TLR4 and is driven by hepatocyte induced chemokines and neutrophil infiltration in M-TLR4KO mice.

21. Ethanol exposure and burn injury in mice results in unique changes in the fecal metabolome suggesting alterations in the gut-brain axis

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Alcohol intoxication at the time of burn injury correlates with worse clinical outcomes, including a higher percentage of confusion assessment method positivity and delirium compared to those who do not drink. In mice, ethanol and burn results in increased neuroinflammation which correlates with heightened intestinal microbial dysbiosis suggesting a role for the gut-brain axis. The gut microbiome influences communication between the intestine and the brain via the production of bacterial metabolites. We hypothesized that ethanol-induced changes in the microbiome after burn cause distinct changes in the microbial metabolite profile that could induce intestinal dysfunction and an altered cognitive response to burn. To test this, C57/BL6 mice were given ethanol (1.25 g/kg) or water by oral gavage 30 minutes prior to receiving a 15% total body surface area burn or sham injury. 24 hours after injury, feces were collected for metabolomic analysis by liquid chromatography-mass spectrometry. Analysis revealed 45 significant changes following burn injury regardless of ethanol exposure (fold change >1.5, $p<0.05$). Compared to burn alone, ethanol and burn resulted in an accumulation of metabolites involved in carnitine and fatty acid metabolism, including L-octanoylcarnitine and hexanoyl-L-carnitine ($p<0.05$), which are linked to increased systemic inflammation and fatty acid oxidation. Ethanol and burn, but not burn alone, resulted in a 3-fold decrease ($p<0.05$) in fecal serotonin levels compared to sham-injured mice. Serotonin can influence both cognitive function and gut motility and intestinal barrier function. Finally, feces from ethanol and burn mice had reduced levels of the gut microbiota-derived anti-inflammatory metabolite, indole 3 acetate (IAA), with a 2-fold reduction in fecal levels compared to burn alone ($p<0.05$). Activation of the aryl hydrocarbon receptor by IAA regulates intestinal immunity and can inhibit microglial activation and neuroinflammation. Overall, ethanol and burn injury in mice altered several metabolites linked to immunomodulation and neurocognitive function. Future experiments will investigate the role of these metabolic signaling pathways in the systemic response to burn. (Supported by R35GM131831 (EJK), R01AG018859 (EJK) and T32AG000279 (KM)).

22. Decreases in microbial derived butyrate may contribute to intestinal inflammation in combined ethanol intoxication and burn injury

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Burn patients who are intoxicated at the time of injury face higher rates of bacteremia and sepsis. Our laboratory has recently profiled the fecal microbiome through 16s rRNA sequencing in both the small intestines (SI) and cecum in a murine model of combined ethanol intoxication and scald burn injury. Ethanol and burn injury (EB) resulted in gut dysbiosis marked by increases in pathobiont bacteria and decreases in many short chain fatty acid (SCFA) producing bacteria. Additionally, the levels of total SCFAs were reduced in ethanol burn mice compared to sham vehicle (SV). The most dramatically reduced SCFA was butyrate, which is known to act in an anti-inflammatory manner and is important for intestinal homeostasis. Combined EB injury leads to increased leakage of intestinal contents; therefore, we assessed plasma levels of endotoxin and found a significant increase in EB mice compared to SV. To assess the impact of butyrate on intestinal inflammation *in vitro*, a mouse small intestinal cell line (MODE-K) cells were pretreated with butyrate prior to the addition of LPS. Butyrate significantly reduced the induction of inflammation marked by IL-6. Further studies are in progress to determine whether SCFA/butyrate supplementation prevents the increase in inflammation of intestinal epithelial cells *in vitro* in response to fecal lysates from EB mice, as well as *in vivo* following combined EB injury. Overall, these findings suggest that changes in the gut microbiome and subsequent decreases in SCFAs may contribute to intestinal inflammation following ethanol and burn injury. (Supported by T32AA013527, and R01GM128242).

23. Alcohol and HIV increase the risk of pneumonia due to impaired bacterial clearance by alveolar macrophages

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People with HIV (PWH) and alcohol use disorder (AUD) have an increased risk for community-acquired pneumonia. Alcohol and HIV synergistically suppress bacterial ingestion (phagocytosis) and digestion (lysosome degradation) in alveolar macrophages (AMs), the primary innate immune effector cells in the lower airway. The mechanisms by which alcohol and HIV impair bacterial clearance in AMs are ill-defined. This study determined how alcohol metabolism increases the risk of pneumonia in PWH and if inhibiting AM oxidative stress by treatment with pioglitazone (PIO) will improve AM immunity. Primary isolated mouse AMs and MH-S cells (a murine AM cell line) were infected with EcoHIV, a chimeric ecotopic HIV, and exposed to the circulating alcohol metabolite, acetaldehyde, via the acetaldehyde generating system (AGS) for 48 h *in vitro*. During the last 24 h of EcoHIV and AGS exposure, cells were treated with PIO. AMs' phagocytic index was determined using fluorescence-labeled *S. aureus*. Lysosomal degradation of phagocytosed bacteria was assessed by cathepsin B activity. Levels of galectin 3 (phagocytosis upregulator), lysosome biogenesis markers, and cathepsin B- malondialdehyde-acetaldehyde (MAA) adducts were analyzed to determine the mechanisms by which acetaldehyde impaired AMs' bacterial clearance. Student's t-test and ANOVA tested significant differences between the groups. EcoHIV-infected AMs exposed to AGS showed depleted galectin 3 and lysosome dysfunction compared to EcoHIV or AGS alone. While AGS and EcoHIV independently impaired lysosome biogenesis, increased adduction of cathepsin B by MAA was observed in AMs exposed to AGS plus EcoHIV. Treatment of AGS-exposed EcoHIV-infected AMs with PIO reinstated galectin 3 expression, increasing AMs' phagocytic index. Further, PIO restored lysosome functions by attenuating cathepsin B-MAA adducts in AGS-exposed EcoHIV-infected AMs. This study implicates galectin 3 depletion and MAA adduction as mechanisms that may contribute to impaired AM bacterial clearance in PWH with AUD. PIO treatment may provide a novel therapeutic strategy to improve bacterial clearance in AMs of PWH with AUD by upregulating galectin 3 and suppressing cathepsin B MAA adduction. (Supported by T32HL116271, R01AA026086).

24. Alcohol administration and fecal microbiota transplantation alter the gut microbiome and metabolome in a murine model of Multiple Sclerosis

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Alcohol is a common dietary factor consumed by Multiple Sclerosis (MS) patients. Yet, despite its widespread use, its effects in modulating MS neuroinflammation are not well understood. We hypothesized that alcohol influences MS neuroinflammation via the gut metabolome, based on our prior research implicating the gut microbiome in Experimental Autoimmune Encephalomyelitis (EAE), a murine MS model. To test this, we performed a Fecal Microbiota Transplantation (FMT) from alcohol-drinking to non-drinking animals. Male and female C57BL/6J donors (N=20) were fed

[*] Denotes equal contribution.

a 2.6% ABV diet or control diet for 3 weeks. Recipients (N=40) received antibiotics to ablate gut microbiota, followed by sex-matched FMT prior to EAE induction. Males receiving FMT from alcohol-fed males showed significantly greater EAE disease remission compared to other groups. 16S rRNA sequencing analysis revealed sex differences in gut microbiome composition at Day 42 post-EAE. Specifically, alcohol FMT female recipients displayed increased levels of *Bacteroidota*, *Verrucomicrobiota*, *Akkermansia*, *Firmicutes* CAG:41 and *Prevotella* sp. CAG:485, whereas alcohol FMT male recipients displayed increased levels of *Actinobacteriota*, *Firmicutes_A*, *Proteobacteria*, *Terrisporobacte*r, *Clostridium* and *Niameybacter*. To further evaluate gut metabolites, we measured volatile organic compounds (VOCs) in fecal samples. VOCs differed significantly among FMT groups, with higher abundance of nitrogen- and oxygen-containing compounds in alcohol FMT recipients. Interestingly, we detected a higher concentration of cyanoacetic acid from alcohol FMT female recipients compared to control groups, a compound known to have anti-inflammatory properties. Our results demonstrate that moderate alcohol FMT directly modulates gut microbiome and metabolome in a sex-dependent manner, contributing to EAE disease amelioration.

25. Mitoquinol improves phagocytosis and glycolysis in ethanol-exposed macrophages via HIF-1α-PFKP axis

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Alcohol use disorder (AUD) is a risk factor for death in sepsis with inability to clear infections. Phagocytosis, the initial step in pathogen clearance, is a high energy demand state. Macrophages easily ramp up the aerobic glycolysis to meet the demand. We reported that acute ethanol exposure lowers glycolysis and phagocytosis by decreased expression of platelet isoform of phosphofructokinase (PFKP), a critical glycolytic enzyme. Ethanol exposure increases oxidative stress and affects the activity of hypoxia-inducing factor (HIF-1α), a transcription factor for glycolysis genes. The involvement of HIF-1α in regulating PFKP remains uncertain. Mitoquinol, a mitochondrial-specific antioxidant, reduces oxidative stress and enhances cellular glycolysis. We hypothesized that acute ethanol exposure leads to oxidative stress that impairs macrophage phagocytosis and glycolysis via the HIF-1α-PFKP axis. We examined our hypothesis in mouse bone marrow-derived macrophages (BMDM) ± ethanol ± E. Coli lipopolysaccharide (LPS) ± MitoQ. We studied 1. Phagocytosis, 2. Glycolysis and oxidative stress by intracellular lactate and reactive oxygen species (ROS) generation, 3. Nuclear translocation HIF-1α by western blot, 4. Chromatin immunoprecipitation-qPCR (ChIP-qPCR) to investigate the transcriptional control of PFKP by HIF-1α, 5. The impact of MitoQ on the 7-day survival in ethanol/vehicle-drinking mice with cecal slurry-induced sepsis. We found that in ethanol-exposed macrophages: 1. Excessive cellular ROS production dampens phagocytosis and glycolysis, impairs nuclear translocation of HIF-1α, leading to decreased PFKP expression via transcriptional control. MitoQ treatment in ethanol-exposed and LPS-stimulated macrophages restrains intracellular ROS levels, facilitates the nuclear translocation of HIF-1α, preserves PFKP mRNA expression, and enhances glycolysis and phagocytosis. MitoQ treatment improves 7-day survival in ethanol with sepsis. In conclusion, excessive ROS generated by ethanol inhibits glycolysis and phagocytosis in macrophages through the HIF-1α-PFKP axis. MitoQ restrains ROS production, maintains PFKP expression, and restores glycolysis and phagocytosis in ethanol-exposed macrophages. MitoQ is a potential therapeutic agent in AUD with sepsis.

26. Ethanol and HBV: unraveling the pathogenic interactions

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Hepatitis B virus (HBV) is a non-cytolytic, hepatotropic virus responsible for approximately 10% of chronic hepatitis B (CHB) cases. Nearly 20% of CHB patients progress to liver cirrhosis, which increases the risk of hepatocellular carcinoma by 100-fold. Despite the availability of a prophylactic vaccine, there are an estimated 250 million CHB carriers worldwide, with an annual death toll of 800,000. CHB remains an incurable infection, and a detailed understanding of its pathogenesis is essential to prevent the severe outcomes associated with HBV infection. Alcohol consumption in the liver exacerbates the progression of viral hepatitis. Previous studies have shown that individuals who abuse alcohol have a higher rate of HBV exposure and a greater likelihood of acquiring chronic infection. However, the complex interactions between alcohol and HBV infection are not fully understood. To explore this further, we utilized HepG2.2.15 and HepAD38 cells, which are HBV-transfected and secrete HBV virions. These cells do not metabolize ethanol, so we exposed them to an acetaldehyde-generating system (AGS), consisting of yeast alcohol dehydrogenase (ADH), NAD+, and 50 mM ethanol, to provide a consistent enzymatic release of acetaldehyde. This system has been successfully used in our previous studies. Our findings, based on RT-PCR and Western blot data, indicate that exposure to ethanol metabolites through AGS increases the expression of all tested HBV markers, including HBV RNA, HBV DNA, and HBV cccDNA. Further mechanistic studies revealed that AGS exposure suppresses STAT1 phosphorylation induced by IFN-α (1000 IU, 30 min) or IFN-λ (50 ng, 30 min), thereby inhibiting type 1 and type 3 signaling pathways essential for anti-viral ISG activation in HBV-transfected cells. We also measured the effect of AGS on APOBEC3G, an ISG that degrades HBV cccDNA, in HepG2.2.15 and HepAD38 cells. These cells were exposed to AGS for 48 hours, followed by ISG induction using human IFN-α (200 IU, 4h). Our results show that ethanol metabolites suppress APOBEC3G induction, leading to increased HBV cccDNA expression in liver cells. Further experiments are ongoing to elucidate the mechanisms and pathways involved in ethanol-mediated suppression of ISG activation, aiming to better understand and control HBV infection in hepatocytes (Supported by 5P50AA0304.

27. Aging promotes liver injury: studies on C57Bl/6 mice

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Many extrahepatic factors determine the liver disease phenotype and outcome. Among these, aging is a predominant risk factor for the development of advanced chronic liver diseases of various etiologies. Since the global population is aging and is predicted to double by 2050, this study was undertaken to establish a baseline of liver injury parameters in the young and old mice. Young (8-10 weeks old) and aged (20-22 months old) C57Bl/6 mice, purchased from the Charles River Laboratories (Wilmington, MA, USA), were housed in an AAALAC-accredited Animal Research Facility at the Omaha VA Medical Center. Metabolic phenotyping was done using the Sable Systems Promethion system. The animals were euthanized, and the serum and liver collected for subsequent analyses. Aged mice exhibited higher body weight compared to their younger counterparts despite aged mice exhibiting higher energy expenditure. Aged mice of both sexes showed higher hepatic triglycerides, lipid peroxidation and lower lysosomal acid lipase activities compared to their younger counterparts. However, only aged females exhibited lower hepatic S-adenosylmethionine levels, methylation potential, trypsin-like proteasome and lysosomal cathepsin B & cathepsin L activities compared to young female mice. Increased hepatic expression of chemokine (C-C motif) ligand 2 and chemokine (C-X-C motif) ligand 2 in aged female mice was associated with a lower liver methylation potential. Age did not affect serum AST or ALT levels nor had any effect on hepatic activities of alcohol metabolizing enzymes or the liver's ability to utilize betaine in aged mice of either sex compared with their younger counterparts. Chow-fed aged mice of both sexes exhibited some parameters of liver injury compared to their younger counterparts. However, more severe liver injury was seen in aged female

[*] Denotes equal contribution