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Alcohol and Inflammatory Responses: Summary of the 2013 Alcohol and Immunology Research Interest Group (AIRIG) meeting

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Abstract

Loyola University Chicago, Health Sciences Campus in Maywood, Illinois hosted the 18th annual Alcohol and Immunology Research Interest Group (AIRIG) meeting on November 22, 2013. This year's meeting emphasized alcohol's effect on inflammatory responses in diverse disease states

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and injury conditions. The meeting consisted of three plenary sessions demonstrating the adverse effects of alcohol, specifically, liver inflammation, adverse systemic effects, and alcohol's role in infection and immunology. Researchers also presented insight on modulation of microRNAs and stress proteins following alcohol consumption. Additionally, researchers revealed sex- and concentration-dependent differences in alcohol-mediated pathologies.

Keywords

alcohol; inflammation; tissue injury; infection

Introduction

In the United States, alcohol is considered to be one of the most commonly abused substances. It is estimated that over 87% of Americans are exposed to alcohol during their lifetimes ("Alcohol Facts and Statistics", 2014). Alcohol consumption has widespread effects on multiple organ systems. Experimental evidence supports that alcohol consumption alters the immune and inflammatory response (Szabo, 1999; Wang, Zakhari, & Jung, 2010). Additionally, alcohol consumption results in immune dysfunction and increases the risk of infection (Choudhry & Chaudhry, 2008; Fan et al., 2010; Karavitis & Kovacs, 2011; Souza-Smith, Kurtz, Molina, & Breslin, 2010). It thus becomes of extreme importance to decipher mechanistic alterations in an attempt to provide effective therapies for alcohol-related pathologies. The focus of the 2013 Alcohol and Immunology Research Interest Group (AIRIG) meeting was on alcohol and the inflammatory response.

The 2013 AIRIG meeting was held at Loyola University Chicago Health Sciences Division in Maywood, IL on November 22, 2013. The meeting was jointly organized by Drs. Elizabeth J. Kovacs and Mashkoor A. Choudhry from Loyola University Chicago, Maywood, IL; Dr. Thomas Waldschmidt from the University of Iowa, Iowa City, IA; Lou Ann Brown from Emory University, Atlanta, GA; and Dr. Pranoti Mandrekar from the University of Massachusetts School of Medicine, Worcester, MA. The meeting consisted of three plenary sessions and one poster session. The focus of the first plenary session was on alcohol and liver inflammation. Speakers in the second session discussed the systemic effects of alcohol. The third and final session was devoted to alcohol as a risk factor for infection. The three plenary sessions consisted of a total of 15 talks including four talks from the invited speakers. The remaining 11 short talks were selected from submitted abstracts. In addition to the three plenary sessions, 35 posters were presented during a poster session. Among the posters presented, 21 posters were from submitted abstracts from trainees and five from junior faculty. The poster session provided additional opportunities to discuss alcohol and its role in the modulation of inflammatory responses.

Alcohol and Liver Inflammation

The first session was chaired by Drs. Pranoti Mandrekar (University of Massachusetts Medical Center) and Brenda Curtis (Loyola University Chicago Health Sciences Division). The focus of the first plenary session was on alcohol and liver inflammation and included two talks from the invited speakers, Drs. Scott L. Friedman and Ian N. Hines. Dr. Friedman

is the Fishberg Professor of Medicine and Chief of Liver Diseases from the Icahn School of Medicine at Mount Sinai; he presented comprehensive data linking fibrosis to hepatocellular carcinoma (HCC). Worldwide, HCC represents the third leading cause of cancer-mediated mortality (El-Serag & Rudolph, 2007; Newell, Villanueva, Friedman, Koike, & Llovet, 2008). The tumor microenvironment contributes to the HCC carcinogenesis (Hernandez-Gea, Toffanin, Friedman, & Llovet, 2013). The biological processes associated with HCC progression include DNA damage and oxidative stress due to inflammatory and stromal cell crosstalk, and survival factor binding to stromal cells, which prevents apoptosis despite DNA damage. The risk of HCC development is increased by telomere shortening, elevation in tissue stiffness, and decreased function and quantity of natural killer cells. The risk of developing hepatocellular carcinoma is also increased by bi-directional hedgehog signaling between the liver parenchyma and tumor cells. Modifications of the gut microbiome and bile acid composition may also promote HCC formation. Other mechanisms of increasing the likelihood of developing HCC include alterations in macrophage polarization and stromal signaling because of hepatic stellate cell alteration of inflammation, creating an environment promoting carcinogenesis. Dr. Friedman emphasized that deciphering the pathways linking fibrosis to hepatocellular carcinoma could ultimately result in therapy and preventative measures for HCC.

Dr. Ian N. Hines, assistant professor in the Department of Nutrition Science at East Carolina University, described the role of chronic alcohol exposure on liver fibrogenesis. Dr. Hines emphasized the role of macrophages in alcohol-induced liver pathology and fibrosis. Tumor necrosis factor alpha (TNF α) and transforming growth factor beta (TGF β) production by macrophages are thought to play a central role in early liver pathogenesis and resulting fibrosis (Hines & Wheeler, 2004; Park, Lee, & Lee, 2014; Yin et al., 1999). Dr. Hines further emphasized the heterogeneity among macrophage populations – particularly in their cytokine production profiles and matrix remodeling repertoire, which play a critical role in regulating the dynamic processes of inflammation, fibrosis, and resolution. Thus, an understanding of the mechanisms of macrophage recruitment and activation are critical to treat or prevent early stage as well as late stage alcoholic liver disease, as well as to potentially resolve tissue damage. Integrins are involved in migration, cell adhesion, and tissue infiltration (Huttenlocher & Horwitz, 2011; Patsenker & Stickel, 2011). Chronic alcohol has been associated with integrin-linked kinase (ILK) activation and increased β -integrin expression in parenchymal and nonparenchymal cells (Patsenker & Stickel, 2011). Mice with macrophages selectively deficient in ILK (M ϕ -ILK mice) exhibit reduced tissue damage associated with chronic alcohol exposure when compared to mice with macrophages that expressed the gene. Furthermore, M ϕ -ILK mice exhibited reduced levels of liver TNF α and IL-6 despite the presence of a normal distribution of F4/80+ cells in the liver after chronic alcohol exposure. Parallel studies demonstrated that Kupffer cells from mice deficient in M ϕ -ILK cultured *ex vivo* with lipopolysaccharide (LPS) showed a decrease in TNF α production. In a separate experiment, *in vivo* LPS administration in mice deficient in M ϕ -ILK also produced decreased hepatic TNF α production. To further elucidate the relationship between macrophage ILK activation and fibrogenesis, wild-type and M ϕ -ILK-deficient mice were treated with carbon tetrachloride (CCl $_4$) for 4 weeks to induce liver fibrosis. Relative to wild-type mice given CCl $_4$, CCl $_4$ -exposed M ϕ -ILK-deficient mice had

comparable hepatic fibrosis. However, mice with M ϕ -ILK deficiency had accelerated resolution. This rapid resolution in the deficient mice was associated with a macrophage population shift (CD11b⁺ and Gr-1^{low}) and a reduced expression of a matrix metalloprotease inhibitor. These observations suggest a relationship between ILK expression and differentiation/function of macrophages following chronic alcohol exposure. Further studies are needed to determine if inhibiting macrophage ILK could be an effective therapy in alcoholic liver disease.

Abhishek Satishchandran, an MD/PhD student in the laboratory of Dr. Gyongyi Szabo at the University of Massachusetts Medical School, discussed findings on chronic alcoholic liver disease and microRNA (miR)-122. MicroRNAs are gene regulators which bind to 3' untranslated region (UTR) sequences resulting in mRNA degradation or translational repression (Mandrekar, 2011). The most abundantly expressed microRNA in hepatocytes is miR-122 (Hsu et al., 2012). The role of miR-122 in liver homeostasis is well established. This microRNA (miR-122) has been implicated in regulating genes involved in mitochondrial function, liver lipid metabolism, cell death pathways, and fibrosis (Szabo & Bala, 2013). To test the hypothesis that miR-122 contributes to alcoholic liver disease (ALD) pathology, miR-122 expression was inhibited by treating mice with an adenoviral vector expressing anti-miR-122 prior to a 4-week period of feeding with an alcohol diet. Along with the standard increase in ALT and weight loss, the alcohol feeding resulted in a significant increase in hypoxia-inducible factor 1- α (HIF-1 α) and inflammatory mediators (IL-1 β , TNF- α , and MCP-1). HIF-1 α is among the predicted targets of miR-122, and inhibition of this miR may modulate the inflammatory response by increasing levels of HIF-1 α . Together, these data support the role of miR-122 in alcoholic liver disease.

Muna Sapkota, a graduate student in the laboratory of Dr. Todd Wyatt at the University of Nebraska Medical Center, spoke next on alcohol-mediated malondialdehyde-deoxyguanosine (M1dG) adduct formation. There is a correlative relationship between alcohol abuse and cigarette smokers; nearly one-third of cigarette smokers also abuse alcohol (Wyatt et al., 2012). Previous work demonstrated that alcohol and cigarette smoke result in the production of malondialdehyde (MDA), which is a product of the CYP2E1 alcohol-metabolism pathway. MDA can cause DNA damage resulting in the formation of various DNA adducts, including M1dG adducts which have been implicated in alcohol-related cancers and cardiovascular disease (McCaskill et al., 2011). The present study tested a hypothesis that CYP2E1 would regulate MDA production, M1dG adduct formation, and single-strand DNA damage in alcohol-exposed and cigarette smoke-exposed lung cells and tissue. To test the hypothesis, mice were exposed to 20% alcohol *ad libitum* for 8 weeks (Meadows-Cook Model); subsequent to the alcohol exposure, mice were exposed to whole body cigarette smoke for 5 weeks (Elliott, Sisson, & Wyatt, 2007). Mice were also treated with diallyl disulfide (DADS), a CYP2E1 inhibitor, in their daily diet. Lung tissue homogenates demonstrated a significant increase in MDA and M1dG adduct formation in alcohol-exposed mice. In contrast, the levels of MDA and M1dG adduct formation were significantly reduced in alcohol-exposed mice treated with DADS. Cigarette exposure did not increase MDA and M1dG adduct formation. These findings reveal that, under these conditions, cigarette smoke does not influence M1dG adduct formation and alcohol may be the sole determinant.

The final speaker in this session was Dr. Sujatha Muralidharan from the laboratory of Dr. Pranoti Mandrekar at the University of Massachusetts Medical School. Dr. Muralidharan discussed the role of stress proteins in the induction of Toll-like receptor 4 (TLR4)-MyD88 tolerance after acute alcohol exposure. Previous work demonstrated that acute alcohol exposure induced stress proteins in monocytes (Mandrekar, Catalano, Jeliaskova, & Kodys, 2008). Dr. Muralidharan hypothesized that TLR4 tolerance following alcohol exposure is mediated by stress proteins, including heat shock factor 1 (HSF1) and heat shock protein 70 (hsp70). Primary human monocytes or murine RAW macrophages treated with LPS showed a reduction in proinflammatory mediator (IL-6, TNF α , IL-1 β) release following 25 mM alcohol exposure, while the expression of HSF1 and hsp70 mRNA was increased subsequent to alcohol exposure. Further analysis of macrophage lysates with chromatin immunoprecipitation (ChIP) assays yielded evidence of HSF1 binding to hsp70 and TNF α promoters. Furthermore, inhibition of HSF1 or hsp70 by siRNA restored LPS-induced TNF α production in alcohol-treated macrophages, suggesting a role for HSF1 and hsp70 in TLR4-MyD88 signaling.

Systemic Effects of Alcohol

The second plenary session, chaired by Drs. Ilhem Messaoudi (University of California, Riverside) and Liz Simon (Louisiana State University Health Science Center) was comprised of speakers addressing questions related to the systemic effects of alcohol. The session began with Dr. Georges E. Haddad, professor from Howard University, demonstrating the role of alcohol on cardiomyopathy. There are conflicting reports on the effects of alcohol on the cardiovascular system. Low alcohol exposure has been clinically defined as beneficial, which contrasts with heavy alcohol consumption having adverse effects (Teos et al., 2008; Zhou, Karliner, & Gray, 2002). Previous work has demonstrated that cardiac inotropy is dependent on the PI3K/Akt pathway (Teos et al., 2008). Current work examined whether the effects of chronic alcohol exposure on cardiac inotropy were dependent on the PI3K/Akt pathway. Rats were given a low (5 mM) or high (100 mM) Lieber-Decarli liquid diet for 3 months and assessed for cardiac function and gene expression. Cardiac function was assessed *in vivo* by left ventricular intra-ventricular catheterization (Scisense/iWorks). Low alcohol consumption improved left ventricular contractility. This was accompanied by reduced hydrogen peroxide (H₂O₂) superoxide dismutase (SOD-3) activity, and elevated nuclear respiratory factors (NRF)1/2, Forkhead box protein O1 (FOXO-1), nuclear factor kappa B (NF- κ B), and Akt expression, as well as an increase in cardiomyocyte survival. In contrast, high blood alcohol led to an increase in H₂O₂ level and SOD-3 activity, and a decrease in NRF1/2, FOXO-1, NF- κ B, and Akt expression, resulting in a decrease in cardiomyocyte survival. Caspase-3/7 was unaltered in rats exposed to low alcohol, whereas high alcohol exposure decreased caspase-3/7. Together, these findings suggest that low and high blood alcohol may produce opposite effects on cardiomyocytes. Improvements in cardiac inotropy mediated by low alcohol exposure are due to oxidative stress reduction mediated by Akt/NRF2 activation, whereas high alcohol exposure produces the opposite effect leading to decreases in cardiac inotropy.

Dr. Alexander R. Mackie, research assistant professor from Northwestern University, continued this session by further describing the effects of acute vs. chronic alcohol exposure

on the cardiovascular system. Moderate alcohol consumption (1–2 drinks per day) has been shown to elicit beneficial effects, whereas higher alcohol exposure (> 2 drinks per day) results in adverse cardiac events (ACE) (Djoussé & Gaziano, 2008; Lee & Regan, 2002). Dr. Mackie utilized a mouse model of acute myocardial infarction (AMI) and examined the effects of alcohol exposure on post-myocardial infarct repair. Mice were exposed isocalorically to an 8-week regimen of Lieber-DeCarli diet of 0, 1% (moderate), or 5% (high) alcohol prior to receiving AMI. Cardiac function was examined 1, 2, and 4 weeks post-AMI. As early as 2 weeks after AMI, mice receiving moderate alcohol exposure (1%) showed an improvement in their cardiac function as determined by end systolic ventricular volume, infarct size, and ejection fraction. In contrast, mice exposed to 5% alcohol showed diminished cardiac function and increased infarct size. To further delineate the mechanisms underlying these opposing responses, murine cardiac fibroblasts were treated with 0.1% and 0.5% (vol/vol) alcohol for 5 days, and the cells were harvested and analyzed for epigenetic alteration. Western blots demonstrated that high alcohol exposure resulted in a decrease in histone 3 lysine 9 (H3K9) methylation, while being significantly increased in cells treated with the moderate dose (0.1% v/v). While more studies are needed to understand the disparity in results obtained with low and high alcohol doses, data obtained from cell culture studies suggest a role for epigenetic alteration in the dimorphic cardiac response following alcohol exposure.

Dr. Flavia Souza-Smith, from the laboratory of Dr. Patricia Molina at Louisiana State University Health Sciences Center, presented findings on the effect of acute alcohol exposure on lymphatic permeability. Rats were exposed to 2.5 g/kg alcohol via intragastric infusion. Thirty minutes subsequent to alcohol exposure, lymphatic permeability was assessed using extravasation administration of Evans Blue dye to the mesenteric perilymphatic adipose tissue (PLAT). There was a significant increase in PLAT permeability in alcohol-exposed mice. PLAT cytokine (IL-6, TNF α , and GM-CSF) expression was unchanged 30 minutes following alcohol treatment; however, 24 hours after alcohol exposure, cytokine expression was higher compared to control animals. PLAT immunohistochemistry data revealed a significant increase in mast cells and neutrophils in rats after alcohol exposure. These results suggest that acute alcohol exposure results in increased mesenteric lymphatic permeability and PLAT inflammation.

Dr. Clare J. Wilhelm from Portland VA Medical Center and Oregon Health & Science University was the final speaker in the second plenary session. Dr. Wilhelm discussed the sex-dependent role of chronic alcohol on immune responses and neurotoxicity. Long-term alcohol abuse has been linked to neurotoxicity. In one study, females were found to be more susceptible to tissue damage in the liver following alcohol exposure (Becker et al., 1996). Additionally, chronic alcohol exposure resulted in different immunological responses based on gender differences (Iimuro et al., 1997). To investigate the gender-specific effect of chronic alcohol exposure on immune response and neurotoxicity, male and female mice were exposed to 72 hours of alcohol inhalation. The inflammatory response was assessed using qPCR of medial prefrontal cortex 8 hours (peak withdrawal) and 72 hours following the alcohol inhalation. Pro-inflammatory mediators were upregulated in females, in contrast to their male counterparts that displayed immunosuppression. Mitogen Concanavalin A (ConA) stimulation of splenocytes was also used to validate sexual dimorphic alteration in

gene expression. The production of IFN γ was unaltered by alcohol exposure but was significantly elevated in female compared to male mice stimulated with ConA. In addition, the levels of IL-2, TNF α , and GM-CSF were higher in ConA-treated splenocytes following alcohol exposure in females while male mice exhibited a reduction in cytokine production. Together, these findings suggest gender specificity in the inflammatory response following alcohol exposure. Female mice displayed a heightened inflammation after alcohol, and this may contribute to increased susceptibility to alcohol-related neurotoxicity in females.

Alcohol: Infection and Immunology

The third and last plenary session was chaired jointly by Drs. Robert Siggins (Louisiana State University Health Sciences Center) and Samantha Yeligar (Emory University/Atlanta VA Medical Center). Dr. Sulie L. Chang, professor and director of NeuroImmune Pharmacology at Seton Hall University, opened the final session with her work on alcohol and HIV. Young adults who consume alcohol are predominantly binge drinkers. Moreover, alcohol abuse is common among the HIV-1 population (Sarkar & Chang, 2013; Sarkar, Mao, Liu, & Change, 2013). Thus, it is important to determine the effects of alcohol exposure following HIV-1 infection. To accomplish this, the laboratory of Dr. Chang utilized HIV-1 transgenic (HIV-1Tg) rats that mimic HIV patients on combined antiretroviral therapy (cART). In short, F344 (control rats) and HIV-1Tg rats underwent a 3-day binge of alcohol (2.0 g/kg) in a low (8%) and high (52%) solution. Data demonstrated that HIV-1Tg rats treated with the high alcohol concentration maintained significantly elevated blood alcohol concentration levels in contrast to F344 rats. Additionally, F344 rats demonstrated a concentration-dependent elevation in alcohol metabolizing enzymes (Adh1, Adh4, and Cyp2e1). In contrast, HIV-1Tg rats exhibited no alcohol concentration-dependent changes in alcohol metabolizing enzymes, contributing to the elevated blood alcohol content in rats exposed to 52% binge alcohol concentration. To further determine alcohol concentration-mediated effects, cytokine levels were evaluated to assess neuroinflammation in the HIV-1Tg rat. IL-1 β , IL-4, IL-5, IL-13, KC-Gro, IFN γ , and TNF α productions were measured and were found to be significantly elevated in the majority of the 52% alcohol-exposed HIV-1Tg rats. Behavior-based deficits were also assessed using locomotor activity 2, 24, and 72 hours following binge exposure, demonstrating a reduction in only the 52% alcohol-treated HIV-1Tg rats at both 2 and 24 hours following binge exposure. These findings suggest alcohol could differentially influence inflammatory and behavioral responses in the HIV patients given cART.

Emily A. Hemann from Dr. Kevin Legge's laboratory at the University of Iowa presented results on the effects of chronic alcohol exposure on CD8 T cell function during influenza A virus (IAV) infection. Chronic alcohol consumption results in increased susceptibility to IAV infection and more severe pulmonary lesions (Meyerholz et al., 2008). IAV-specific CD8 T cells play a critical role in clearance of IAV infection. Mice with chronic alcohol exposure fail to mount an optimal IAV-specific CD8 T cell response. Additionally, IAV-specific CD8 T cells produce significantly less IFN- γ compared to IAV-infected, water-consuming controls. However, TNF α and IL-2 levels assessed 8 days following infection were unaffected by chronic alcohol exposure. Although there were no significant differences in number (or amount) of granzyme B-expressing pulmonary CD8 T cells between water-

and alcohol-consuming mice, alcohol exposure resulted in a reduction of degranulation. Lungs from mice chronically exposed to alcohol demonstrated a reduction in expression of TRAIL and FasL in CD8 T cells. Together, these results highlight how alcohol-mediated changes in CD8 T cells can contribute to increased susceptibility to IAV infection.

Dr. Samantha Simet, postdoctoral fellow from the University of Nebraska Medical Center, presented her findings on the impact of alcohol on tight junction proteins. Alcohol increases permeability in airway epithelial cells (Simet et al., 2012). Pulmonary infections are common following prolonged alcohol exposure (Jerrells et al., 2007). Similarly, respiratory syncytial virus (RSV) is also known to result in airway epithelial injury. Thus, prior alcohol exposure is likely to exacerbate RSV-induced wounds in the airway epithelium and increase the time it takes for migrating airway epithelial cells to heal the wound. Dr. Simet examined the role of the PKC α /ROCK (Rho-associated protein kinase) pathway as an underlying mechanism by which alcohol potentiates RSV-induced airway injury. Primary mouse tracheal epithelial cells and normal human airway epithelial cells were exposed to 100 mM alcohol for 24 hours prior to RSV infection. Data demonstrate that the combination of alcohol exposure and RSV infection increased tight junction permeability considerably more than RSV or alcohol alone. She further observed that inhibitors of PKC α (Gö6976) and Rho-associated protein kinase ROCK (Y27632) prevented the leakiness associated with alcohol-induced tight junction disruption. These results suggest that the PKC α /ROCK (Rho-associated protein kinase) pathway is likely involved in alcohol-induced tight junction disruption leading to the airway leakiness after alcohol exposure and RSV infection.

Jill Ippolito, a graduate student in the laboratory of Dr. Elizabeth J. Kovacs at Loyola University Chicago Health Sciences Campus, presented data examining the effect of alcohol on the phenotype of alveolar macrophages during burn injury. Fifty percent of burn-injured patients are intoxicated at the time of injury, which leads to increased risk of pulmonary infections and mortality (McGill, Kowal-Vern, Fisher, Kahn, & Gamelli, 1995; Silver et al., 2008). Previous work from Dr. Kovacs' laboratory has demonstrated that combined insult leads to significant pulmonary inflammation, relative to either insult alone (Bird, Morgan, Ramirez, Yong, & Kovacs, 2010). Pulmonary inflammation is partially regulated by alveolar macrophages, which have been shown to both initiate and resolve inflammation (Herold, Mayer, & Lohmeyer, 2011). Their ability to shift from an M1, pro-inflammatory macrophage to an M2, anti-inflammatory macrophage is a necessary transition for the resolution of inflammation. Using a mouse model of alcohol exposure and burn injury, current studies examined the effect of alcohol on alveolar macrophage expression of M2 marker CD206 during the inflammatory (24 hours) and resolution (72 hours) phases of inflammation. There was no change in the expression of CD206 at 24 hours; however, at 72 hours an increase in CD206 was observed with burn injury. Mice exposed to alcohol prior to burn injury had higher levels of CD206 than burn injury alone, indicating an amplified M2 phenotype during combined insult. This suggests that the heightened pro-inflammatory response observed with combined injury may result in an elevated anti-inflammatory response during the resolution phase. Overall, these data may highlight a mechanism responsible for increased susceptibility to pulmonary infections observed in intoxicated burn patients.

Dr. Majid Afshar from the University of Maryland Medical Center used ethyl glucuronide (EtG) and ethyl sulfate (EtS) as urine biomarkers to compare the effects of binge alcohol exposure in recent and non-recent drinkers in human volunteers. EtG and EtS are direct alcohol metabolites whose appearance in urine can be used to positively assess recent alcohol intake (Lostia, Vicente, & Cowan, 2013). Urine samples were collected before binge alcohol exposure and 24 hours after binge alcohol exposure (0.89 g/kg males and 0.81 g/kg females). Blood was also collected before alcohol dosing, and 20 minutes, 2 hours, and 5 hours subsequent to alcohol dosing. The collected blood was incubated with or without phytohemagglutinin (PHA) for 24 hours and cytokine release was measured. As compared to non-recent drinkers, there was an elevation in EtG and EtS levels in the urine collected from recent drinkers after alcohol dosing. There was no difference in blood alcohol levels between recent and non-recent drinkers. Similar elevations in PHA-induced IFN γ and IL-8 levels were noted after binge alcohol dosing. Additionally, IL-2 levels were elevated in recent drinkers when compared to non-recent drinkers. This suggests that recent drinkers of alcohol may have an altered immune response after binge drinking compared to non-recent drinkers.

The final speaker was Dr. Samantha M. Yeligar from Emory University/Atlanta VA Medical Center, who provided additional evidence of the mechanism of alcohol-mediated increase in pulmonary infection susceptibility. Alcohol consumption results in an increase in oxidative stress resulting in dysfunction of the phagocytic ability of alveolar macrophages. NADPH oxidases are the main reactive oxygen producers in alveolar macrophages. Previous work has demonstrated that NADPH oxidases (Nox) are upregulated following chronic alcohol exposure (Yeligar, Harris, Hart, & Brown, 2012). C57BL/6J mice were used to test the hypothesis that pioglitazone (peroxisome proliferator-activated receptor gamma [PPAR γ] ligand) could reduce alcohol-mediated oxidative stress by upregulating Nox4-regulating microRNAs. Mice were exposed to 20% alcohol in drinking water in combination with 10 mg/kg/day pioglitazone treatment for a total of 12 weeks. Following this regimen, alveolar macrophages were harvested. A similar experiment was carried out using MH-S cells (alveolar macrophage mouse cell line) by treating the cells with 0.08% alcohol for 3 days, and 1 day of 10 mM pioglitazone treatment. Expression of Nox4-related microRNAs (miR-25, miR-32, miR-92a, miR-92b, miR-363, and miR-367) was determined. The ability of alveolar macrophages to phagocytose *Staphylococcus aureus* was determined. Alcohol induced an increase in Nox4 expression both *in vivo* and *in vitro*, which correlated, with a decrease in Nox4-related microRNAs (miR-92a, miR-92b, and miR-363). Alcohol also reduced phagocytic ability while increasing oxidative stress. The pioglitazone treatment attenuated the oxidative stress and dysfunction associated with alcohol exposure, suggesting beneficial effects of pioglitazone in alcohol-induced alveolar macrophage dysfunction.

Summary

The 2013 AIRIG meeting focused on alcohol's role in liver inflammation, alcohol's systemic effects, and alcohol's participation in infection and immunology. Both clinical and experimental data demonstrated that alcohol, in conjunction with other injuries, results in more adverse outcomes than either alone. The studies presented demonstrated alcohol-mediated epigenetic modifications (microRNA and histone modification). The studies also

provided insight concerning infectious and immunological alterations that occur following alcohol exposure. The findings presented at the 2013 AIRIG meeting elaborated further on alcohol's widespread effects while proposing mechanistic relationships and demonstrating sex- and concentration-dependent differences.

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