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Alcohol and Inflammation & Infection: Clinical and Experimental Systems Summary of the 2010 Alcohol and Immunology Research Interest Group (AIRIG) Meeting

Anita Zahs¹, Robert T. Cook², Thomas J. Waldschmidt², Mashkoor A. Choudhry¹, Elizabeth J. Kovacs^{1,*}, and Melanie D. Bird¹

¹Alcohol Research Program, Department of Surgery, Burn and Shock Trauma Institute, Loyola University Medical Center, Maywood IL

²Department of Pathology, University of Iowa, Iowa City, IA

Abstract

The 15th annual meeting of the Alcohol and Immunology Research Interest Group (AIRIG) was held on November 19, 2010 at Loyola University Medical Center in Maywood, Illinois. This year, the focus of the meeting was on alcohol's effect on the immune system in both clinical and experimental systems. The event consisted of three sessions, which featured plenary talks from invited speakers along with oral presentations from selected abstracts, in addition to a poster session. Participants presented a variety of information on ethanol-induced effects on infection susceptibility and resolution, oxidative stress, and organ inflammation. Specifically, speakers presented new insights on the mechanism of alcohol-mediated deleterious effects in the lung, liver, skin, and neuroendocrine system as well as on immune cells in both *in vivo* and *in vitro* systems. Additional oral presentations suggested possible mechanisms for how alcohol-induced reactive oxygen species promote immune dysregulation both locally and systemically.

Keywords

alcohol; immune response; inflammation; infection; reactive oxygen species; lung; skin; musculoskeletal; skin; human

Introduction

Alcohol exposure has long been associated with immunosuppression. This dysregulation of the immune system includes decreased activation of lymphocytes following antigen stimulation, reduced cytokine production by macrophages and T cells, as well as alterations in neutrophil infiltration and phagocytic ability (Cook, 1998; Jerrells et al., 1990; Kawakami et al., 1990; Messingham et al., 2002; Sibley et al., 2001; Szabo et al., 1998). These immune alterations increase susceptibility to infection and inhibit the ability to heal following traumatic injury or other health challenges. Alcohol is also known to cause an elevation in

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*Corresponding Author: Elizabeth J. Kovacs, PhD, Loyola University Medical Center, 2160 South First Avenue, Maywood, IL 60153, 708-327-2477, ekovacs@lumc.edu.

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oxidative stress, which if not resolved can further promote inflammation and tissue damage (Nordmann et al., 1992).

To further discuss and examine these topics, the 2010 Alcohol and Immunology Interest Group (AIRIG) meeting was held on November 19, 2010 at Loyola University Medical Center in Maywood, Illinois. This meeting was sponsored by the Alcohol Research Program and the Department of Surgery at Loyola University Medical Center in Maywood, Illinois, the Society for Leukocyte Biology, and the National Institute on Alcohol Abuse and Alcoholism. Meeting organizers were Drs. Melanie D. Bird, Mashkoor A. Choudhry, and Elizabeth J. Kovacs (Loyola University Medical Center), Robert T. Cook and Thomas J. Waldschmidt (University of Iowa). There were three plenary sessions, which consisted of talks from invited speakers as well as presentations of selected abstracts. Thirty-six posters were presented during a separate session allowing further discussion on alcohol's effect on inflammation, oxidative stress, and susceptibility to infection, which was the focus of this year's meeting.

Alcohol and Infection

Alterations in pulmonary innate immunity in Alcohol Use Disorders and their relevance to pneumonia

Ellen L. Burnham, M.D., University of Colorado at Denver—Alcohol use disorders (AUDs) and the development of pneumonia have been associated for decades (Boffetta et al., 1990), and pneumonia in AUD patients is frequently associated with increased morbidity (Fernandez-Sola et al., 1995; Saitz et al., 1997). AUDs likely affect pulmonary innate immunity, however these specific effects are not well characterized in humans. The effect of alcohol on maintaining airway sterility has been proposed as a potential mechanism underlying the increased predisposition for pneumonia. Using bronchoalveolar lavage samples from otherwise healthy subjects with AUDs and normal volunteers, alcohol's effects were examined on acellular and cellular components of innate immunity that could affect airway sterility, specifically the antimicrobial proteins, lysozyme and lactoferrin. AUDs are associated with decreased lactoferrin levels, less lysozyme activity in bronchoalveolar lavage as well as a reduction in alveolar macrophage (AMs) produced lactoferrin. Gene expression by bronchoalveolar lavage cells (>90% AMs) was examined, and an association between AUDs and expression of genes involved in apoptosis was observed. This suggests that the pulmonary immune status of individuals with AUDs is not optimally suited to meet a pathogen challenge. Investigations in animal models of AUD have demonstrated a decreased ability of AMs to respond to infection with impaired phagocytosis of both pathogens and apoptotic cells, as well as AM hypo-responsiveness to microbial by-products (e.g. lipopolysaccharide (LPS). Furthermore, human AMs from those with AUDs stimulated with LPS produced less interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), and granulocyte macrophage colony stimulating factor (GM-CSF); TNF- α -treated AMs phagocytosis also was diminished. Overall, these observations could explain the elevated incidence of bacteremia and acute lung injury reported in those with AUDs following pneumonia, and each of these observed abnormalities could contribute to increased severity of illness in the setting of pulmonary infections. Moreover, they suggest that AUDs alter specific components of innate immunity that could be targeted for prophylactic therapy to decrease the incidence of pneumonia. Alternatively, strategies to normalize AM function in the setting of pneumonia could improve morbidity observed in patients with AUDs.

Transcriptomics and functional analysis of the effects of acute ethanol exposure on early events of sepsis in a mouse model

Minny Bhatti, PhD, Stephen Pruett, PhD, Bindu Nanduri, PhD Mississippi State University—

Acute ethanol exposure is known to significantly increase the risks and mortality associated with sepsis (McGill et al., 1995), which is a major cause of death worldwide. To understand the underlying mechanisms of this association, the effect of acute ethanol administration on the global gene expression profiles of peritoneal macrophages was evaluated during early events of sepsis. RNA was isolated from peritoneal macrophages collected from ethanol treated (6 g/kg, gavage) mice 2 hours after challenge with non-pathogenic *E. coli* (2×10^8 CFU/mouse). Gene expression profiling was done using an Affymetrix platform, and these genes, which were differentially expressed in response to ethanol treatment, were identified using Genesifter. Pathways and networks associated with these differentially expressed genes were identified using Ingenuity Pathway Analysis. The results indicate that ethanol inhibits the early innate immune response to *E. coli*, including Toll-like receptor (TLR) signaling, cytokine/chemokine production, macrophage mobilization and bactericidal activity. In addition, novel molecular pathways like an interferon amplification loop, protein translation initiation, phospholipase signaling and CDC42 activation were also inhibited in response to ethanol. The effects of acute ethanol exposure and TLR4 on bacterial clearance, spleen cell numbers, peritoneal macrophage numbers, and cytokine production by peritoneal macrophage 2 hours after the bacterial challenge were also analyzed. Wild type and TLR4 hypo-responsive mice were treated with ethanol and *E. coli* as described above. Ethanol-treated mice had decreased bacterial clearance and produced less pro-inflammatory cytokines in both strains of mice, thus confirming the transcriptomics data. Neither ethanol treatment nor a hypo-responsive TLR4 had significant effects on the cell numbers in the peritoneal cavity and spleen 2 hours post infection. The suppressive effect of acute ethanol exposure on cytokine and chemokine production was more pronounced in the wild type mice as compared to the TLR4 mutant mice. Overall the study indicates that acute ethanol administration significantly affects the early innate immune response to sepsis, which might be an important factor for the adverse outcome of sepsis in individuals exposed to alcohol.

Effects of chronic alcohol on macrophages in vivo and in vitro

Tanya R Johnson, PhD, Jay K Kolls, PhD Louisiana State University Health Sciences Center—

Liver injury and fibrosis from chronic alcohol have been linked to increases in TNF- α , which promotes a robust immune response in the liver, possibly induced by Kupffer cells (KCs), specialized liver macrophages (Tilg & Diehl, 2000; McClain et al., 1999). The goals of this study were to find regulatory changes in the cells that contribute to liver damage, and to establish whether the cytokine response in chronic alcohol-treated primary bone marrow derived macrophages (PBMMs) can emulate KCs *in vivo*. For *in vivo* studies, C57BL/6 mice were administered the Lieber-DeCarli liquid diet (35.5% calories from ethanol), while control diet mice were given a comparable non-alcohol diet (calories from maltose-dextrin). Mice were fed the Lieber-DeCarli or the control diet for 9 weeks then injected with LPS (1 μ g/g mouse weight) or with PBS (vehicle). Three hours after LPS treatment, mice were sacrificed by cardiac puncture, and serum cytokine levels were evaluated by Luminex cytokine multiplex assay. For *in vitro* studies, PBMMs from C57BL/6 mice were cultured with ethanol (0, 25 or 50 mM) for 5 days followed by 6-hr stimulation with LPS, after which supernatants were collected for multiplex assay. C57BL/6 mice fed the Lieber-DeCarli and inoculated with LPS, displayed increases in serum levels of TNF- α , IL-22, IL-10, MIP-1 α , MIP-2, KC and RANTES. Dramatic decreases in LIF, M-CSF, MIG, IL-9, IL-13, IL-15, IP-10, IL-12p40 and IL-12p70 were also seen in these mice. As observed with mice given the Lieber-DeCarli diet, cell supernatants from LPS stimulated PBMMs also displayed increases in TNF- α , MIP-1 α , MIP-2 and KC. In contrast to the *in vivo* data,

chronic alcohol and LPS-treated PBMMs expressed elevated levels of IP-10 and IL-6, and modestly expressed, but decreased levels of G-CSF. Treatment of PBMMs is a useful tool to study regulatory changes strictly related to chronic alcohol exposure; however, to acquire a more accurate and comprehensive understanding of regulatory changes in the liver from chronic ethanol, the full cytokine milieu and signaling pathways that affect the alcohol response must be studied in the organism.

Alcohol and Oxidative Stress

Spreading the oxidative stress: circulating bioactive phospholipids in chronic alcohol exposure

T.M. McIntyre, PhD, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University—Ethanol metabolism by the liver generates reactive oxygen species, and peroxidized lipids circulate in alcoholic individuals (Adachi et al., 2004). Chronic alcoholism damages organs besides liver, but how liver oxidant stress promotes distal pathologies is obscure. Tandem mass spectrometry was used to search for circulating biologically active lipids that could fulfill this role in rats fed a Lieber-DeCarli ethanol diet. A several fold increase in plasma levels of peroxidized phosphatidylcholines in rats chronically ingesting ethanol was observed. While these lipids themselves are not bioactive, they are precursors for oxidatively truncated phospholipids, some of which are pro-inflammatory and pro-apoptotic. Accordingly, bioactive oxidation products of peroxidized phospholipids along with Platelet-activating Factor—a remarkably potent and pleiotropic inflammatory mediator—were also increased in the circulation in ethanol-fed animals. However, reactive oxygen species generated by ethanol catabolism were not responsible for these circulating oxidized phospholipids as accumulation of circulating oxidized phospholipids did not correlate with the onset of ethanol exposure, hepatic oxidative insult, or the early increase in circulating alanine transaminase. Rather, circulating oxidized lipids appeared after 4 weeks of ethanol feeding, correlating with steatohepatitis and hepatic centrilobular TNF α accumulation. A progressive hepatic and systemic inflammatory response, rather than oxidant production associated with ethanol catabolism, underlies an increase in circulating oxidized phospholipids. Circulating oxidized phospholipids are biomarkers of an unappreciated process temporally distinct from the direct oxidant stress associated with hepatic ethanol catabolism or hepatocyte damage. More importantly, circulating oxidatively truncated phospholipids are pro-inflammatory and pro-apoptotic mediators with the potential to systemically distribute the effect of chronic ethanol exposure.

Sources of oxidative stress in the lung during chronic alcohol ingestion

Michael Hart, MD, Emory University, Atlanta VA Medical Center—Chronic alcohol ingestion causes oxidative stress in the lung and increases susceptibility to lung injury. Previous studies have determined that precursors of the antioxidant glutathione can attenuate oxidative stress and alcohol-induced susceptibility to lung injury in experimental animals (Guidot et al., 2000). The mechanisms by which alcohol causes pulmonary oxidative stress remain to be completely defined; however, evolving evidence in rodent models indicates that chronic alcohol ingestion increases the expression and activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases in the lung. Based on findings that activation of the nuclear hormone receptor, peroxisome proliferator-activated receptor gamma (PPAR γ), attenuates NADPH oxidase expression in other models (Hwang et al., 2007), recent studies have confirmed that short term treatment with ligands for PPAR γ reverse alcohol-induced increases in NADPH oxidase expression and activity in the lung. These findings suggest that targeting PPAR γ may represent a novel strategy to prevent or treat alcohol-induced oxidative stress and related derangements in lung cell function. One

hypothesis is that alcohol stimulates oxidative stress in lung cells thereby activating pro-inflammatory transcription factors that increase NADPH oxidase expression. NADPH oxidase-derived reactive oxygen species then contribute to maintenance of altered cell responses and phenotype. PPAR γ , by attenuating activation of redox-responsive transcription factors, reduces the upregulation of NADPH oxidases thereby lessening the deleterious effects of alcohol on the lung.

Upregulation of NADPH oxidases in alveolar type II cells in response to chronic alcohol consumption

Angelica Trumer, Lou Ann S Brown, PhD Emory University—Previous studies demonstrate that chronic ethanol consumption stimulates oxidative stress in alveolar type II cells (Holguin et al., 1998). However, the underlying mechanisms for this chronic generation of reactive oxygen species remain undefined. NADPH oxidases (Noxes) are key producers of reactive oxygen species and are known to be expressed in alveolar type II cells. However, the role of NADPH oxidases (Noxes) in ethanol-induced oxidative stress is unknown. It is possible that alcohol induces Nox upregulation in alveolar type II cells. To examine this hypothesis, the ethanol content of the drinking water for the C57BL/6 mice was incrementally increased over a 2-week period to a final concentration of 20%. The mice were then maintained on the 20% ethanol in the drinking water for an additional twelve weeks. Alveolar type II cells were isolated from the control and ethanol-fed mice, followed by RNA purification and quantitative RT-PCR for Nox gene expression. Ethanol-fed mice had robust increases in Nox1, gp91phox (also known as Nox2), and Nox4. Ethanol also induced elevations in the Nox regulators p22phox, p47phox and p67phox. Additional confocal microscopic studies demonstrated a significant increase in Nox4 expression in type II cells. These results suggest that Noxes may significantly contribute to the chronic oxidant stress associated with chronic ethanol ingestion, particularly for Nox4 as it is constitutively active. Future studies are to determine the role of the Nox isoforms in the ethanol-induced oxidative stress observed in type II cells.

The effects of alcohol-induced oxidative stress on bone fracture repair

Philip M Roper, John J Callaci, PhD, Loyola University Medical Center—Alcohol abuse is associated with many detrimental health effects including damaging effects on skeletal health and bone metabolism which are characterized by decreased bone mineral density, decreased bone strength, and inhibition of fracture repair and fracture malunion (Chakkalakal, 2005). Recent studies suggest that these harmful effects can be attributed to induction of oxidative stress (Muller et al., 2007). Mesenchymal stem cells (MSC), located in the bone marrow, periosteum, and endosteum of bone, as well as many other places, can give rise to bone, cartilage, and adipose tissue. MSCs are highly important in fracture healing as they mediate the immense anabolic response of osteoblasts and chondrocytes, both of which are necessary for fracture union. Differentiation of MSCs into either osteoblasts or chondrocytes is regulated by the Wnt signaling pathway in which the transcription factor β -catenin up-regulates specific target genes. However, β -catenin is also an important co-factor for a group of transcription factors called Forkhead box (FoxO) transcription factors. FoxO's, specifically FoxO3a, are activated by oxidative stress and compete with the Wnt pathway for a limited supply of β -catenin. In times when Wnt signaling is extremely important, such as fracture repair, the slightest perturbation can have devastating effects. We hypothesized that alcohol induces oxidative stress in the fracture callus, promoting FoxO's binding to and competing for β -catenin and ultimately dysregulating Wnt signaling. Using both histology and functional strength testing, our laboratory has shown deficient fracture healing in animals receiving alcohol administration, and this deficiency can be attenuated with MSC administration via tail vein injection. Our studies indicate that FoxO3a and its downstream target genes are up regulated in the fracture

callus of animals receiving alcohol, as well as in cultured MSC exposed to alcohol. These studies support the idea that alcohol can inhibit proper fracture healing through this crosstalk mechanism involving FoxO3a and β -catenin.

Alcohol and Organ Inflammation

Cyclodextrin and alcohol have adverse effect on the expression of cyclooxygenase 2 (COX-2) and other inflammatory proteins in BMDM macrophages

Anser C. Azim, PhD, Chicago State University—Toll-like receptors are highly conserved trans-membrane proteins that play an important role in the detection and recognition of microbial pathogens. During acute inflammation, resident macrophages in the lung are rapidly replaced by infiltrating peripheral blood monocytes derived from bone marrow precursors in response to endotoxemia or bacterial pneumonia. Monocytes are recruited to the lung along a positive concentration gradient of monocyte chemotactant protein-1 (MCP-1) through an interaction with the chemokine receptor, CCR2, on the surface of the infiltrating monocytes. This work discusses the role of alcohol and cyclodextrin and their roles in the pathogenesis of acute lung injury and acute respiratory disease syndrome (ALI/ARDS). Ethanol and cyclodextrin are known to affect lipid rafts present in the plasma membrane and the subsequent organization of TLRs (Szabo et al., 2007). In the present study we show the effect of alcohol and cyclodextrin on the expression of COX-2, TNF- α , and inducible nitric oxide synthase (iNOS). These findings suggest that these membrane-destabilizing reagents regulate Toll like receptors in an opposite manner to how they regulate inflammatory genes in MHS (mouse alveolar macrophage cell line), Raw 264.7(transformed macrophage-like cells), and bone marrow derived macrophages. These data suggest that alcohol and cyclodextrin mediate Toll-like receptor clustering and expression of inflammatory genes.

Interferon regulatory factor 3 and Type I interferons are protective in alcoholic liver injury in mice via cross-talk of parenchymal and myeloid cells

Jan Petrasek, PhD, Gyongyi Szabo, PhD, University of Massachusetts Medical School—Alcoholic liver disease (ALD) features increased hepatic exposure to bacterial LPS, which is recognized by Toll-like receptor-4 (TLR4) and activates signaling pathways depending on MyD88 or TRIF adaptors. It has previously been shown that in ALD, TLR4 induces Type-I interferons (IFN) in a MyD88-independent manner that involves interferon regulatory factor-3 (IRF3) (Hritz et al., 2008). The role for IRF3 in ALD is currently unknown. Alcohol or control diets were given orally to wild type (WT), IRF3 knock-out (IRF3 KO) mice, and to mice with a selective IRF3 deficiency in liver parenchymal and bone marrow-derived cells. Whole-body IRF3-KO mice were protected from alcohol-induced liver injury, steatosis and inflammation (Petrasek et al., 2011). In contrast to WT or bone-marrow specific IRF3-KO mice, only IRF3 deficiency in parenchymal cells aggravated alcohol-induced liver injury, as indicated by increased pro-inflammatory cytokines, lower anti-inflammatory cytokine IL-10 and lower Type-I IFNs as compared to WT mice (Petrasek et al). Co-culture of WT primary murine hepatocytes with liver mononuclear cells resulted in higher LPS-induced IL-10 and IFN- β , and lower TNF- α levels compared to liver mononuclear cells alone. Type-I IFN was important since co-cultures of hepatocytes with liver mononuclear cells from Type-I IFN receptor KO mice showed attenuated IL-10 levels compared to control co-cultures from WT mice. Furthermore, type-I IFNs potentiated LPS-induced IL-10 and inhibited inflammatory cytokine production in both murine macrophages and human leukocytes, indicating preserved cross-species effect (Petrasek et al., 2011). These findings suggest that liver parenchymal cells are the dominant source of Type-I IFN in TLR4/IRF3-dependent manner, and parenchymal cell-derived Type-I IFNs increase anti-inflammatory and suppress pro-inflammatory cytokines production by liver mononuclear

cells in a paracrine manner. Furthermore, these results indicate that IRF3 activation in parenchymal cells and resulting type I IFNs have protective effects in ALD via modulation of inflammatory functions in macrophages and may represent potential therapeutic targets in ALD.

Inhibition of Hsp90 attenuates chronic alcohol induced pro-inflammatory cytokine TNF α in Kupffer cells: role for molecular chaperones

Aditya Ambade, PhD, Pranoti Mandrekar, PhD. University of Massachusetts Medical School—Gut-derived endotoxin and oxidative stress play significant roles in the pathogenesis of alcoholic liver disease via increased production of the pro-inflammatory cytokine, TNF α . Heat shock protein 90 (Hsp90), an oxidative stress-inducible molecular chaperone is essential for proper LPS signaling. Earlier studies indicated that chronic alcohol augmented LPS induced hsp90 in macrophages (Mandrekar et al., 2008). This led to the hypothesis that inhibition of Hsp90 using a specific inhibitor, 17-DMAG [17-Dimethylamino-ethylamino-17-demethoxygeldanamycin] would prevent chronic alcohol-induced TNF α in hepatic macrophages. Kupffer Cells [KCs], isolated from mice fed Lieber-DeCarli diet with 5% v/v ethanol or iso-caloric control diet for 5 weeks, were treated with 17-DMAG (0.5 μ M) and LPS (100 ng/ml) *in vitro* and TNF α levels were analyzed. LPS treatment induced increases in both TNF α mRNA and protein (49% up-regulation) in KCs from alcohol-fed mice; however, inhibition of hsp90 prevented these inductions. To delineate the mechanism of TNF α inhibition during hsp90 inactivation in chronic alcohol exposed macrophages, hsp90 client proteins, CD14, TLR4 and NF- κ B activity were examined. RAW macrophages, a surrogate model of KCs, were exposed to 50mM alcohol for 5 weeks, treated with DMAG (0.5 μ M) and LPS (100 ng/ml) then analyzed for TNF α , NF- κ B activity, as well as CD14 and TLR4 surface expression. Similar to observations in KCs, 17-DMAG significantly decreased LPS-induced TNF α mRNA ($p < 0.05$) and protein ($p < 0.05$) in alcohol-exposed RAW macrophages. Interestingly, CD14 and TLR4 expression were not altered; however, NF- κ B activity was significantly reduced ($p < 0.05$). Hsp90 inhibition activates its own transcription factor, HSF-1 and induces hsp70, which inhibits LPS-induced NF- κ B activity in stressed macrophages (Mandrekar et al., 2008). HSF-1 activity and Hsp70 mRNA and protein levels were significantly increased after 17-DMAG treatment in macrophages exposed to alcohol. Collectively, these results suggest that hsp90 inhibition prevents chronic alcohol-induced TNF α in macrophages by inhibition of NF- κ B DNA binding and hsp70 induction. Thus, targeting Hsp90, a LPS pathway molecular chaperone, can be a unique strategy to significantly alleviate elevated production of TNF α in liver after chronic alcohol.

Chronic ethanol feeding results in lasting alterations to murine skin T cell populations

Corey Parlet, Annette J Schlueter, PhD, University of Iowa Health Center—Ethanol-induced epithelial dysfunction is made evident by the diminished ability of alcoholics to fight skin infections and heal wounds; however, the precise mechanism responsible for these changes has not been completely elucidated. Acute ethanol exposure in a rodent model induces alterations in inflammation and innate immunity detrimental to both host defense and wound healing responses in the skin (Radek et al., 2005; Fitzgerald et al., 2007). In addition, the skin is home to numerous immune cell populations including dendritic cells (DCs) and T cells. Using a murine model of chronic alcoholism, we previously reported that chronic ethanol feeding imposes both numeric and functional defects upon skin DCs (Ness et al., 2008), however it is unknown how skin T cells are affected by chronic ethanol. In the present work the effect of chronic ethanol on skin T cell populations was evaluated. Ethanol exposure decreased the number of epidermal and dermal T cells expressing the $\gamma\delta$ T cell receptor (TCR), while a trend towards increased numbers of dermal T cells co-expressing the $\alpha\beta$ TCR and the immunosuppressive integrin CD103 was

observed. These numeric alterations were lasting as they were not resolved five weeks after ethanol withdrawal. Given the established roles for $\gamma\delta$ T cells in innate immunity and tissue repair, their deficiency in ethanol-fed mice may help explain why alcoholism is associated with both impaired skin immunity and wound healing. Future studies hope to define the identities, mechanism(s) for numeric changes, and potential alterations in functional properties of skin T cells due to ethanol exposure.

Attenuated neuroendocrine response to hemorrhage in acute alcohol intoxication potentially mediated by decreased IL-6 and increased nitric oxide production in the paraventricular nucleus of the hypothalamus

Annie M Whitaker, Patricia E. Molina, PhD, Louisiana State University Health Sciences Center—Acute alcohol intoxication (AAI) impairs hemodynamic counter-regulation to hemorrhagic shock including greater hypotension to blood loss and impaired blood pressure recovery during hemorrhage and fluid resuscitation. This is associated with a blunted neuroendocrine response characterized by decreased circulating catecholamines and arginine vasopressin (AVP), suggesting that acute alcohol intoxication disrupts signaling mechanisms controlling AVP release (Molina et al., 2004). Nitric oxide (NO) and IL-6 exert opposing effects on arginine vasopressin (AVP) release and are both altered by acute exposure to alcohol (Goral et al., 2004; Seo & Rivier, 2003). This led to the hypothesis that intoxication disrupts the NO/IL-6 balance in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) contributing to the attenuated AVP response to hemorrhagic shock. Chronically-catheterized, adult male Sprague-Dawley rats (250–300g) received a primed-constant (2.5g/kg + 300mg/kg/h) 15h intragastric infusion of alcohol (blood alcohol ~250 mg/dl) or isocaloric/isovolumic dextrose (control). Acute alcohol intoxication significantly increased levels of NO (~30%) in the PVN and SON of the hypothalamus as well as nitric oxide synthase (NOS) activity in the SON as compared to DEX-treated controls. Additionally, alcohol intoxication attenuated the rise in circulating AVP levels, increased NO in the PVN, and augmented the NOS response in the PVN at the end of hemorrhagic shock, as compared to controls. Moreover, IL-6 expression in the PVN was lower at the end of hemorrhagic shock in after alcohol exposure compared to controls without altering IL-6 levels in the SON. These results suggest that acute alcohol intoxication disrupts NO/IL-6 regulatory control of AVP release, and it is possible that increased central NO production in the PVN and SON and decreased IL-6 signaling in the PVN contributes to the attenuated neuroendocrine response to hemorrhage-shock after acute alcohol intoxication.

Summary

The meeting focused on alcohol's effects on susceptibility to infection and elevated inflammation. Acute and chronic alcohol exposure alters numerous immune cell types, as well as stem cells and organ-specific cells. Macrophages, type II alveolar cells, Kupffer cells, T cells, keratinocytes, and stem cells all react adversely to alcohol exposure. In addition, alcohol promotes formation of peroxidized lipids and activation of various enzymes, all of which increases reactive oxygen species formation and inflammation. Alcohol also alters the neuroendocrine response. Clinical studies presented support an association between alcohol exposure and susceptibility to infection as alveolar macrophages from alcohol use disorder patients have decreased phagocytosis and pro-inflammatory cytokine production following stimulation. Moreover, this clinical evidence parallels the results found in animal models. Overall, the findings presented at the meeting show some salient mechanisms by which alcohol contributes to enhanced inflammation and susceptibility to infection and highlighted the importance of translational research in the field. Furthermore, these studies indicate that researchers are beginning to discover the key

mechanisms involved in immune dysregulation following alcohol exposure, which will aid in the better treatment of patients in the future.

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