survival. **Results:** CXCL12 inhibition reduced splenic MDSC expansion in CLPs by 50% at days 5, 7 and 10 (p<0.005). The CMP and GMP populations were reduced by 60% and 80%, respectively (p<0.001) with anti-CXCL12 treatment. Anti-CXCL12 therapy had no impact on splenic or bone marrow stem cell, CMP, GMP, or MDSC proliferation on cell cycle analysis. Anti-CXCL12 treatment during sepsis increased mortality by 40% (p<0.001). **Conclusion:** Complete splenic MDSC expansion depends on CXCL12 signaling and splenic CMP-GMP development during sepsis. The splenic expansion of MDSCs depends on CXCL12 mediated transmigration rather than replication in situ. CXCL12 inhibition reduced survival suggesting a crucial role for CXCL12 and splenic CMP, GMP and MDSC expansion during sepsis.

INCREASED LOCAL NEUTROPHIL RECRUITMENT IMPROVES SEPSIS SURVIVAL & BACTERIA CLEARANCE. F. Craciun*, M. Osuchowski, B. Belikoff*, E. Schuller*, D. Remick. Boston University, Boston, MA 02118.

Synthesis of CXC chemokines and neutrophil (NE) recruitment in the cecal ligation and puncture (CLP) model of sepsis occur within a few hours after injury. We hypothesized that earlier peritoneal local recruitment of NE will result in increased clearance of bacteria, reduced organ injury and improved survival. To investigate the correlation of outcome in sepsis with NE recruitment, bacterial clearance and organ function ♀ ICR mice subjected to CLP were sacrificed at 24 hours (plasma- P, peritoneal lavage- PL and tissue samples were collected) and separated in predicted to live (Live-P) or die (Die-P) based on P IL-6 levels. To test if increased local NE recruitment improves

outcome, mice received 500ng MIP-2+50ng KC or saline, injected i.p. right after CLP; mice were followed for survival at 28 days or sacrificed at 24 hours and samples collected. Die-P mice had 8 times more PL bacteria than Live-P and more PL NE, but the bacteria/NE ratio was 4 times higher. P and PL levels of both pro and anti-inflammatory cytokines were higher in Die-P mice, but their PL/P concentration gradient for MIP-2 was lower. Die-P mice showed more organ injury

(e.g. BUN). MIP-2+KC mice had improved 28 day survival (52% vs. 27% for saline, $p=0.02$), lower PL bacterial count at 24 hours (0.6×10^6 vs. 1.4×10^6 for saline), lower P and PL cytokine levels (including MIP-2) and less organ distress. Dying septic mice fail to control bacterial invasion because of insufficient NE recruitment to the site of inflammation leading to increased release of inflammatory mediators and organ dysfunction. Increasing local NE recruitment early in the evolution of sepsis improves survival, reducing both the bacterial load and the inflammatory response.

	Die-P	Live-P
PL Bacteria	1×10^7	1.3×10^6
PL NE *	9×10^6	5×10^6
PL MIP-2 (pg/ml)	13,182	1,928
P MIP-2 (pg/ml)	20,315	1,065
PL/P MIP-2	0.65	1.81
BUN mg/dL	90.2	18.2
All significantly different, except *		

THE EFFECTS OF BETA-GLUCAN ON ENDOTOXEMIA AND SEPSIS. C. Newsome*, B. LeBlanc*, A. Ayala, J. Reichner*. Rhode Island Hospital, Providence, RI 02903.

Beta-glucans, a class of long-chain glucose polymers found in the fungal cell wall, stimulate innate immune cells. The soluble beta-glucan, PGG-beta-glucan has been shown to stimulate immune cell functions without inducing cytokines. The aims of this project were 1) to evaluate the effect of beta-glucan pretreatment on the LPS-induced TNF production *in vivo* and 2) to evaluate the effect of beta-glucan on the mortality of and IL-6 production in septic mice induced by cecal ligation and puncture (CLP) when it is administered after the onset of infection. The age and sex of the mice as well as the time of beta-glucan administration were varied. 90 minutes after the administration of LPS or 6 hours after CLP, serum was isolated and TNF and IL-6 levels were measured by ELISA. The results indicate that beta-glucan pretreatment (50mg/kg) for 96 hours prior to LPS stimulation significantly reduced the LPS-induced TNF production in 15-19 week old male mice (556.5 +/-547.98 in saline + LPS treated mice vs. 6172.7pg/ml +/- 1122.8 in beta-glucan + LPS treated mice), but not in 6-8 week old mice. The results also indicate that beta-glucan (10mg/kg) given 1 hour after CLP enhanced 10 day survival in female mice (52% in beta-glucan-treated mice vs. 25% in dextran-treated mice) and had a moderate enhancement on 3 day survival in male mice. The IL-6 values of the mice that went on to die were higher than the IL-6 values of the mice that survived CLP. beta-glucan significantly reduced IL-6 levels in male mice (11947.2pg/ml in beta-glucan-treated mice vs. 27928.3pg/ml in dextran-treated mice) and reduced IL-6 levels (though not significantly) in female mice (32613.28pg/ml in beta-glucan-treated mice vs. 56717.95pg/ml in dextran-treated mice). The development of beta-glucan as a therapeutic agent could aid patients with uncontrolled inflammation. Supported by NIH GM066194 and NIH F31-GM086069

THE RELATIONSHIP BETWEEN ARGINASE, NOS, AND ADMA TO ACUTE LUNG INJURY IN SEPSIS. L.Sousse*, C.Jonkam*, D.Traber, L.Traber, D.Herndon, P.Enkhbaatar, University of Texas Medical Branch, Galveston TX 77555.

More than 750,000 patients in the United States develop sepsis annually. Previously we have demonstrated variant arginase activity that is dependent on the nature of the causative agent such as *Pseudomonas aeruginosa* (PA) or Methicillin-resistant *Staphylococcus aureus* (MRSA). We hypothesize that asymmetrical dimethyl-arginine (ADMA) causes a decrease in nitric oxide (NO) production, increasing arginine availability and resulting in increased arginase activity in PA sepsis. METHODS: Ewes were operatively prepared and randomized after a 7-day recovery period into control, MRSA, and PA groups (n=6). Injury consisted of instillation of $2-5 \times 10^{11}$ CFU of live MRSA or PA into the airway, and the animals were sacrificed after 24 hours. In addition, groups of C57Bl/6J, iNOS and nNOS knockout mice (n=8) were nasally inoculated with $2-5 \times 10^5$ CFU of live MRSA or PA and were sacrificed after 8 hours. RESULTS: PA induced a more severe lung injury compared to MRSA (PaO₂/FiO₂: 319 ± 82 vs 205 ± 72). PA-treated sheep had a larger increase in arginase activity compared to MRSA-treated sheep (1.55 ± 0.16 uM urea/uG protein vs 1.33 ± 0.11) and had significantly higher ADMA ($1.79 \mu\text{M} \pm 0.14$ vs 1.30 ± 0.28 , p<0.05). PA-treated sheep had significantly lower plasma NOx compared to MRSA-treated sheep. iNOS knockout mice treated with PA vs MRSA had significantly higher arginase activity (10.96 ± 1.43 vs 4.18 ± 0.32 , p<0.05). nNOS knockout mice treated with PA had significantly higher arginase activity as well (14.12 ± 1.48 vs 5.07 ± 0.59 , p<0.05). CONCLUSION: The results strongly suggest that the severity of acute lung injury in PA sepsis is due to the increased activity of ADMA and arginase. Treatment strategies for PA and MRSA should consider their different host responses. For possible therapeutic intervention, effects of arginase inhibitors should be tested.

ADIPOCYTE RESPONSE TO S.AUREUS-INDUCED PULMONARY INFLAMMATION.

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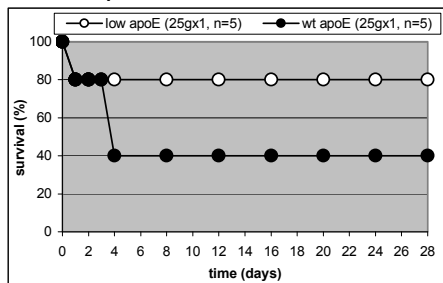
Sepsis is associated with a high mortality particularly in older individuals. We have found that during sepsis, the lung can release inflammatory mediators that affect cardiac function and survival. Here, we have examined possible effects of sepsis from a pulmonary origin on adipose tissue, since abdominal visceral adiposity (which increases with age) is associated with significantly increased inflammatory circulating mediators. *S.aureus*, a major pathogen in pneumonia, is associated with a high mortality, particularly in the elderly. Furthermore, *S.aureus* lung infection in older individuals is the most common cause of sepsis in European intensive care units. Our model used intratracheal instillation, into C57/BL6 mice, of staphylococcal cell wall components lipoteichoic acid (LTA) and peptidoglycan (PGN) or saline (controls). 6h later, the mice were sacrificed and blood collected. The lungs were harvested, and total mRNA isolated to examine the global transcriptome response. To examine the possible influence of pulmonary infection on adipose tissue, murine adipocytes (3T3-L1) were treated with either LTA/PGN or plasma collected from the mice. Analysis of lung transcriptomes revealed a robust inflammatory response to LTA/PGN including increases in *il6*, *ccl2*, *tlr2*, *cxcl1*, 2, and 5. Plasma IL-6 was significantly elevated in LTA/PGN instilled mice compared to controls. The 3T3-L1 adipocytes responded dose dependently to LTA/PGN, significantly increasing MIF in the culture medium. Treating 3T3-L1 with plasma from LTA/PGN instilled mice also induced a significant release of MIF. The data suggest that staphylo-coccal cell wall components within the lung can induce a profound inflammatory response that can be disseminated, via plasma borne factors, to other sites including adipose tissue, inducing further release of inflammatory mediators such as MIF. This may be of particular importance in older individuals where abdominal visceral adiposity is increased.

SIALIDASE TREATMENT SENSITIZES CELLS FOR TLR LIGANDS. L. Doughty, C. Yin*, V. Wolfe*, E. Stalets, B. Zingarelli, and H. Wong. Cincinnati Children's Hospital, Cincinnati, OH 45229

The surfaces of many cell types contain a multitude of glycoproteins and glycolipids which are rich in sialic acid. Sialic acid content on the cell surface can be decreased by inducible endogenous sialidases and pathogen expressed neuraminidases. Desialylation can modulate the activity of many cell types. Our objective was to explore the impact of desialylation by exogenous sialidase on sensitivity to TLR ligands. We used a mouse monocyte/macrophage cell line (J774 cells) and a human respiratory epithelial cell line (BEAS-2B). Cells were treated with NANase which hydrolyzes $\alpha(2\rightarrow3)$, $\alpha(2\rightarrow6)$, and $\alpha(2\rightarrow8)$ -glycosidic linkages of terminal sialic residues of various glycomolecules for 1 hour then by flow cytometry we quantified the amount of desialylated sites via the density of binding of PNA, a lectin that binds to the galactose moiety on surface glycoconjugates after removal of sialic acid residues. Our results demonstrate that NANase treatment of BEAS-2B cells increased the density of PNA binding demonstrating the impact of NANase on sialic acid content of the cell surface molecules (control MFI 408 +/- 17 vs NANase 50 mU 712 +/-26, $p<.001$). NANase treated J774 and BEAS-2B cells were stimulated with a dose curve of TLR4 or TLR2 ligands and incubated overnight. Supernatants were analyzed for cytokine production by ELISA. Our results show that removal of desialylation sensitized J774 cells and BEAS-2B cells to LPS and Pam3CysK as indicated by exaggerated $TNF\alpha$ and IL-8 production. In summary, our data show that the desialylation of cell surfaces may contribute to the magnitude of responses to TLR ligands. This may be a critical regulator of the magnitude of immune responses to neuraminidase-expressing pathogens such as Influenza.

APOLIPOPROTEIN E REGULATES SEPTIC MORTALITY IN MICE. K. Chuang*, O. Kattan*, B. Leung*, N. Presser*, H. Harris. Department of Surgery, University of California, San Francisco. San Francisco, CA 94143

Apolipoprotein E (apoE), a component of plasma lipoproteins, plays an important, but poorly defined role in sepsis. Our lab has shown that injecting apoE increases septic mortality in a rat model of sepsis. We questioned whether the observed hyperactive inflammatory response induced by supra-physiologic concentrations of apoE represents an epiphenomenon. Alternatively, it may represent the concentration-dependent, apoE-mediated activation of host immunity. Thus, we sought to determine the effect of normal versus sub-physiologic concentrations of apoE on septic mortality and systemic cytokine levels. Conditional apoE knock down mice (hypomorphic apoE), which express 2-5% of wild-type levels of apoE yet remain normolipidemic, were subjected to cecal ligation and puncture (CLP). Serum cytokines were measured at 24 h and survival monitored for 28 days after CLP. Hypomorphic apoE mice consistently had a lower CLP-induced mortality rate than their induced, wild-type counterparts (Figure below). The serum concentration of apoE in septic mice also corresponded to higher levels of T_H1 cytokines, as detected by multiplex analysis (IFN- γ , IL-1 β , TNF- α ; $p < 0.05$ for CLP hypomorphic versus wild-type). These findings indicate that the serum concentration of apoE directly correlates with the magnitude of the T_H1 cytokine response and mortality rate following CLP in mice. Further, these data provide support for apoE as a genuine regulator of the host response to infection.



THE ROLE OF VAV GUANINE NUCLEOTIDE EXCHANGE FACTOR IN DECTIN-1 MEDIATED PHAGOCYTOSIS. J. Johnstone*, N. Morin*, J. Reichner*, (A. Ayala).
Department of Surgical Research, Rhode Island Hospital, RI 02903.

Phagocytosis is an important mechanism of the innate immune system that involves the cellular recognition and engulfment of foreign particles by a diverse number of cell surface receptors on phagocytes. Particle recognition by cell surface receptors is accompanied by polymerization of the actin cytoskeleton, which has regulatory pathways that are complex and may be receptor dependent. VAV is a guanine nucleotide exchange factor (GEF) that regulates Rho GTPases, which in turn regulate cytoskeleton rearrangement. Dectin-1 is a receptor shown to have a role in mediating the immunological responses to beta-glucan, a carbohydrate found in the cell wall of yeast. Using macrophages from wild type and VAV-knockout mice with *in vitro* phagocytic assays, we found that VAV GEF's are essential for Dectin-1 mediated phagocytosis. We then studied the function of VAV *in vivo* by infecting both wild type and VAV-knockout mice intravenously with *Candida albicans* SC5314 as a model of systemic candidiasis. At day four after injection the mice were sacrificed and their internal organs were obtained and sectioned for histopathological evaluation. The VAV-knockout mice were found to have considerably greater fungal burdens and fungal dissemination, which is consistent with a defect in the innate immune system's ability to recognize and control a fungal pathogen. These findings advance our knowledge of the role of VAV GEF's in innate immunity and phagocytosis. A better understanding of the regulation of phagocytosis can help us to target drug therapies to improve immune responses to invading pathogens or help to suppress detrimental hyperactive inflammatory responses.

BLOCKADE OF IP-10 INHIBITS ADJUVANT-INDUCED PROTECTION DURING NEONATAL SEPSIS

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Globally, greater than one million newborns die every year as a result of neonatal sepsis. Current clinical management includes antimicrobial therapy and supportive treatment as attempts at immunomodulation have not yielded significant improvements in survival. Further investigation is needed to characterize biological markers of neonatal sepsis that can be used for earlier detection and/or more efficacious treatment modalities to improve survival. IP-10 is an important chemokine and diagnostic marker in adult and neonatal sepsis. We have recently demonstrated that pretreatment of mice with specific TLR agonists improves survival in neonatal sepsis from 30% to 70 % ($p < 0.05$), in sham vs TLR-agonist (LPS) treated animals. Neonatal mice pretreated with TLR agonists also exhibited increased recruitment of neutrophils and B1 cells to the peritoneum. Using a blocking antibody to IP-10, we were able to abrogate the sepsis survival benefit following TLR4 pretreatment to 35%, which was not statistically different than the 30% survival observed in sham or non-TLR saline-pretreated septic group. Of note, blocking IP-10 had no adverse effect on survival when given prior to neonatal sepsis. IP-10 blockade also resulted in a 55% reduction in recruitment and CXCR3 expression on peritoneal neutrophil and B1 cells as compared to isotype TLR4 agonist pretreated septic animals. These data demonstrate that the enhanced survival and recruitment of peritoneal neutrophil and B1 cells seen following adjuvant TLR4 pretreatment of neonatal mice during polymicrobial sepsis is IP-10 dependent.

IL-15 PREVENTS APOPTOSIS OF CD8+ T CELLS, NK CELLS AND DENDRITIC CELLS AND IMPROVES SURVIVAL IN SEPSIS

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Background IL-15 is a potent anti-apoptotic and immunomodulatory cytokine with unique effects on cells of the innate and adaptive immune system. IL-15 is listed as the most promising immunomodulatory agent by the National Cancer Institute. **Objective** Determine if IL-15 prevents sepsis-induced lymphocyte apoptosis, modulates cytokines, and improves survival in a mouse peritonitis sepsis model. **Methods:** CD1 mice underwent 2x25 gauge cecal ligation puncture (CLP) or sham surgery. After 30min, 1.5µg mouse IL-15 was administered S.Q. Spleens were harvested 24 hrs later and apoptosis quantitated using FACS analysis of the TUNEL assay. Serum cytokines were also quantitated. Survival studies were conducted in a separate cohort of mice. **Result** The degree of lymphocyte apoptosis in IL-15 treated mice was markedly decreased compared to control. TUNEL analysis demonstrated 10.4% CD8+ T cell apoptosis, 14.8% NK cell apoptosis, and 36.5% dendritic cell apoptosis in control vs 4.9%, 7.1% and 17.1% in IL-15 treated mice ($p < 0.01$). IL-15 also significantly increased anti-apoptotic proteins, Bcl-2 and Bcl-xL in CD8+ T cells, NK cells, and dendritic cells. IL-6, IL-10, and INF- γ in serum were 22.1µg/ml, 1.2µg/ml, and 18.2 pg/ml in control vs 9.8µg/ml, 0.6µg/ml, and 41.5pg/ml in IL-15 treated mice ($p < 0.05$). Finally, IL-15 caused a marked improvement in survival. At 7days survival was 70% in IL-15 treated mice vs 18% overall in control ($p = 0.0018$). **Conclusion** IL-15 represents an exciting novel approach to sepsis because it not only blocks apoptosis but modulates the immunosuppression.

RELEASE OF APOPTOTIC FACTORS IN MULTIPLE INJURED PATIENTS - DOES C5A REGULATE PMN APOPTOSIS?

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In multiple injured patients (MIP) marked complement activation can be observed. However, it is unclear whether this is accompanied by an increase of proapoptotic factors in the blood and whether - on a cellular level - complement factors regulate neutrophil (PMN) apoptosis (Ao). Serum of MIP (ISS>18, n=8) was analyzed by ELISA on hospital admission and 4, 12, 24, 48, 120, 240hrs after trauma to quantify Fas-Ligand (FasL), Fas-receptor (CD95), C5a, C3a. PMNs from volunteers (n=5) were incubated with C5a (100ng/ml, 60min) and mRNA was extracted for gene-array analysis. PMNs were incubated with C5a (100ng/ml) or C3a (1000ng/ml) for 6hrs with or w/o 12 μ M of camptothecin to induce Ao and active Caspase-3 was assessed via western blotting. As early as 4hrs after trauma a marked and persisting increase of FasL and CD95 was observed in patients' serum (ISS 35 \pm 3), when compared to healthy volunteers, paralleling increased serum C5a and C3a concentrations. Incubation of isolated PMN with C5a *in vitro* altered mRNA-expression levels of apoptotic molecules, such as Bcl-2 (5fold), FasL (4fold), Bcl-xL (3fold). C5a, but not C3a, markedly reduced camptothecin induced caspase-3 activation in PMN. Early after multiple injury a parallel increase of apoptotic serum markers and activated complement can be observed. C5a regulates PMN expression of apoptotic molecules and negatively regulates caspase-3 activation in PMN. Thus, C5a might be involved in posttraumatic modulation of activated PMN via negative regulation of apoptosis. This might contribute to the prolonged survival of PMN after trauma. (Supported by KFO-200 and DFG-PE 908).

PREVENTION OF ALVEOLAR EPITHELIAL CELL (AEC) APOPTOSIS BY IL-6-ACTIVATED STAT3 IN TRAUMA/ HEMORRHAGIC SHOCK (T-HS): ROLE OF AEC INTRINSIC AND EXTRINSIC PATHWAYS. Moran A., M. Mastrangelo, T. Eckols, H. Lin, and D.J. Tweardy*. Baylor College of Medicine, Houston, TX 77030.

Acute lung injury (ALI) occurs in up to 37% of patients after T-HS. AEC apoptosis is a key contributor to ALI; strategies to reduce it are needed. We have shown that T-HS induced ALI and apoptosis in up to 15% of lung cells of which >80% were AECs. Importantly, T-HS-induced AEC apoptosis was prevented by IL-6-mediated Stat3 activation initiated at the start of resuscitation. To identify genes downstream of Stat3 critical for mediating protection from apoptosis, we performed microarray analysis using whole lung RNA from sham (S) rats, and 3 groups of T-HS rats: 1) rats resuscitated with placebo (T-HS/P), 2) rats treated with IL-6 (10 ug/ml) at the start of resuscitation (T-HS/I) and 3) rats pretreated with the Stat3 inhibitor, T40214, and resuscitated with IL-6 (T-HS/I/T). T-HS altered 72% of known apoptosis-related genes. IL-6 treatment normalized 75% of these genes, while Stat3 inhibition blocked normalization by IL-6 in 65% of them. Apoptosis-related genes altered the most by T-HS and normalized by IL-6-mediated Stat3 activation were anti-apoptotic intrinsic (AAI) genes expressed within epithelial cells—*Glp1r* and *Pik3r1*—and pro-apoptotic extrinsic (PAE) genes expressed by infiltrating leukocytes—*Gzmb* and *Prf1*. To confirm their site of expression and pattern of modulation, we performed Q-RT-PCR using amplified RNA from laser capture microdissection-isolated AECs and non-AEC cells from S, T-HS and T-HS/I rat lungs. AAI gene transcripts were enriched up to 630 fold in AECs and PAE genes were depleted by $\geq 99\%$. T-HS decreased AAI gene transcripts in AECs by up to 14-fold and increased PAE gene transcripts up to 137-fold. Remarkably, IL-6 treatment normalized both sets of genes—AAI genes were increased up to 32 fold and PAE genes were decreased by up to 1,200 fold. Thus, AAI and PAE genes are implicated in T-HS-induced AEC apoptosis and its prevention by IL-6-activated Stat3 and, if confirmed at the protein level, would suggest novel interventions that may reduce the incidence and severity of T-HS-mediated ALI.

SCAVENGER RECEPTOR-A (SR-A) PLAYS A CRITICAL ROLE IN MEDIATING THE PATHOPHYSIOLOGY OF MYOCARDIAL ISCHEMIA/REPERFUSION INJURY.

C. Li*, Y. Hu*, T. Ha*, J. Kalbfleisch*, J. Kelley*, R. Kao* and D. Williams.

Pattern recognition receptors in the innate immune system play a pivotal role in the pathology of myocardial ischemia/reperfusion (I/R) injury. Recent evidence indicates that the macrophage scavenger receptor-A (SR-A) is an innate immune receptor, but its role in heart I/R injury has not been fully delineated. In this study, we examined the role of SR-A in myocardial I/R injury. SR-A deficient mice (SR-A^{-/-}, n=10) and age-matched wild type (WT) mice (n=10) were subjected to myocardial ischemia (45 min) followed by reperfusion (4 hrs). Infarct size was assessed by TTC staining. Cardiac myocyte apoptosis was evaluated by the TUNEL assay. The ratio of infarct area/risk area in SR-A^{-/-} mice was reduced by 43.5% (p<0.01) compared with WT mice (13.0 ± 0.01% vs. 0.23 ± 0.01%) after myocardial I/R. In SR-A^{-/-} mice I/R induced cardiac myocyte apoptosis was decreased by 53.4% (p<0.05) compared to WT I/R (7.5 ± 0.13% vs 16.1 ± 0.21%) mice. Ischemia/reperfusion increased (p<0.05) myocardial FasL levels and caspase-8 activity in WT mice. In contrast, the levels of these apoptosis associated molecules was reduced (p<0.05) in SR-A^{-/-} myocardium following I/R. In addition, the levels of p-IRAK, p-IκBα and NFκB binding activity increased (p<0.05) in the myocardium of I/R WT mice, when compared to SR-A^{-/-} I/R mice. These data indicate that SR-A plays a critical role in mediating the pathophysiology of myocardial I/R injury. Our data suggest that SR-A-mediated activation of NFκB and apoptotic signaling contribute to the pathophysiology of myocardial I/R. Based on these data, we speculate that modulation of SR-A-mediated signaling may be a useful approach for ameliorating myocardial injury in heart attack patients.

IL-7 INCREASES BCL-2, DECREASES APOPTOSIS, PREVENTS LOSS OF IMMUNITY, AND IMPROVES SURVIVAL IN SEPSIS.

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Sepsis is a lethal disorder that is characterized by widespread apoptosis-induced depletion of immune cells and development of an immunosuppressive state. This suggests that lymphocyte-protective anti-apoptotic therapies may reduce morbidity and mortality in patients with sepsis. IL-7 is a potent Bcl-2-inducing, anti-apoptotic cytokine that restores impaired immunity and is essential for lymphocyte survival and expansion. **Hypothesis:** IL-7 will block sepsis-induced apoptosis, prevent the immune suppression that occurs in sepsis, and improve survival. **Methods:** C57BL6 male mice underwent cecal ligation and puncture (CLP) or sham surgery. 90 minutes later mice received 5 μ g of IL-7 or saline. At different time points splenocytes and blood were harvested and apoptosis of lymphocytes, cytokine responses, expression of pro- and anti-apoptotic proteins, the delayed type hypersensitivity (DTH) response, and survival studies were determined. **Results:** IL-7 significantly increased intracellular Bcl-2 and prevented the sepsis-induced 2-3 fold increase in the pro-apoptotic protein PUMA. These mechanistic effects of IL-7 reduced the sepsis-induced death of naïve T cells by 50 % and preserved the DTH response, a key measure of global immunity. Furthermore, animals treated with IL-7 showed significantly reduced bacteremia as well as a ~ 2 fold improvement in survival. **Conclusion:** In conclusion, IL-7 reverses fundamental defects in sepsis, i.e., the loss of critical immune effector cells and functional immunity. The significant potential utility of IL-7 is illustrated by the fact that IL-7 is currently in 4 multi-national clinical trials. Therefore, IL-7 could move rapidly into therapeutic trials in sepsis.

CARDIAC SPECIFIC EXPRESSION OF PI3K P110 α ATTENUATES SEPSIS INDUCED CYTOKINE EXPRESSION AND LUNG NEUTROPHIL INFILTRATION, BUT NOT SPLENOCYTE APOPTOSIS.

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Activation of PI3K/Akt signaling prevents cardiac dysfunction and improves survival outcome in CLP sepsis. We have presented evidence that cardiac myocyte specific expression of constitutively active PI3K isoform p110 α maintains cardiac function and enhances survival in CLP sepsis. In this study, we examined serum cytokine levels, tissue neutrophil infiltration, organ NF κ B activation and tissue apoptosis in transgenic mice with cardiac myocyte specific expression of constitutively active PI3K p110 α (caPI3K Tg). caPI3K Tg mice and age-matched wild type (WT) control mice were subjected to CLP induced sepsis. At 12 hrs post-CLP serum cytokine levels (IL-1 α , IL-5, IL-6, IL-12, IL-17, TNF α , IFN- γ , KC, IP-10, MCP-1, MIG and MIP-1 α) were increased ($p < 0.01$) in WT mice. With the exception of KC, serum cytokine levels in caPI3K Tg mice did not increase in response to sepsis. Tissue MPO levels increased in the heart and lung of WT CLP mice at 12 hrs after CLP. Heart and lung MPO levels were attenuated ($p < 0.05$) in caPI3K CLP mice. Lung NF κ B activation and induction of apoptosis was not observed in caPI3K CLP mice. CLP sepsis increased ($p < 0.05$) splenocyte apoptosis in WT mice. caPI3K mice showed comparable levels of splenocyte apoptosis, indicating that cardiac PI3K p110 α expression did not alter CLP induced splenocyte apoptosis. The data indicate that constitutive activation of PI3K p110 α is protective in CLP and caPI3K Tg mice show a predominantly normal inflammatory phenotype in response to sepsis, but the beneficial effect of PI3K p110 α did not extend to splenocyte apoptosis. We conclude that modulating specific PI3K isoforms may be beneficial in the prevention and/or management of sepsis/septic shock.

THE HYPOXIA INDUCIBLE FACTOR 1A DECREASES T CELL IFN-GAMMA PRODUCTION AND APOPTOSIS DURING SEPSIS.

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Previously, we observed that septic mice with a T cell specific Hypoxia Inducible Factor 1a (HIF1a) deficiency demonstrated decreased: a) mortality, b) bacterial load, and, c) tissue damage. Here, we hypothesized that during sepsis, HIF1a deficient T cells would exhibit increased functionality and survival. To induce sepsis, mice were subjected to a cecal ligation and puncture (CLP) using a 23-gauge needle (single punch) followed by an 80% ligation of the cecum. Using an *in vivo* capture assay, we determined that systemic IFN γ concentrations were increased in the T cell specific HIF1a-deficient mice. Additionally, we found that HIF1a-deficient CD4 and CD8 T cells taken from septic mice produced increased IFN γ as compared to wild type mice. Associated with the increased IFN γ production was increased T-bet expression, active ERK and NF- κ B. Surprisingly, we found that thymic and splenic HIF1a-deficient T cells isolated from septic mice underwent increased apoptosis as compared to wild type T cells. These HIF1a-deficient T cells showed increased expression of BIM and decreased expression of Bcl-2. Altogether, these results suggest that during sepsis, HIF1a-deficient T cells have increased IFN γ production during their decreased life span as compared to wild type controls. Further, these data suggest that a sharp pro-inflammatory response by T cells that is quickly terminated is beneficial during sepsis.

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THE ENDOGENOUS BACTERIA MEDIATE SEPSIS-INDUCED INCREASES IN GUT EPITHELIAL APOPTOSIS.

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Introduction: Sepsis induces increased gut epithelial apoptosis, decreased villus length, and increased gut permeability. Sepsis-induced gut apoptosis is even higher in mice that lack lymphocytes (Rag mice). *In vitro* studies suggest that under systemic stress, the endogenous flora mediate increased gut apoptosis. Our objective was to determine if the endogenous bacteria mediate sepsis-induced gut apoptosis. Methods: Conventional (conv) C57Bl/6 mice and germ-free (GF) mice that lack all endogenous flora received intratracheal *P. aeruginosa* (3×10^7 vs 3×10^5 CFU; 58% vs 64% mortality, p=ns) and were sacrificed 24hrs later. Another cohort of GF and GF Rag mice were handled similarly. Gut apoptosis was quantified in 100 crypts/mouse by caspase-3 staining. Villus length was measured from the crypt neck to villus tip in 12 villi/mouse. Permeability was measured by gavaging mice with 22mg/ml FITC-dextran 19hrs post-injection. The FITC concentration in the plasma was determined at 24hrs. Data were compared using Mann-Whitney tests. Results: Septic GF mice had lower apoptosis than septic conv mice (2.6 ± 0.6 vs 6 ± 1 cells, $p=0.02$, $n=5/\text{grp}$). Septic GF mice had longer villi than septic conv mice (409 ± 17 vs $320 \pm 16 \mu\text{m}$, $p<0.01$). Septic GF mice had a trend toward lower permeability than septic conv mice (0.007 ± 0.004 vs 0.021 ± 0.009 pg/ml, p=ns). Septic GF Rag and septic GF mice did not differ in levels of gut apoptosis (0.8 ± 0.5 vs 2.5 ± 0.3 cells, p=ns, $n=4/\text{grp}$). Conclusions: Germ-free mice had longer villi and a trend towards lower permeability than conventional mice. Sepsis-induced apoptosis was not seen in germ-free mice nor was an augmentation of apoptosis seen in septic germ-free Rag mice. The endogenous bacteria may mediate sepsis-induced increases in gut apoptosis and may be necessary for the crosstalk responsible for lymphocyte control of gut apoptosis.

REDUCED MYELOID DENDRITIC CELLS AFTER TRAUMA IS ASSOCIATED WITH INCREASED APOPTOSIS

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Introduction: Dendritic cells are potent antigen presenting cells and represent an important linkage between the innate and adoptive immune system. The myeloid dendritic cells (MDC) and the plasmacytoid dendritic cells (PDC) are two major subtypes that can be distinguished according to their surface receptors and function. Severe trauma initiates an inflammatory response that might lead to subsequent complications. Apoptosis might relevantly contribute to this observation. In this study apoptotic and anti-apoptotic genes are investigated and related to the subpopulation of DC after severe trauma.

Material and Method: On day 1 and day 4 after admission 25ml blood was withdrawn from 10 severely traumatized patients (ISS 27 ± 12) and compared with the blood samples of 10 healthy volunteers. Purified mononucleated cells were incubated over 6 h and subsequently apoptosis of MDC and PDC was assessed by annexin-V-staining using flowcytometry. The expression of genes involved in apoptosis (Caspase 8, c-Flip, bcl-2, bax, GADD45) in DC was assessed by means of realtime RT-PCR. For statistical evaluation the Kruskal-Wallis-test was used ($p < 0.05$).

Result: Concerning MDC the number of apoptotic cells increased significantly from $44\% \pm 9$ on day 1 to $86\% \pm 28$ on day 4 after trauma (control: $31\% \pm 6$) while PDC did not relevantly change. The ratio of bcl-2/bax was significantly increased on day 1 (0.92 ± 0.26) and day 4 (0.88 ± 0.25) after trauma compared to controls (0.27 ± 0.07).

Conclusion: An increase of apoptosis might lead to the reduced number of MDC observed after severe trauma. However, anti-apoptotic genes like Bcl-2 are also significantly increased during the following days indicating the complex regulation of this cell type during the posttraumatic inflammatory response.

ROLE OF ENDOTHELIAL SPECIFIC NF- κ B SIGNALING IN ENDOTOXEMIC COAGULATION IN THE MICE. X. Ye, D. Song, S.F. Liu (E. Miller). Feinstein Institute for Medical Research, NHP, NY 11040.

We have previously demonstrated that selective blockade endothelial intrinsic NF- κ B pathway ameliorates septic multiple organ injury without impairing host defense response (J. Exp. Med 205:1303). In this study, we examined whether endothelial selective NF- κ B blockade abrogates coagulation, another major component of septic pathology. Transgenic mice (TG) that conditionally overexpress a mutant I- κ B α , an inhibitor of NF- κ B, selectively on endothelium and their wild type (WT) littermates were injected with saline (1 ml/kg, i.p.) or *E coli* LPS (5 mg/kg, i.p.). Plasma levels of fibrinogen, D-dimer and thrombin-antithrombin (TAT) were measured at 6 hours post-LPS. For WT-Con, TG-Con, WT-LPS and TG-LPS groups, respectively, plasma fibrinogen was 159.9 \pm 10.4, 163.9 \pm 9.2, 32.9 \pm 20.9 and 161.9 \pm 10.3 mg/dl (P < 0.01, between WT-LPS and any other group); Plasma TAT was 3.3 \pm 1.2, 1.7 \pm 0.2, 48.6 \pm 12.2 and 15.2 \pm 8.3 ng/ml (P < 0.01, between WT-LPS and any other group); and Plasma D-dimer was 81.4 \pm 10.2, 83.3 \pm 4.4, 341.8 \pm 27.8 and 214.7 \pm 12.9 (P < 0.01, between WT-LPS and any other group). Immunohistochemical staining of tissue sections of heart, kidney, liver and spleen from WT-LPS mice showed a widespread tissue fibrin deposition. Fibrin deposition was not evident in those tissue sections of TG-LPS mice. TG mice showed an improved survival. The 2-day and 7-day survival rate was 55% and 30% for WT-LPS mice, and 86% and 48% for TG-LPS mice. Our data demonstrates that endothelial intrinsic NF- κ B signaling plays a critical role in the development of coagulation in endotoxemic mice, and provides further evidence for endothelial selective NF- κ B blockade as a useful approach to develop sepsis therapies. (Supported by NIH R01GM063907).

NRF2 IS ESSENTIAL IN INNATE IMMUNE CELLS FOR SURVIVAL IN SEPSIS. X. Kong^{*1}, R. Thimmulappa^{*1}, P. Kombairaju^{*1}, D. Blake^{*1}, F. Craciun², D. Remick² and S. Biswal¹. ¹Johns Hopkins University, Baltimore, MD; ² Pathology and Laboratory Medicine, Boston University, Boston, MA.

Rationale: Nuclear factor-erythroid 2 p45-related factor 2 (Nrf2), is a bZIP transcription factor that dissociates from its cytosolic inhibitor, Keap1 to regulate a stress response transcriptional program including antioxidant defenses. Global disruption of Nrf2 enhances cecal ligation and puncture (CLP) induced mortality as compared to wild-type litter mates. Whether Nrf2 dependent regulation of innate immune response alone is central in mediating sepsis induced mortality and if enhancing Nrf2 pathway in macrophages and neutrophils protects from sepsis pathogenesis was unclear. **Method:** Mice with myeloid cell specific deletion of Nrf2 (Lyzm-Nrf2^{-/-}) and Nrf2-inhibitor, Keap1 (Lyzm-Keap1^{-/-}) that enhances Nrf2 activity, Nrf2^{fllox/fllox} and Keap1^{fllox/fllox} were subjected to CLP. Survival, bacteremia and systemic inflammation were measured. TLR4 signaling was investigated in macrophages. **Results:** (a) Lyzm-Keap1^{-/-} mice showed low bacteremia and dramatic improvement in survival compared to Keap1^{fllox/fllox} while, Lyzm-Nrf2^{-/-} mice showed poor survival with high blood bacteremia compared to Nrf2^{fllox/fllox}. (b) Serum levels of inflammatory cytokines (IL-6, TNF α , IL-1 β , IL-17, IFN λ , MCP-1, IL-10, IL-13, IL-1RA) as well as HMGB1 were significantly higher in Lyzm-Nrf2^{-/-} mice when compared to Lyzm-Keap1^{-/-} and Nrf2^{fllox/fllox} mice. (c) LPS stimulus induced greater ROS production, TLR4 surface expression, recruitment of MYD88, and TRIF to TLR4, phosphorylation of IKB and IRF3, and cytokine expression in macrophages from Lyzm-Nrf2^{-/-} compared to Lyzm-Keap1^{-/-} mice. **Conclusion:** Nrf2 in the innate immune cells is a critical nodal point in redox modulation that limits lethal systemic inflammation and preserves antibacterial defenses in sepsis. (Supported by NIGMS R01GM079239)

GHRELIN'S INHIBITORY EFFECT ON PRO-INFLAMMATORY CYTOKINES IN SEPSIS IS MEDIATED BY MITOGEN ACTIVATED PROTEIN KINASE PHOSPHATASE-1 (MKP-1). A. Jacob*, D. Rajan*, B. Pathickal*, I. Balouch*, A. Hartman*, R. Wu, M. Zhou, Y. Ji*, W. Dong*, P. Wang. Department of Surgery, North Shore University Hospital and Long Island Jewish Medical Center, Manhasset, NY 11030.

Ghrelin's beneficial effect in sepsis is mediated by the inhibition of the sympathetic nervous system (SNS), as evidenced by the reduced gut-derived norepineprine (NE) release in sepsis after ghrelin treatment. Recent data suggested that MKP-1, a protein phosphatase, is involved in the innate immune responses. We hypothesized that ghrelin's beneficial effect in sepsis is mediated by MKP-1. To test this, rats were subjected to sepsis by cecal ligation and puncture (CLP), treated with ghrelin, beginning at 5h post-CLP, and liver tissues were harvested and examined for MKP-1 gene and protein expression. CLP produced a significant decrease in MKP-1 gene expression in liver tissues at 20h after CLP ($P < 0.05$). The levels were decreased by approximately 40% at 2-10h after CLP. MKP-1 protein expression was significantly decreased as early as 2h after CLP and remained low at 5-20h after CLP. Ghrelin treatment in septic rats restored the MKP-1 gene and protein expression as similar to sham rats while vehicle treated rats showed an average 42.4% and 57.5% decrease from sham rats (1.0 ± 0.06 vs. 0.576 ± 0.07 $P < 0.05$; 0.322 ± 0.02 vs. 0.137 ± 0.02 ; $P < 0.001$), respectively. Since ghrelin's inhibitory effect is mediated by the SNS, NE treatment in Kupffer cells may downregulate MKP-1. Kupffer cells treated with NE for 1h showed an average 45.6% decrease in MKP-1 gene expression compared to untreated cells ($P = 0.02$). Interestingly, MKP-1 gene expression remained low for a further 2h even after the NE withdrawal. Therefore, we concluded that ghrelin's inhibitory effect on gut-derived NE release in sepsis leading to the downregulation of pro-inflammatory cytokines is mediated by MKP-1.

SEPSIS-INDUCED INFLAMMATION IS NEGATIVELY REGULATED BY $G_{\alpha_{i2}}$ PROTEIN COUPLED SIGNALING PATHWAYS. H. Fan, B. Zingarelli, K. Borg, P. Halushka, L. Birnbaumer, J. Cook Medical Univ. of SC, Charleston, SC, 29425

Previous studies have implicated heterotrimeric G_i proteins in signaling leading to inflammatory mediator production induced by lipopolysaccharide. However, the role of $G_{\alpha_{i2}}$ protein in cecal ligation and puncture (CLP) -induced sepsis has not been investigated. We hypothesized that $G_{\alpha_{i2}}$ protein is a critical modulator of inflammatory responses in experimental sepsis. $G_{\alpha_{i2}}$ knockout (KO) and wildtype (WT) mice were subjected to CLP. Eighteen hours after CLP, mice were sacrificed, blood and tissue samples were collected. CLP-induced plasma TNF α , IL-6 were significantly increased (14 ± 7 and 33 ± 15 fold, $p<0.05$) in $G_{\alpha_{i2}}$ KO compared to WT mice. CLP-induced lung and liver myeloperoxidase (MPO) activity were also increased (2 ± 0.3 and 7 ± 2 fold, $p<0.05$) in $G_{\alpha_{i2}}$ KO compared to WT mice. Another group of mice were subjected to CLP and mouse survival was monitored every 24 hours until 120 hours. The survival rate post-CLP was significantly decreased (33% decrease, $p<0.05$) in the $G_{\alpha_{i2}}$ KO mice compared to WT mice. These data demonstrate that $G_{\alpha_{i2}}$ protein signaling pathways are activated in sepsis either directly or through autocrine pathways and negatively regulate polymicrobial sepsis-induced inflammation. Understanding the role of $G_{\alpha_{i2}}$ protein in regulation of TLR signaling pathways will provide novel insights into regulation of inflammatory gene expression. (Supported by NIH GM27673 and GM67202)

	Plasma TNF (pg/ml)	Plasma IL-6 (ng/ml)	Lung MPO U/100mg	Liver MPO U/100mg
WT Sham	6 \pm 1	0 \pm 0	84 \pm 20	4 \pm 1
KO Sham	19 \pm 8	0 \pm 0	122 \pm 42	4 \pm 2
WT CLP	92 \pm 8*	1.4 \pm 0.4*	160 \pm 60	9 \pm 5
KO CLP	1318 \pm 678*#	45.5 \pm 20.0*#	271 \pm 50*#	61 \pm 21*#

* $p<0.05$ vs Sham, # $p<0.05$ vs WT CLP. N=7/group

CAVEOLIN-1 (CAV-1) IS REQUIRED FOR EXPRESSION OF HEPATIC VASCULAR INFLAMMATORY RESPONSE IN ENDOTOXEMIA. S.H. Lee, C. Culberson, W. Kwok and M.G. Clemens. Dept of Biology, Univ. North Carolina at Charlotte, Charlotte NC, 22823.

Cav-1 is a scaffolding protein that gives structure to caveolae which sequester receptors and signaling molecules at the cell surface. We have shown that overexpression of Cav-1 in hepatic sinusoidal endothelial cells is associated with decreased endothelin (ET)-stimulated eNOS activity resulting in excessive constrictor response and vascular dysregulation. Therefore we tested whether deletion of Cav-1 affected expression of vascular regulatory proteins. Cav-1^{-/-} (KO) or wild type (WT) mice (n=10 each) were injected with 10 mg/kg E. coli LPS or saline (control) and 6 hrs later livers were harvested for analysis by Western blot. Control KO mice overexpressed ET-A receptors (185%), calmodulin (148%) and eNOS (222%) but under expressed eNOS pSer1177 (61%; all changes p<.01) suggesting an overall enhanced ability to activate eNOS in the Cav-1 KO mice. Hsp90, ET-B receptor, iNOS and eNOS pThr495 were not different. Following stimulation with LPS, WT increased iNOS expression by 900% while KO only doubled. Although ET receptor expression did not change with LPS in WT, both ET-A and ET-B receptors decreased in KO. eNOS expression was not significantly changed by LPS in either WT or KO mice with eNOS remaining overexpressed in KO; however, eNOS-pThr495 which decreases eNOS activity was significantly increased in WT but not in KO mice. This suggests a release of eNOS inhibition in the KO mice. Taken together, these results demonstrate that expression of Cav-1 is an important regulator of expression of stress genes associated with hepatic vascular regulation. Quite notable was the dramatic decrease in iNOS expression in KO suggesting a critical role for Cav-1 in the proinflammatory response. Supported by DK38201.

SEVERE BURN IS ASSOCIATED WITH ALTERATIONS IN THE EF2K/EEF2 PATHWAY.

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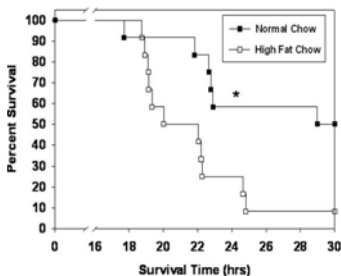
Severe burn has been shown to cause vast protein catabolism and marked alterations in protein synthesis for a prolonged period of time post-burn. **Eukaryotic elongation factor 2** (eEF2) plays a key role in protein translation and regulation of protein synthesis and breakdown. Recently we found that **severe burn deactivated** eEF2 by phosphorylation of eEF2K in animal models. In the current study, we investigated the role of eEF2 in response to severe burn in our pediatric patients. Eight pediatric burn patients (TBSA > 40%) were enrolled in this study. Muscle and skin biopsies were collected in the OR and immediately snap-frozen. Specimens were collected at early (day 0-10), middle (day 11-49) and late (day 50-365) time points post-burn. Three specimens from non-burned pediatric patients were used as controls to determine normal expression. Protein was extracted from the tissue samples according to our standard laboratory protocol and 30µg of protein samples were analyzed by SDS-PAGE and Western blotting. Our results showed that Burn caused a compensatory elevation of Ca²⁺/calmodulin-dependent protein kinase III and eEF2k phosphorylation at serine 366 site, which leads to deactivation of eukaryotic translation elongation factor 2 (eEF2). Phosphorylation of eEF2k deactivated eEF2 at the early and middle time points in muscle, while in skin there was a delay in modulating the EF2K pathway until the middle time point. In summary, severe burn injury leads to vast alterations in the EF2k/ eEF2 pathway in skin and muscle. These alterations last up to one year post-burn and could contribute to the dramatic protein catabolism observed in severely burned patients.

A HIGH FAT DIET INCREASES THE INFLAMMATORY RESPONSE IN SEPSIS.

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Obesity is associated with low grade chronic systemic inflammation however the mechanism underlying this inflammatory response is unknown. Here we investigated the effects of a high fat diet on sepsis. Six-week old C57BL/6 male mice were randomized to a high fat diet (HFD) (60% kcal fat) or a standard diet (control) (13% kcal fat) for 3 wks. Sepsis was induced in mice by cecal ligation and puncture (CLP) and monitored for survival for 24h (n=12/group). In a separate experiment mice underwent CLP and were sacrificed at 0, 3, 6, and 18h thereafter. Plasma and tissue were obtained for subsequent analysis. A p value of <0.05 was considered significant. Animals fed a HFD had a significantly lower percent survival at 24h following CLP compared to control mice (Fig). Analysis of myeloperoxidase activity indicated significant neutrophil infiltration in the lung in HFD mice vs controls at 18h after CLP (177 ± 24 vs. 80 ± 25 U/100 mg tissue, $p<0.05$). Adiponectin levels were similar at time 0 in both groups however at 18h after CLP the HFD mice had a decrease in adiponectin levels (6122 ± 393 ng/ml) compared to time 0 ($13,438\pm 2,980$ ng/ml) ($p<0.05$). In contrast, resistin levels were increased 18h after CLP in HFD mice. $\text{TNF}\alpha$ levels were lower in HFD mice at 3h after CLP compared with controls ($p<0.05$) however both diet groups had increased levels compared to time 0. Animals on a HFD had higher lung $\text{PPAR}\gamma$ protein levels at time 0 vs controls and had a larger decrease in $\text{PPAR}\gamma$ protein levels at 6h after sepsis. High fat feeding alters $\text{PPAR}\gamma$ and increases the susceptibility to sepsis. (NIH K12HD028827; R01GM067202).



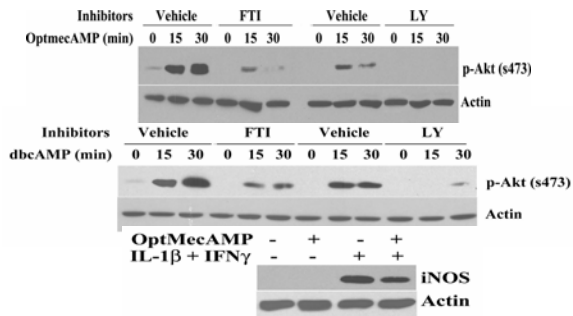
HEMORRHAGIC SHOCK INCREASES VASCULAR SMOOTH MUSCLE TONE PREFERENTIALLY AT LOW PRESSURES VIA HEME OXYGENASE ACTIVITY R. Johnson, T. Craig*, F. Johnson. University of Texas Health Science Center at San Antonio, San Antonio, TX 78229.

Heme oxygenase-1 (HO-1) is a shock protein that degrades heme. Recent studies suggest HO products can impact on vascular functions. **Objective:** Determine if untreated hemorrhage directly alters myogenic vascular tone through increased vascular heme oxygenase activity. **Methods:** Rats with chronic indwelling catheters underwent 45% hemorrhage without replacement. Twenty-four hours later, 1st order gracilis muscle arterioles were mounted in a microvessel chamber. Vessels from unbled animals served as controls. Vessel luminal diameters were measured in response to decreased intraluminal pressures (80-20mmHg, no flow); subsets were treated with an inhibitor of HO (15 μ M chromium mesoporphyrin, CrMP). In a complementary series, blood pressures were measured in awake bled and unbled rats in response to nitroprusside (2-50 μ g Kg⁻¹ min⁻¹, IV) infusions. **Results:** With respect to unbled controls, vessels from bled animals expressed higher levels of HO-1 and heightened generation of HO products. In response to decreased intraluminal pressures, vessels from shock animals displayed a 2-4 fold higher degree of tone (vs. unbled) at pressures below 60 mmHg; but, when treated with CrMP, vascular myogenic responses were indistinguishable from unbled controls. The same phenomenon was observed in vessels denuded of endothelium. Consistent with our findings in isolated vessels, nitroprusside induced vasodepressions in awake shock animals were markedly attenuated when compared to unbled controls. **Conclusions:** Hemorrhagic loss drives HO activity in peripheral vascular smooth muscle to heighten vascular tone at reduced pressures. This local increase in peripheral tone might limit the peripheral distribution of circulating blood to attenuate vasodepression and delay circulatory collapse.

CYCLIC AMP ACTIVATES AKT/PROTEIN KINASE B IN HEPATOCYTES THROUGH A GUANINE NUCLEOTIDE EXCHANGE FACTOR.

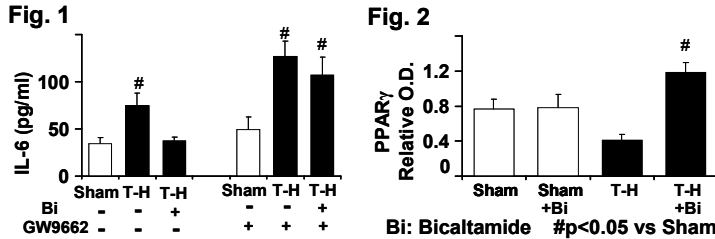
B. Harbrecht, S. Li*, B. Zhang*, University of Louisville, Louisville, KY 40292

cAMP is a ubiquitous intracellular second messenger that activates several intracellular signaling pathways. The best characterized signaling pathway activated by cAMP in hepatocytes is the cAMP-induced activation of PKA that regulates the expression of PEPCK to control hepatocyte gluconeogenesis. The liver contains guanine nucleotide exchange factors (GEF II or Epac) but their role in cAMP signaling in the liver and their role in hepatic function are both unknown. We therefore evaluated the role of Epac in mediating hepatocyte signaling. Rat hepatocytes were isolated and cultured overnight and exposed to 0.5 mM dbcAMP and 0.2 mM OptMecAMP (Epac analogue) in the presence of the Ras inhibitor FTI and the PI3K inhibitor LY294002. Activation of Akt/PKB, a known pathway of cAMP signaling, was assessed by western blot analysis. Both dbcAMP and OptMecAMP increased Akt activation (Figure). Inhibition of Ras with FTI and inhibition of PI3K with LY294002 decreased cAMP- and Epac-induced Akt activation. OptMecAMP also decreased cytokine-induced iNOS expression in cultured hepatocytes (Figure). These data suggest that Epac mediates some of the effects of cAMP on cellular signaling in cultured hepatocytes and does so through a Ras- and PI3K-mediated effect on Akt activation.



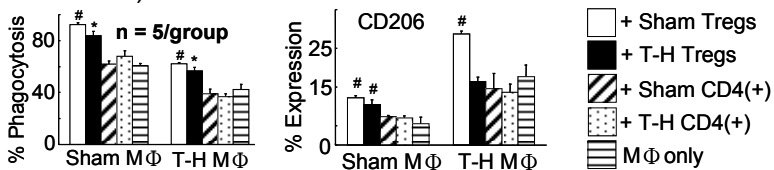
PPAR γ PLAYS A ROLE IN PRODUCING THE SALUTARY EFFECTS OF BICALUTAMIDE ON KUPFFER CELL CYTOKINE PRODUCTION FOLLOWING TRAUMA-HEMORRHAGE. T Suzuki*, T Kawasaki*, MA Choudhry, KI Bland*, IH Chaudry. Center for Surgical Research and Department of Surgery, University of Alabama at Birmingham, AL

Since the activation of peroxisome proliferator-activated receptors (PPAR) γ has been shown to be protective following ischemic conditions, we examined if PPAR γ plays any role in the salutary effects of bicalutamide on Kupffer cell cytokine production following trauma-hemorrhage (T-H). Male rats underwent T-H (mean BP 40 mmHg for 90 min, then resuscitation). Bicalutamide (5 mg/kg) or vehicle with or without PPAR γ antagonist (GW9662) was injected subcutaneously at the middle of resuscitation. At 2 hrs after resuscitation, plasma IL-6 and TNF- α levels, Kupffer cell IL-6 and TNF- α production and mRNA expression, and PPAR γ , NF- κ B and AP-1 DNA binding activity were determined. Results are presented as mean \pm SEM of 5-6 rats in each group. Plasma IL-6 and TNF- α levels, Kupffer cell IL-6 (**Fig. 1**) and TNF- α production and mRNA expression, and NF- κ B and AP-1 activity increased following T-H. However, bicalutamide administration following T-H normalized these parameters. In addition, PPAR γ activity was increased by bicalutamide administration following T-H (**Fig. 2**). Co-administration of GW9662, however, abolished the effects of bicalutamide on plasma cytokines and Kupffer cells. Thus, activation of PPAR γ appears to play an important role in the salutary effects of bicalutamide on plasma cytokine levels and Kupffer cell cytokine production after T-H and this is likely mediated via NF- κ B and AP-1. (NIH R01 GM39519)



THE FUNCTION OF REGULATORY T CELLS ON MACROPHAGE ACTIVITY IS DYSREGULATED FOLLOWING TRAUMA-HEMORRHAGE. C-H Hsieh*, WJ Hubbard*, KI Bland*, IH Chaudry. Center for Surgical Research and Department of Surgery, University of Alabama at Birmingham, Birmingham, AL

Regulatory T cells (Tregs) are potent immune suppressors. Studies have shown that Treg activities are enhanced and contribute to the T cell suppression following trauma-hemorrhage (T-H). However, their effects on macrophages (MΦ) are less clear. Male C3H/HeN mice were subjected to sham operation or T-H (laparotomy, 90 min hemorrhagic shock, mean arterial pressure 35 mmHg, followed by resuscitation with Ringer's lactate, 4x the shed blood volume), and were sacrificed 3 days later. Splenic MΦ phagocytosis, CD206, CD14, MHC II and iNOS expression as well as NO and cytokine production were examined with or without the presence of Tregs. Sham Tregs were more potent than T-H Tregs in enhancing MΦ phagocytosis (# $p < 0.05$ compared to other groups of the same condition; * $p < 0.05$ compared to sham Tregs). MΦ phagocytosis was decreased if Tregs were depleted. In contrast, adoptive transfer of sham Tregs enhanced MΦ phagocytosis. Sham Tregs suppressed the MΦ expression of CD14, MHC II and iNOS but increased the expression of CD206; however, T-H Tregs did not possess such capability. T-H induced increased production of NO by MΦ, while Tregs attenuated such effects. Finally, Tregs suppressed MΦ TNF and IL-6 production but enhanced IL-10 production. T-H Tregs also had more pronounced influence in affecting MΦ cytokine production than sham Tregs. Thus, Tregs modulate MΦ via their activation; however, such regulatory ability of Tregs was altered after T-H and may contribute to the immunodepression observed under those conditions. (NIH RO1 GM37127)



ROLE OF SOCS-1 AND SOCS-3 PROTEINS IN CARDIAC INFLAMMATION AFTER SHOCK. C.S. Chung, Y. Chen*, and A. Ayala. RI Hospital, Providence, RI 02903

Cytokines are one of the important mediators of cardiac pathophysiology. Studies have shown that the suppressor of cytokines signaling (SOCS)-1 and -3, which are potent negative feed back regulators of JAK/STAT, mRNA are upregulated in the heart after injury. However, little is known about the role of SOCS proteins in the cardiac pathophysiological responses to shock. The aim of this study was to understand the involvement of SOCS-1 and/or -3 in the regulation of cytokine-mediated cardiac injury/inflammation following shock. To do this, male C57BL/6 mice were subjected to sham or hemorrhage (HEM, 90 min at 30±5mmHg and resuscitated with Ringers, 4X shed blood vol.) with or without SOCS siRNA treatment. At different times post-surgery, blood and heart were harvested. SOCS-1, -3 or phosphorylated STAT-3 proteins were detected by immunoblotting and gene expression was measured by RT-PCR. Cytokine levels were assessed by cytometric bead array or ELISA. Our data show that SOCS-1 and -3 gene expressions were up regulated after HEM compared to shams. Two hrs after HEM, which is the peak time for gene expression, there was a general increase in IL-12, TNF- α , MCP-1, IL-6 and IL-10 in the heart. However, these cytokines in the heart were further increased when SOCS-1 or -3 was knock-down by siRNA treatment. Similar results were observed in the plasma cytokine levels after HEM. Moreover, a marked increase in heart IL-10 level was correlated with increased STAT-3 phosphorylation in mice treated with SOCS-1 siRNA. Taken together, these results suggest that the SOCS proteins potentially play an important role in regulating cytokine activation in the heart after shock.

Group/Cytoki	IL -12	TNF- α	MCP-1	IL -6	IL -10	
Hem	Cont	9+0.6	6+0.7	148+46	5+0.6	33+9
+	SOCS-	16+2	20+6*	447+19*	36+2*	90+13*
	SOCS-	15+0.9	14+4	366+49*	31+8*	42+12

[§] heart lysates. *p<0.05 vs. control siRNA, n=4-6/gp, ANOVA.
(Shock-Novo Nordisk Fellowship & NIH GM46354)

HMGB1 IN TRAUMA PATIENTS: MORTALITY, ORGAN DAMAGE, AND INFLAMMATION

R. Namas, A. Ghuma*, R. Zamora, J. Ochoa, T.R. Billiar, Y. Vodovotz. University of Pittsburgh, PA 15213

Objective: Trauma/hemorrhagic shock (T/HS) induces an inflammatory response that includes damage-associated molecular patterns, chief among them HMGB1. We sought to correlate HMGB1 with outcome, organ damage, and inflammation in T/HS patients.

Methods: Serum samples from 10 T/HS patients, 9 males and 1 female (age: 54 ± 3 , ISS: 30 ± 3 , 4 non-survivors [NS] and 6 survivors [S]) were collected at <6h, day 1, 2, 3, 4, 7 and 14 and assayed for HMGB1 and other cytokines using a specific ELISA (Shino Test) and Luminex™, respectively. $\text{NO}_2^-/\text{NO}_3^-$ was assayed by nitrate reductase. At each time point, Marshall Score was calculated along with lactate, base deficit, creatinine, BUN, AST, and ALT. HMGB1 was related to the above factors using Pearson product moment correlation (significance set at $P < 0.05$).

Results: Mean post-T/HS HMGB1 values at 6h were significantly higher in NS vs. S ($P = 0.02$). HMGB1 and Marshall Scores were correlated positively ($P < 0.002$) across all samples, as well as in NS ($P = 0.003$) but not S. HMGB1 levels correlated positively with lactate ($P < 0.03$) and AST ($P < 0.006$) in all samples. When examining the patient group as a whole, HMGB1 was correlated with IL-1Ra ($P = 0.008$), IFN- γ ($P = 0.005$), IL-8 ($P = 0.019$), IL-17 ($P = 0.04$), IL-4 ($P = 0.03$), and IL-10 ($P = 0.04$). When examined as a function of outcome, HMGB1 in S correlated with IFN- γ ($P = 0.007$), IL-17 ($P = 0.002$), MIP-1 α ($P = 0.04$), and IL-4 ($P = 0.001$). In NS, HMGB1 correlated with IL-12p70/p40 ($P = 0.01$), IP-10 ($P = 0.048$), IL-1Ra ($P = 0.02$), IL-8 ($P = 0.04$), and $\text{NO}_2^-/\text{NO}_3^-$ ($P = 0.03$).

Conclusion: Non-survivors of T/HS exhibit a rapid increase in serum HMGB1 not seen in survivors, and in these two groups HMGB1 is associated with a different array of inflammatory markers.

TLR4-DEPENDENT MYOCARDIAL CHEMOKINE RESPONSE TO ISCHEMIA/REPERFUSION: ROLE OF EXTRACELLULAR HSC70 L Ao, N Zou, JC Cleveland, DA Fullerton and X Meng Department of Surgery, University of Colorado Denver, Denver, CO 80045, USA

Background: Myocardial ischemia and reperfusion (I/R) injury causes an inflammatory response involving the up-regulation of multiple pro-inflammatory cytokines and chemokines. While chemokines have a pivotal role in the overall inflammatory response through inducing leukocyte infiltration and activation, the mechanisms underlying chemokine expression during myocardial I/R are incompletely understood. Several studies suggest that Toll-like receptor 4 (TLR4) signaling can be activated by endogenous agents released by injured cells and contributes to myocardial inflammatory response to I/R injury. Our recent study demonstrated in an isolated mouse heart model of I/R injury that the 70 kDa heat shock cognate protein (HSC70) released from the myocardium contributes to cardiac dysfunction through a TLR4-dependent mechanism. The current study was undertaken to test the hypothesis that extracellular HSC70 and myocardial TLR4 mediate the cardiac chemokine response to I/R injury. Methods and Results: We subjected hearts isolated from C3H/HeJ (TLR4-defective) and C3H/HeN (TLR4-competent) mice to I/R via the Langendorff technique and assessed myocardial neutrophil accumulation and expression of the chemokines KC and MCP-1. TLR4-defective hearts exhibited less neutrophil infiltration and chemokine expression than TLR4-competent hearts after I/R. Treatment with a HSC70 antibody reduced myocardial chemokine expression induced by I/R, and perfusion of recombinant HSC70 increased chemokine expression in TLR4-competent hearts but not in TLR4-defective hearts. Recombinant HSC70 also induces the chemokine response in isolated macrophages in a TLR4-dependent fashion. Conclusions: This study demonstrates that myocardial TLR4 mediates chemokine expression and neutrophil infiltration, and that extracellular HSC70 is involved in the TLR4-mediated myocardial chemokine expression. These results imply the HSC70-TLR4 pathway as a novel mechanism underlying the myocardial chemokine response to I/R injury.

GR1+ MACROPHAGES CONTRIBUTE TO THE DEVELOPMENT OF THE TWO-HIT RESPONSE FOLLOWING BURN INJURY.

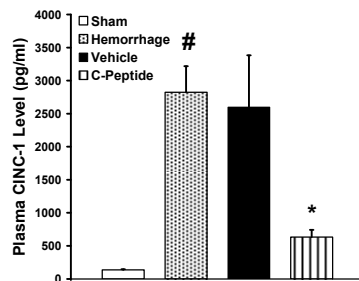
F O'Leary*, G Tajima, A Delisle, C Stallwood*, K Harris*, J Mannick, J Lederer., Harvard Medical School, Boston MA 02115.

Excessive activation of the innate immune system contributes to mortality in major trauma or sepsis. GR1⁺ macrophages are a subset of macrophages called myeloid suppressor cells that have been reported to suppress immune surveillance in murine cancer models. We studied the contribution of macrophage subsets (GR1⁺ vs. GR1⁻) to the production of pro-inflammatory cytokines following burn injury. WT B6 mice, CD4^{-/-} mice, anti-CD25 antibody and control antibody treated WT B6 mice were used in these experiments. Mice underwent sham or burn injury and were killed at day 7. Spleen cells were prepared and surface stained with F4/80, GR1 and CD11b. Spleen cells were stimulated with LPS (TLR4). Bead array assays and intracellular cytokine stains were used to determine TNF production. Two-hit injury mortality studies were carried out where mice underwent sham/burn injury followed by knockdown of Gr1⁺ macrophages with α Ly6G antibody 1A8 or control immunoglobulin at day 7 and LPS challenge at day 10. Burn-injury caused a significant increase in the percentage of GR1⁺ macrophages. We found that GR1⁺ macrophages were the primary source of LPS-induced TNF production. The absence of CD4 T cells or Tregs had no significant influence on the burn-induced increase in GR1⁺ macrophages. Two-hit mortality studies showed a reduction in mortality in mice depleted of GR1⁺ cells compared with control mice. These results suggest that GR1 macrophages represent the primary innate cell type that expand after injury, develop a pro-inflammatory phenotype, and contribute to the development of the two-hit response following burn injury. A better understanding of the source of excessive innate immune activation after major injury may provide therapeutic insights for patients in the critical care setting.

C-PEPTIDE REDUCES THE INFLAMMATORY RESPONSE FOLLOWING SEVERE HEMORRHAGE. R. Chima, G. Piraino*, T. LaMontagne*, P. Hake*, B. Zingarelli. Division of Critical Care Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229

C-peptide is a 31-amino acid peptide cleaved from pro-insulin during insulin synthesis. Recent data suggest that it may modulate the inflammatory response in endotoxemia. Our objective was to study the effect of C-peptide on the inflammatory response following severe hemorrhage. Hemorrhagic shock was induced in rats (age 3-4 months) by withdrawing blood from the femoral artery to a mean arterial pressure of 50mmHg. Animals were kept in shock for 3h at which time they were rapidly resuscitated by returning their shed blood. At the time of resuscitation and every hour thereafter, one group of animals received C-peptide (280 nm/kg IV) while another group received vehicle. Hemorrhage significantly increased plasma CINC-1 (cytokine-induced neutrophil chemoattractant-1) levels and this was associated with a significant increase in lung neutrophil infiltration (Fig1). Following resuscitation, C-peptide treated rats had a significant reduction in plasma CINC-1 levels and lung neutrophil infiltration when compared to vehicle-treated rats (Fig1). In the lung this effect was associated with a reduction in ERK phosphorylation and increased expression of PPAR γ . Thus, our data

Figure 1.

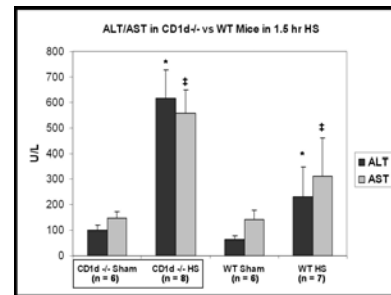


shows that C-peptide administration following severe hemorrhage reduces the inflammatory response and effects ERK and PPAR γ signaling in the lung. (#P<0.05 vs. sham, *P <0.05 vs. vehicle treated group, n=3-5 in each group)

Supported by the Shock Society/Novo Nordisk and NIH RO1 AG-027990

INVARIANT NKT CELLS ARE PROTECTIVE IN MICE DURING HEMORRHAGIC SHOCK. M. Chapman*, J. Brumfield*, L. Kohut*, T. Billiar, M. Scott University of Pittsburgh, Pittsburgh, PA 15213.

Introduction: Invariant NKT (iNKT) cells are known important mediators of both innate and adaptive immune responses, and can be induced to produce a wide range of pro- and anti-inflammatory cytokines. iNKTs have been implicated in both organ injury and organ protection depending on the model investigated. We investigated the role of iNKT cells in mice during hemorrhagic shock (HS). **Methods:** C57BL/6 (WT) mice and iNKT-deficient (CD1d^{-/-}) mice were subjected to HS via femoral artery cannulation and bleeding to 25 mmHg MABP for either 1.5h or 2.5h followed by resuscitation with lactated ringers solution. Sham animals underwent cannulation only. Plasma ALT and AST were measured 6h post initiation of HS, as well as liver cytokine mRNA expression measured by quantitative PCR. **Results:** CD1d^{-/-} mice had significantly higher AST(556±94 vs. 313±68 IU/L) and ALT(617±112 vs. 232±53 IU/L) compared with WT mice after 1.5h HS (p<0.05 Student's t-test)(n=7 or 8/gp). In WT mice, liver IL6 and IL17 mRNA expression were elevated over 50-fold over sham after 1.5h HS, however, CD1d^{-/-} mice were unable to mount a significant IL6 or IL17 response. (p <0.05 n = 4/gp). 2.5 HS was lethal in 55% of CD1d^{-/-} mice (n=20) compared to 15.3% (n=13) WT mice (p=0.03 Fisher's exact test). **Conclusion:** iNKT cells play a key protective role in HS both in terms of liver injury and overall survival. Early production of cytokines by, or stimulated by, iNKTs in the liver appears important to this protective function. Mediation of iNKT responses may provide future therapies for trauma related organ damage.



SYMPATHETIC MODULATION OF THE HOST DEFENSE RESPONSE TO INFECTIOUS CHALLENGE DURING RECOVERY FROM HEMORRHAGE. A. Whitaker*, J. Sulzer*, E. Walker*, K. Mathis*, P. Molina. LSUHSC New Orleans, LA 70112.

Hemorrhagic shock results in pronounced activation of the sympathetic nervous system (SNS). Previously we have demonstrated that noradrenergic depletion accentuates the early pro-inflammatory response to hemorrhagic shock, suggesting that hemorrhage-induced SNS activation attenuates the pro-inflammatory cytokine response. Whether this blunted pro-inflammatory response persists during the post-hemorrhage period affecting host-response to an infectious challenge has yet to be determined. We examined the contribution of SNS activity to the host-defense response to an infectious challenge following trauma/hemorrhage (TxHem). Male Sprague-Dawley rats underwent chemical sympathectomy (SNSx; 6-hydroxydopamine; 6-OHDA i.p. daily for 3 days) prior to vascular catheter implantation. Conscious, unrestrained rats were subjected to traumatic injury (muscle crush) prior to a fixed-pressure hemorrhage (40mmHg for 60min) followed 24 hr later by cecal ligation and puncture (CLP). Lung TNF- α content and MPO activity of TxHem animals was significantly higher ($p < 0.05$) than sham controls 48hr post-TxHem. CLP produced an increase in lung IL-1 and spleen IL-10 ($p < 0.05$) in sham and TxHem animals. SNSx did not alter unstimulated cytokine content in lung and spleen, but produced an accentuated tissue-specific cytokine response to CLP. In the lung, SNSx significantly ($p < 0.05$) enhanced the IL-1 content and MPO activity in sham and TxHem animals. In addition, there was a significant ($p < 0.05$) increase in spleen expression of IL-1 and IL-10 in sham animals and IL-6, IL-10 and TNF- α in TxHem animals. These results provide additional evidence for the regulatory role of SNS activation during TxHem and its contribution to modulation of host defense responses following an infectious challenge. DOD PR-054196, and NIAAA-AA7577.

HIGH-DOSE INTRAVENOUS ANTITHROMBIN III REDUCES PULMONARY VASCULAR PERMEABILITY AND NEUTROPHIL COUNT IN THE LYMPH AFTER COMBINED BURN AND SMOKE INHALATION INJURY.

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The University of Texas Medical Branch, Galveston, Tx

Objectives: We hypothesized that intravenous (i.v.) antithrombin (ATIII) reduces the transition of neutrophils into the lymph and thereby pulmonary vascular permeability. Therefore, this randomized, controlled study was designed to investigate the effects of i.v. high-dose ATIII on pulmonary lymph neutrophil count and flow as well as pulmonary function in an established ovine model of combined burn and smoke inhalation injury.

Methods: After 5 days of recovery, chronically instrumented female sheep (n=4) were randomly assigned to receive either an i.v. bolus of 48 U/kg ATIII followed by a continuous infusion of $12 \text{ U}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ (n=2) or only the vehicle (normal saline; n=2). Following tracheostomy, a 40% total body surface area 3rd degree cutaneous burn and smoke inhalation injury (48 breaths of cotton smoke $<40^{\circ}\text{C}$) were performed under deep anesthesia. All sheep were mechanically ventilated and fluid resuscitated during the 48h-study period. The ATIII treatment was started 1h after injury. Permeability index was calculated according to the formula: lymph flow*lymph protein/plasma protein. Data are expressed as mean \pm SE. Results: No statistical differences in PO_2/FiO_2 -ratio could be demonstrated. After 24 h, lymph flow was $27\pm 3 \text{ mL/h}$ in the ATIII and $49\pm 9 \text{ mL/h}$ in the control group. Pulmonary permeability and transvascular protein flux and were lower in ATIII treated animals 24 h post injury ($p<0.01$ each). The neutrophil count in the lymph was reduced in the ATIII group ($1.9\pm 0.3\times 10^6\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$) compared with the control group ($7\pm 1\times 10^6\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$; $p=0.04$). Conclusion: These preliminary data suggest that i.v. ATIII reduces pulmonary vascular permeability after combined burn and smoke inhalation injury by reducing neutrophil activation.

EFFECTS OF NEUTROPHIL AND MACROPHAGE DEPLETION ON LUNG INJURY FOLLOWING T/HS LYMPH INJECTION. D. Palange*, W. Dong, R. Bonitz*, D. Xu, Q. Lu, E.A. Deitch, UMDNJ-NJMS Newark, NJ 07103

Objective: Neutrophils and macrophages play an important role in the inflammatory response which causes lung injury. This study tested the effects of neutrophil and macrophage depletion on lung injury following trauma-hemorrhagic shock (T/HS) lymph injection. Methods: Neutrophils were depleted in CD-1 mice via injection of rabbit anti-mouse PMN antibody, while macrophages were partially depleted via tail vein injection of gadolinium chloride. 24 hours later mice were injected with either rat or pig lymph (T/HS or T/SS) over 3 hours via internal jugular vein. After lymph injection, lung permeability was assessed via bronchoalveolar lavage with Evan's blue dye. Lung tissue was also assessed for PMN sequestration (MPO and lung injury). Results: Rat T/HS lymph-induced increases in lung permeability and MPO levels were totally abrogated by neutrophil depletion and partly abrogated by macrophage reduction.

Neutrophil Depletion	Lung Permeability	MPO
Antibody+T/SS	4.2 ± 1.6	11.3 ± 7.3
Antibody+T/HS	4.7 ± 1.2	4.5 ± 2.5
Serum+T/HS	16.9 ± 4.1 ***	26.9 ± 7.1 **
Macrophage Depletion	Lung Permeability	MPO
Gadolinium+T/SS	3.8 ± 1.9	11.5 ± 3.7
Gadolinium+T/HS	9.3 ± 3.2 *	10.9 ± 5.0
Naive+T/HS	15.3 ± 4.8 #	9.3 ± 5.1

* P < 0.05, ** P < 0.01, *** P < 0.001 compared to all groups, # P < 0.001 compared to Gadolinium + T/SS

The degree of morphologic lung damage was similarly decreased (data not shown). Similar results were observed with pig T/HS and T/SS lymph specimens. Conclusion: These results suggest that neutrophils and to a lesser extent macrophages play a key role in lung injury induced by T/HS lymph injection from both rats and pigs.