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Summary of the 2016 Alcohol and Immunology Research Interest Group (AIRIG) meeting

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Abstract

On November 18th, 2016 the 21st annual Alcohol and Immunology Research Interest Group (AIRIG) meeting was held at the Center for Translational Research and Education at Loyola University Chicago's Health Sciences Campus in Maywood, IL. The 2016 meeting focused broadly on alcohol and inflammation, epigenetics, and the microbiome. The four plenary sessions of the meeting were: Alcohol, Inflammation, and Immunity; Alcohol and Epigenetics; Alcohol, Transcriptional Regulation, and Epigenetics; and Alcohol, Intestinal Mucosa, and the Gut Microbiome. Presentations in all sessions of the meeting explored putative underlying causes for chronic diseases and mortality associated with alcohol consumption, shedding light on future work and potential therapeutic targets to alleviate the negative effects of alcohol misuse.

Keywords

Alcohol; Inflammation; Epigenetics; Immunity

Introduction

Alcohol use is attributed to over 80,000 deaths per year in the United States (National Institute on Alcohol Abuse and Alcoholism, 2017). Furthermore, alcohol consumption is associated with increased prevalence of chronic diseases, such as cancers, cardiovascular disease, lung disease, and liver disease (National Institute on Alcohol Abuse and Alcoholism, 2003). Underlying many of these chronic diseases is altered inflammation and immunity imparted by the amount and duration of alcohol consumed (Goral, Karavitis, & Kovacs, 2008). One of the major challenges of delineating how alcohol misuse drives enhanced chronic disease is that many organ systems, signaling pathways and regulatory mechanisms are affected, including alteration of cellular epigenetic landscapes and durable changes in the microbiome and mucosal surfaces of the body (Curtis, Zahs, & Kovacs, 2013; Engen, Green, Voigt, Forsyth, & Keshavarzian, 2015; Hammer, Morris, Earley, & Choudhry, 2015). Therefore, the 2016 meeting of the Alcohol and Immunology Research Interest Group (AIRIG) touched upon these subjects. Specifically, the meeting was broken into four plenary sessions with oral presentations, each with a focus on alcohol and immunology research, including the impact of alcohol on inflammation, epigenetic mechanisms that regulate immunity, and host-microbiome interactions in the gut.

Alcohol, Inflammation and Immunity

Dr. Elizabeth J. Kovacs, from the University of Colorado Denver, opened the 2016 AIRIG meeting by welcoming and thanking all the attendees for their presence and willingness to share their data and advancements in the field of alcohol and immunology research. Her welcome was followed by the first plenary session chaired by Drs. John J. Callaci (Loyola University Chicago) and Kristina Bailey (University of Nebraska Medical Center), which was entitled "Alcohol, Inflammation, and Immunity." Dr. Cynthia Ju from the University of

Colorado Denver opened the session with her talk entitled “Role of macrophages in alcoholic liver disease.” Dr. Ju established the clinical problem surrounding alcohol misuse and liver disease, in that alcohol-attributable liver cirrhosis was responsible for 493,300 deaths globally in 2010, and alcohol-attributable liver cancer was responsible for 80,600 deaths (National Institute on Alcohol Abuse and Alcoholism, 2017). Moreover, macrophages are implicated in the pathogenesis of alcoholic liver disease due to their increased infiltration (Christoffersen, Braendstrup, Juhl, & Poulsen, 1971; Davis & Culpepper, 1948; McClain, Hill, Song, Deaciuc, & Barve, 2002), heightened activation (Luna-Casado et al., 1997), and enhanced production of pro-inflammatory mediators (Hanck, Glatzel, Singer, & Rossol, 2000; Hill, Barve, Joshi-Barve, & McClain, 2000; McClain & Cohen, 1989). In the mouse model of chronic alcohol feeding employed by Dr. Ju’s laboratory, there is an increase in infiltrating inflammatory macrophages in the liver, which can be divided into two different populations based on their expression of Ly6C. Ly6C^{hi} cells express high levels of pro-inflammatory genes, including *Tnfa*, *Il1b*, and *Ccl2*, whereas Ly6C^{lo} infiltrating inflammatory macrophages express genes associated with anti-inflammatory functions, such as *Ym1* (M. Wang et al., 2014). Interestingly, a heightened proportion of Ly6C^{hi} compared to Ly6C^{lo} infiltrating inflammatory macrophages resulted in more severe liver injury (as measured by alanine aminotransferase (ALT) levels). To understand a putative underlying mechanism that may be driving altered macrophage function in the context of alcoholic liver disease, Dr. Ju’s lab has begun to explore the role of Gp91^{phox}, which is the active subunit of the NOX2 enzyme (Kleniewska, Piechota, Skibska, & Goraca, 2012). She hypothesized that NOX2 serves as an important regulator of macrophage efferocytosis (phagocytosis of apoptotic cells), which in turn is critical for macrophages to downregulate pro-inflammatory actions and upregulate pro-resolving/anti-inflammatory functions. In support of her hypothesis, macrophages from gp91^{phox-/-} mice are reduced in their ability to efferocytose, leading to an increase in apoptotic cells present in the liver of gp91^{phox-/-} mice. Furthermore, if gp91^{phox-/-} mice were on the chronic ethanol diet, their macrophage efferocytosis was further impaired compared to pair-fed gp91^{phox-/-} mice. In addition, ethanol fed gp91^{phox-/-} mice had heightened expression of pro-inflammatory markers and reduced levels of receptors that recognize apoptotic cells, which culminated in higher ALT levels in ethanol fed gp91^{phox-/-} mice compared to pair-fed gp91^{phox-/-} mice. Dr. Ju finished her talk with a discussion of the ways in which macrophage polarization could be targeted for therapeutic use, including antagonizing the chemokines responsible for recruiting the infiltrating macrophages, and altering the microbiome which is thought to lead to increased systemic inflammation in chronic alcoholics.

Next, Dr. Lisbeth A. Boule, a postdoctoral fellow in the laboratory of Dr. Elizabeth Kovacs at the University of Colorado Denver presented her work examining biomarkers of healthy aging in populations of alcohol misusers in her talk entitled “Assessment of biomarkers of healthy aging in alcohol consumers.” She measured levels of analytes in the plasma of two cohorts: burn patients and healthy adults. Alcohol consumption was measured differently in both cohorts based on access to the research subjects; the burn patients’ alcohol levels were measured by blood alcohol concentration (BAC) and the healthy adults’ alcohol use was determined by the Alcohol Use Disorders Identification Test (AUDIT) (Saunders, Aasland,

Babor, de la Fuente, & Grant, 1993). Based on BAC or AUDIT score, subjects were grouped into young non-drinkers, young drinkers, and older non-drinkers. There was not an older drinker group due to the fact that the great majority of older drinkers die prematurely, and therefore it is hard to find enough subjects for research studies (Agahi, Kelfve, Lennartsson, & Kareholt, 2016). For the burn patients, subjects were considered young if they were 19–40 years old, and older if they were over the age of 50. The average BAC of the drinking groups was 220 mg/dL. In these patients, the cytokines measured followed three patterns: (1) unaffected, (2) increased with advanced age alone, or (3) increased with both advanced age and by alcohol misuse. The cytokines that were unaffected included interferon (IFN) γ , interleukin (IL)-5, IL-10, IL-12, and macrophage inflammatory protein (MIP)-1 α . Those that were increased by advanced age alone were Eotaxin, IL-9, IL-15, IFN γ -induced protein (IP)-10, monocyte chemoattractant protein (MCP)-1, and tumor necrosis factor (TNF) α . There were only three that were elevated in the plasma by advanced age and alcohol misuse, and those were IL-6, IL-8, and granulocyte colony stimulating factor (G-CSF). To examine potential underlying mechanisms that may result in heightened pro-inflammatory cytokines in healthy older individuals and young drinkers, she looked at circulating intestinal fatty acid binding protein (iFABP) levels in the second cohort of healthy subjects (i.e., no burn injury). Plasma levels of iFABP have been correlated with increased gut permeability associated with the aging process (Steele et al., 2014). For this cohort, young individuals ranged from 30–40 years, and older individuals were greater than 50 years of age. Drinking status was determined by AUDIT scores, and an AUDIT score less than 5 was considered a non-drinker, whereas the average AUDIT score for drinkers was 32. Dr. Boule showed that even in a small cohort of individuals (6 per group) there was an approximate 1.5-fold increase in iFABP levels in young drinkers and older non-drinkers as compared to young non-drinkers. Thus, her data were encouraging in that they suggest that decreased gut barrier integrity could be one contributing factor in higher circulating pro-inflammatory cytokine levels associated with advanced age and alcohol misuse. Future directions include expanding upon these preliminary findings with a larger number of healthy subjects and examining iFABP levels in burn patients. Dr. Boule finished her talk by discussing the implications of the work thus far, including the idea that young drinkers might have an earlier onset of diseases associated with aging, and the fact that diseases associated with inflammation due to the aging process may be exacerbated in older individuals who drink.

The third talk of the session was given by Jonathan Eby, a PhD student in the laboratory of Dr. Matthias Majetschak at Loyola University Chicago. This presentation was entitled “Regulation of vascular function by ethanol: A possible role of heteromeric CXCR4: α 1-adrenergic receptor complexes.” Mr. Eby first set the stage with the clinical relevance for his research; globally there are over 800,000 cardiac related deaths associated with alcohol consumption each year (Rehm, Shield, Roerecke, & Gmel, 2016), and moderate to heavy alcohol consumption leads to hypertension in people and in rodent models (Marchi, Muniz, & Tirapelli, 2014; Mukamal, Chen, Rao, & Breslow, 2010; Rehm et al., 2014). Yet, the mechanism by which this occurs remains unknown. One potential link is increased vasoconstriction responses upon vascular α 1-adrenergic receptor (AR) activation in ethanol-induced hypertension (Marchi et al., 2014). He explained that recently their laboratory determined that α 1-ARs and CXCR4 are constitutively expressed as heteromeric complexes

on vascular smooth muscle cells, and upon stimulation with catecholamines, $\alpha 1$ -ARs are activated and induce vasoconstriction in vascular smooth muscle, suggesting that CXCR4 and $\alpha 1$ -AR complex formation is required for $\alpha 1$ -AR function (Evans et al., 2016; Tripathi et al., 2015). Based on these findings, Mr. Eby hypothesized that ethanol may sensitize intrinsic vascular function through effects on heteromerization of $\alpha 1$ -AR with CXCR4 in vascular smooth muscle cells due to ethanol-mediated enhanced membrane fluidity. To test this hypothesis, they used pressure myography, which measures mesenteric resistance artery $\alpha 1$ -AR-mediated vasoconstriction (Akhtar, Sherratt, Cruickshank, & Derby, 2011). Ethanol sensitized the potency of activating the $\alpha 1$ -AR potency 3- to 4-fold, suggesting that ethanol is sensitizing intrinsic $\alpha 1$ -AR function. Next, they used PRESTO-Tango, which measures G-protein coupled receptor function by assaying β -arrestin recruitment following agonist stimulation (Kroeze et al., 2015). They found that ethanol does not change the intrinsic function of the individual receptors (CXCR4 and $\alpha 1$ -AR). Thus, they assayed whether ethanol affects expression of the individual receptors or the formation of the heterodimeric complex using the Proximity Ligation Assay. This technique allows for visualization of protein-protein interactions at single molecule resolution (Soderberg et al., 2006). Using this technique, they found that while ethanol does not affect the expression of either receptor, there was a significant dose-dependent increase in heteromeric complex formation on human vascular smooth muscle cells. To conclude, Mr. Eby explained that the CXCR4- α -AR heteromeric complexes exist in equilibrium at baseline, and upon consumption of alcohol the equilibrium shifts due to an increase in membrane fluidity, which could contribute to the vasoconstriction associated with alcohol-induced hypertension observed in people.

The final talk in Session 1 was entitled “Analysis of the respiratory tract microbial biogeography in alcohol-use disorder populations” given by Dr. Derrick Samuelson in the laboratory of Dr. David Welsh from Louisiana State University Health Sciences Center. Dr. Samuelson explained that the lung microbiome can be altered by many environmental insults, including viral infections, cigarette smoke, and cystic fibrosis (Faner et al., 2017). Moreover, he explained that the specific microbiome in each section of the lung (bronchial mucosa, oropharyngeal compartment, and distal alveolar spaces in the lung) is important in maintenance of homeostasis (Faner et al., 2017). He hypothesized that not only will the lung microbiome differ between healthy subjects and those that misuse alcohol, but that the very specific biogeographical localization of commensals in the lung would not be properly maintained in populations of alcohol abusers. To test this hypothesis, he collaborated with Dr. Ellen Burnham and the Colorado Pulmonary Alcohol Research Collaborative (CoPARC). Through the CoPARC, oropharyngeal, bronchial mucosa, and distal lung alveolar microbial communities were sampled and 16s rDNA sequencing was performed for all subjects (healthy and alcohol abusers – as measured by AUDIT scores). Thirty-seven healthy control subjects and forty-one alcohol use disorder subjects were used, and all subjects were matched on the basis of age, gender, smoking status, and self-reported race. The average AUDIT score for the subjects with alcohol use disorders was 30, and healthy controls was < 3. In support of his hypothesis, Dr. Samuelson showed that alcohol use disrupts the respiratory tract microbial community composition, and alcohol use changes the relative taxa abundances in the respiratory tract. Interestingly, the proportion of Gram negative organisms increases with alcohol use. In addition, alcohol use drives the

convergence of microbial communities between biogeographical sites, leading to increased co-abundances of specific types of bacteria in distinct anatomical locations. Dr. Samuelson concluded his talk explaining the implications of his results. He postulated that the increased risk of bacterial pneumonia seen in people with alcohol abusers may be, in part, mediated by the respiratory tract microbial communities that shift with alcohol misuse. Furthermore, alterations in the lung microbiome might lead to increased pathogen availability in the lung, cause chronic immune stimulation leading to immune hyporesponsiveness, decrease colonization resistance, and alter cytokine and chemokine responses in the lung. Thus, microbial diversity and biogeographical stratification could be important for normal physiological and immune function in the lungs, and changing the lung microbiome may be responsible for some of the health complications associated with alcohol use disorders.

Alcohol and Epigenetics

The second session of the day was “Alcohol and Epigenetics” and it was chaired by Dr. Paul Drew of the University of Arkansas and Dr. Anser Azim of Chicago State University. Dr. Subhash C. Pandey of the University of Illinois at Chicago started this session with his talk “Epigenetic reprogramming and synaptic remodeling during alcoholism.” Dr. Pandey explained that adolescence is a particularly vulnerable time in which environmental factors, such as alcohol consumption, can affect brain development; during adolescence the brain undergoes neuromaturation, which is characterized by changes in neurotransmission, gene expression, and synaptic remodeling, including formation and pruning of axons, dendrites, and synapses in various brain regions (Keshavan, Giedd, Lau, Lewis, & Paus, 2014; Kyzar et al., 2016). Binge drinking is prevalent during adolescence (Center for Disease Control and Prevention, 2016), and evidence reveals that adolescents who first used alcohol at age 14 or younger were more than 7 times as likely to be diagnosed with alcohol dependence or abuse than those who had their first drink at age 21 or older (15.2 vs. 2.1 percent)(SAMSA, 2016). Dr. Pandey hypothesized that this relationship is due to alcohol-mediated epigenetic changes in the brain (Kyzar et al., 2016). To model adolescent binge drinking, adolescent rats were given intermittent (2 grams of ethanol per kilogram body weight) ethanol (AIE) or intermittent saline (AIS) intraperitoneally every other day between postnatal day 28 and 41. From postnatal day 41 until postnatal day 90, rats were ethanol-free. At postnatal day 90, measurements of anxiety-like behavior were taken, and epigenetic modifications were assayed (Pandey, Sakharkar, Tang, & Zhang, 2015). Dr. Pandey found increased anxiety-like behavior & increased preference for ethanol in AIE adult rats as compared to control AIS adult rats (Pandey et al., 2015). Additionally, Dr. Pandey found an increase in nuclear histone deacetylase (HDAC) activity in the amygdala of AIE adult rats compared to controls, and higher amounts of HDAC2 mRNA and protein levels in the amygdala of the same ethanol treated animals compared to controls. HDAC activity leads to deacetylation of histones, resulting in reduced gene transcription (Gong & Miller, 2013; Krishnan et al., 2014), suggesting that increased HDAC activity will lead to lower levels of important genes being expressed. To this end, AIE adult rats treated with an HDAC inhibitor had their anxiety-like behavior reduced to normal levels (i.e., AIE adult rats behaved like control rats). This decrease in anxiety-like behavior was due to a reversal of the decreased acetylation at promoters of synaptic plasticity-associated genes in the amygdala of AIE adult rats.

Thus, reduced gene transcription due to enhanced HDAC activity and deficits in histone acetylation in the amygdala due to AIE is responsible for the increased anxiety-like behavior and alcohol intake in adulthood. To further examine other epigenetic modifications that may play a role in alcohol-mediated changes in the brain imparted by alcohol misuse in adolescence, Dr. Pandey explored changes in histone methylation mechanisms. Unlike histone acetylation, methylation of histones can either repress or promote gene transcription depending upon the residue modified (Mosammaparast & Shi, 2010; Krishnan et al., 2014). Dr. Pandey's group found in their rat model of adolescent drinking that there was a reduction in histone demethylases, specifically lysine-specific histone demethylase 1A (LSD1), in the amygdala of the AIE adult rats compared to AIS adult rats (Kyzar, Zhang, Sakharkar, & Pandey, 2016).

Additionally, Dr. Pandey reported that levels of neuron specific *Lsd1+8a* mRNA levels, are decreased in the amygdala of AIE adult rats as compared to AIS adult rats. Acute ethanol challenge of AIE adult lead to attenuated anxiety like behaviors, which was associated with normalization of decreased *Lsd1+8a* expression and increased levels of histone H3 dimethylation of brain-derived neurotrophic factor (*bdnf*) gene promoter in the amygdala. Dr. Pandey then shared some of his data involving synaptic plasticity in the amygdala of rats that were exposed to ethanol during their adolescence. He showed that there was decreased synaptic plasticity-related gene expression such as *bdnf* and *Arc* in the amygdala of adult rats exposed to ethanol as adolescents when compared AIS adults rats. These genes with reduced expression in the amygdala have been implicated in anxiety and alcohol drinking behaviors (Moonat, Sakharkar, Zhang, & Pandey, 2011; Pandey, Ugale, Zhang, Tang, & Prakash, 2008; Prakash, Zhang, & Pandey, 2008). Dr. Pandey then emphasized the implications of these results by confirming them in an animal model of alcoholism using alcohol-preferring rats (P) and comparing them to non-alcohol-preferring rats (NP). P rats serve as a useful animal model to study the epigenetic basis for the co-morbidity between anxiety and alcoholism because they orally self-administer ethanol in significantly greater amounts than NP rats, leading to pharmacologically relevant BAC levels, alcohol dependence and signs of withdrawal, and heightened anxiety-like behaviors (Bell, Rodd, Lumeng, Murphy, & McBride, 2006). P rats have higher HDAC2 levels and deficits in histone acetylation compared to NP rats in the amygdala, which is reduced by acute alcohol consumption (Moonat, Sakharkar, Zhang, Tang, & Pandey, 2013; Sakharkar et al., 2014). Moreover, when HDAC2 levels are reduced using HDAC2 siRNA or using an HDAC inhibitor, the anxiety-like behaviors of P rats are diminished and they consumed less alcohol when made available to them compared to those given the control siRNA or vehicle (Moonat et al., 2013; Sakharkar et al., 2014). Thus, Dr. Pandey concluded that the higher levels of HDAC2 in the amygdala of P rats results in the increased anxiety-like behaviors and preference for alcohol consumption due to reduced histone acetylation and therefore lower synaptic plasticity. Therefore, HDAC2 along with LSD1 are likely examples of many epigenetic targets within the epigenome that may be involved in the pathophysiology of alcoholism.

The second speaker of session 2 was Suhas Sureshchandra, a graduate student from the laboratory of Dr. Ilhem Messaoudi at the University of California Irvine. Mr. Sureshchandra gave a talk entitled "Chronic ethanol consumption alters the transcriptional program in

splenic monocytes by modifying chromatin accessibility.” He noted that of the 60–70% of people that drink alcohol, about 10% of them develop alcohol use disorders, and this 10% of people have poor wound healing and increased risk for infection, cardiovascular disease, and cancers (Trevejo-Nunez, Kolls, & de Wit, 2015). According to Mr. Sureshchandra, the difficulty in studying these individuals lies mainly in measuring exact drinking quantities and patterns, other risky behaviors, performing longitudinal studies, and access to tissues. Thus, his laboratory collaborates with Dr. Kathy Grant to use a rhesus macaque model of voluntary ethanol self-administration. This model is advantageous because the animals have close genetic and physiological homology to humans, a natural propensity for alcohol consumption, researchers can precisely record fluid and food intake and perform studies with a longer duration, and they have longitudinal access to several tissues (Grant et al., 2008). Using this model, Mr. Sureshchandra showed that chronic ethanol consumption results in robust transcriptional changes in peripheral blood mononuclear cells, and many of those changes were in monocytes (Sureshchandra, Rais, Stull, Grant, & Messaoudi, 2016). Other groups have also reported similar findings of altered functions in monocytes after alcohol consumption in other animal models and human studies (Goral et al., 2008; Karavitis & Kovacs, 2011; Mandrekar & Szabo, 2009) (Choudhry, Fazal, Goto, Gamelli, & Sayeed, 2002; Szabo & Saha, 2015). To determine the specific genes changed in monocytes and the associated epigenetic changes driving differential gene expression after alcohol misuse, Mr. Sureshchandra used splenic monocytes from control or ethanol-consuming macaques, isolated their RNA for RNA sequencing (RNA-seq) and DNA for Assay for Transposase-Accessible Chromatin sequencing (ATAC-seq), which will give information regarding the accessibility of genes for transcription (Buenrostro, Giresi, Zaba, Chang, & Greenleaf, 2013). The data show that the alcohol-consuming macaques have greater chromatin accessibility at promoter regions and exons in their genome. Moreover, upon gene ontology analysis, many of the genes with enhanced chromatin accessibility are innate immune genes. These more accessible genes correlated with increased expression of genes associated with innate immune cell function. Next, he isolated monocytes from the spleens of both drinking and non-drinking macaques, treated them with TLR ligands, and then measured cytokine production in the culture supernatants and performed RNA-seq. He found that the monocytes from the alcohol consuming animals produced significantly higher levels of IL-6, MCP-1, Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES), MIP-1 α , IL-8, G-CSF, and IL-1RA. In addition, RNA-seq analysis suggested that chronic ethanol consumption results in the establishment of an exaggerated inflammatory environment following TLR stimulation in monocytes. He concluded his talk with his working model, which suggests that chronic alcohol consumption results in more open chromatin, which in turn allows for heightened gene transcription and subsequent cytokine production upon TLR stimulation in monocytes. His data suggest that innate immune cells from alcoholics are poised to be pro-inflammatory, and could lead to heightened inflammation associated with many chronic diseases that plague this population.

Alcohol, Transcriptional Regulation, and Epigenetics

The third session of the meeting was entitled “Alcohol, Transcriptional Regulation, and Epigenetics” and it was chaired by Dr. Brenda Curtis of the University of Colorado Denver

and Michael Price of the University of Nebraska Medical Center. The first speaker was Dr. Marisela Agudelo of Florida International University, and her talk was titled “Alcohol induces epigenetic immunomodulation of human monocyte-derived dendritic cells.” Dr. Agudelo is interested in understanding how epigenetic mechanisms may play a role in alcohol use disorders, with the eventual goal of using these mechanisms as targets for potential therapeutics. In particular, Dr. Agudelo’s laboratory focuses on the role of epigenetics in alcohol-induced oxidative stress and inflammation in human primary monocyte-derived dendritic cells (MDDCs). She has analyzed the role of histone deacetylases (class I HDACs) and histones H3 and H4 both *ex vivo* and *in vitro*. Her group has found that MDDCs from alcohol abusers have higher mRNA and protein levels of HDACs compared to control subjects, with a particularly notable increase in HDAC2 and HDAC8. Moreover, when she treated MDDCs with HDAC inhibitors, there was a reduction in the alcohol-induced oxidative stress-related genes expressed by the cells. To delve further into the mechanism by which the HDAC inhibitors restore a normal oxidative state in MDDCs treated with ethanol, she examined the acetylation status of H4. She found that H4K12 acetylation regulates expression of IL-15, RANTES, Transforming growth factor (TGF) β , TNF α , and MCP-2, all of which can contribute to alcohol-induced inflammation. In summary, she explained that elucidating the ability of alcohol to induce epigenetic changes in genes associated with inflammation and oxidative stress in MDDCs will help to further our understanding of the immunomodulatory power of alcohol, and point to putative epigenetic markers that could be targeted to reduce the negative effects of alcohol misuse.

The second talk of this session was given by Booker Davis, a graduate student in the laboratory of Dr. Ali Keshavarzian at Rush University Medical Center, and was titled “Mechanistic role of Per2 and PPAR-gamma in ethanol-mediated hyperpermeability.” Mr. Davis began his talk with a summary of alcoholic liver disease, citing that only 30% of alcoholics get the disease. He explained that one theory as to the differential disease susceptibility is variations in the increased permeability of intestinal lumen between alcoholics (Szabo & Bala, 2010). In fact, prior data from his laboratory associates alcohol-mediated hyperpermeability of the intestine and endotoxemia with the development of steatohepatitis (Keshavarzian et al., 2009). In addition, he pointed out that circadian rhythms regulate the intestine, and that circadian rhythms run on a transcriptional-translational feedback loop which induces the production of cellular timekeeper protein Per2 (Forsyth, Voigt, Burgess, Swanson, & Keshavarzian, 2015). Prior work from the laboratory demonstrated that alcohol causes an increase in Per2 levels, and knocking down Per2 in a colonic epithelial cell line (Caco-2 cells) leads to increased permeability (Keshavarzian et al., 2009). In addition, they linked changes in circadian rhythm to the metabolism of alcohol mediated by CYP2E1 (Keshavarzian et al., 2009). Specifically, knockdown of CYP2E1 blocks the alcohol-induced increase in Per2, and therefore rescues the alcohol-induced permeability of Caco-2 cells (Keshavarzian et al., 2009). Based on these findings, he hypothesized that oxidative stress, mediated by alcohol metabolism via the CYP2E1 enzyme, induces Per2 protein expression and leads to intestinal hyperpermeability in Caco-2 monolayers. To test this hypothesis, he treated cultures with the antioxidant N-Acetylcysteine (NAC) and measured permeability and Per2 levels. He found that treatment with NAC prevented the increase in Per2 protein and mRNA levels and returned

permeability levels to non-alcohol treated levels, suggesting that NAC helps to rescue the alcohol-induced permeability of colonic epithelial cells at the transcriptional level. In other experiments, he extended this finding by showing that NAC treatment prevented the alcohol-mediated increase in cAMP response element binding protein (CREB) phosphorylation, which is upstream of Per2 transcription. He next wanted to determine exactly how Per2 is regulating intestinal permeability, and hypothesized a role for peroxisome proliferator-activated receptor (PPAR) γ due to its ability to regulate tight junction complexes (Varley et al., 2006). Mr. Davis showed that alcohol leads to increased PPAR γ -Per2 interactions, and showed that PPAR γ stimulation using pioglitazone, which prevents its interaction with Per2, rescued the increased permeability after alcohol exposure. Alcohol also had no effect on the mRNA or protein levels of PPAR γ , suggesting that its interactions with other proteins, like Per2, is likely regulating its effects on alcohol-induced intestinal permeability. Therefore, he concluded that oxidative stress misdirects circadian mechanisms in alcohol-induced intestinal permeability.

The final talk of this session was given by Tiyaash Parira, a graduate student in the laboratory of Dr. Marisela Agudelo at Florida International University and was titled “Histone H4 modification landscape due to chronic alcohol treatment in human monocyte-derived dendritic cells (MDDCs).” Her talk extended their laboratory’s findings that histone modifications are altered by alcohol in MDDCs. Ms. Parira’s presentation focused mainly on H4 post-translational modifications in MDDCs after chronic alcohol use. For her studies, MDDCs from human donors were treated with alcohol for 5 days, and 10 H4 modifications sites were examined. She found that chronic alcohol exposure lead to a significant decreased in H4K5acetylation(ac), H4K8^{ac}, H4K20^{me2}, H4K20^{me3}, H4arginine3^{me2}, and H4serine1phosphorylation. Yet, only one marker was significantly upregulated by almost 400%, H4K12^{ac}. Next, they confirmed that the histone acetyltransferase (HAT) inhibitor NU9056 significantly blocked the upregulation of H4K12^{ac} imparted by chronic alcohol exposure in MDDCs. Taking this finding one step further, Ms. Parira analyzed the cytokine secretion of MDDCs after chronic alcohol exposure both in the presence and absence of the HAT inhibitor. She found that there was an increase in IL-15, RANTES, TGF β 1, and TNF α , and a decrease in MCP-2, when the MDDCs were treated with alcohol and the inhibitor compared to alcohol alone. This work suggests that H4 modifications in the context of chronic alcohol exposure may be a putative epigenetic mechanism by which alcohol leads to increased inflammation mediated by innate immune cells. Therefore, her work is an important first step in identifying epigenetic markers in the periphery that are correlated with the negative immunomodulatory effects of chronic alcohol use.

Alcohol, Intestinal Mucosa, and the Gut Microbiome

The final session of the day was entitled “Alcohol, Intestinal Mucosa, and the Gut Microbiome” and was chaired by Drs. Ilhem Messaoudi (University of California Irvine) and Adam Hammer (Loyola University Chicago Health Sciences Campus). The first speaker was Dr. Bernd Schnabl from the University of California San Diego who presented his work in a talk titled “Mucosa-associated bacteria as therapeutic target of alcoholic liver disease.” He began his talk with a discussion of how liver steatosis, experienced by 90–95% of chronic alcohol abusers, transitions into cirrhosis and hepatocellular carcinoma in 8–20 and

3–10% of cases, respectively (European Association for the Study of Liver, 2012). Given the interactions between the liver and the gut, his main research question surrounds whether changes in the gut microbiota due to chronic alcohol consumption mediate the negative effects found in the liver. He showed data that supported the basis for his experimental question, which demonstrated that patients with alcoholic liver disease have intestinal bacterial overgrowth using bacterial culture techniques (Bode, Bode, Heidelberg, Durr, & Martini, 1984). These data were also replicated in a mouse model of alcoholic liver disease (Yan et al., 2011), supporting his use for a pre-clinical model to interrogate the changes in the gut that may affect alcoholic liver disease progression. With further culture-independent deep sequencing analyses of the bacteria found in the gut of the mice used in this model of alcoholic liver disease, he has found intestinal dysbiosis (Yan et al., 2011). Moreover, after abstinence from alcohol, the dysbiosis was partially reversible (Leclercq et al., 2014). Dr. Schnabl then transitioned into a discussion of whether intestinal dysbiosis was mechanistically responsible for the pathogenesis of alcoholic liver disease. Patients with alcoholic liver disease exhibit higher levels of bacterial translocation, as demonstrated by higher levels of bacterial products in the circulation, supporting the idea that the permeability of the intestine and release of bacterial products may influence alcoholic liver disease (Parlesak, Schafer, Schutz, Bode, & Bode, 2000). In addition, abstinence from alcohol leads to a restoration of gut barrier integrity in humans (Leclercq et al., 2014). Using his mouse model of alcoholic liver disease, he treated mice with non-absorbable antibiotics (i.e., its action is solely in the gut and not in peripheral tissues) and found that alcoholic liver disease was reduced in mice treated with the non-absorbable antibiotics compared to those given alcohol alone (Chen, Starkel, Turner, Ho, & Schnabl, 2015). Therefore, host-microbiome interactions are altered with chronic alcohol use, leading to increased inflammation which results in the pathogenesis of alcoholic liver disease. The next section of Dr. Schnabl's talk involved understanding what factors modulate the composition of the gut microbiome. He showed data revealing that in mice given alcohol or alcoholic humans, the amount of the antimicrobial peptide Regenerating islet-derived protein (Reg3 γ) is reduced compared to nondrinkers (Yan et al., 2011). This is an important finding, as Reg3 γ is a critical antimicrobial peptide involved in maintaining homeostasis and preventing bacterial dissemination from the gut (Yan et al., 2011). Thus, to test whether the lack of Reg3 γ influences inflammation and alcoholic liver disease progression in a mouse model of chronic alcohol use, Dr. Schnabl used *Reg3b*^{-/-}, *Reg3g*^{-/-} and *Reg3g*-transgenic(Tg) mice (which overexpress Reg3 γ specifically in intestinal epithelial cells) in his alcoholic liver disease model. He found that Reg3 γ restricts the mucosa-associated microbiota. *Reg3g*^{-/-} mice given alcohol showed an increased number of mucosa adherent bacteria compared to wildtype mice given alcohol. In addition, *Reg3g*^{-/-} mice given chronic alcohol had a higher amount of bacterial translocation, and worsened liver pathology. All of the negative effects of chronic alcohol exposure that were worsened in *Reg3*^{-/-} mice were alleviated when the mice were treated with antibiotics, underlining the importance of host-microbiome interactions mediating the progression of alcoholic liver disease. To address whether increased Reg3 γ levels were sufficient to promote healthier host-microbiome interactions in the context of chronic alcohol consumption, Dr. Schnabl treated *Reg3g*-Tg mice with chronic alcohol or vehicle control and found that the increased liver damage and bacterial translocation were no longer present in the *Reg3g*-Tg mice given alcohol. Therefore,

increased Reg3 γ levels were sufficient to reduce the bacterial translocation and subsequent inflammation that promotes alcoholic liver disease in the alcohol fed mice. Dr. Schnabl ended his talk by emphasizing that chronic alcohol use suppresses Reg3 γ levels, leading to increased numbers of mucosa-adherent bacteria and bacterial translocation, which in turn increases hepatic inflammation and enhances the progression of alcoholic liver disease (L. Wang et al., 2016).

The next talk of this session was given by Abigail Cannon, a PhD student in the laboratory of Dr. Mashkoor Choudhry at Loyola University Chicago Health Sciences Campus, and was titled “Does alcohol exacerbate an ulcerative colitis flare period?” Her work focuses on inflammatory bowel disease (IBD), which is diagnosed in over 1.4 million Americans each year, and is the most diagnosed disease in people under 30 years of age (Crohn’s & Colitis Foundation of America, 2014). This disease encompasses both Crohn’s Disease and Ulcerative Colitis (UC), with UC making up more than half of the diagnoses (Crohn’s & Colitis Foundation of America, 2014). The exact cause of UC is unknown, but it is thought to be due to a combination of alterations in the immune system, environmental factors, and genetic influences, all of which are believed to impact one another to lead to the disease (Crohn’s & Colitis Foundation of America, 2014). However, what is known is that one of the main mediators of disease are T cells, and in particular the types of T cells elicited in the intestine. In the context of UC, TNF α , IL-23 and IL-6 are released and cause increased differentiation of two types of CD4⁺ T helper cells, Th2 and Th17 cells, which then produce even more pro-inflammatory cytokines, including IL-6, TNF α , and IL-17, leading to inflammation in the gut (Neurath, 2014). Since there is no cure for UC, patients are advised on disease maintenance (i.e., taking pharmaceuticals to reduce symptoms and avoiding behaviors that will exacerbate or cause a flare in disease). UC patients are specifically told to not consume alcohol due to its inflammatory effects that may lead to flares or exacerbated symptoms for the patient. However, specific studies examining the exacerbation of UC symptoms due to alcohol are almost nonexistent. The one study that looked at IBD symptoms and alcohol consumption did show that 75% of IBD patients reported a worsening of GI symptoms with alcohol consumption (Swanson, Sedghi, Farhadi, & Keshavarzian, 2010). Therefore, Ms. Cannon’s project used a mouse model of colitis to determine the mechanism by which alcohol consumption leads to worsened colitis symptoms and/or flares. The mouse model she used is dextran sulfate sodium (DSS)-induced colitis, and she gave the mice 2% DSS daily for 5 days via the water, followed by daily alcohol treatment for 3 days. She found that weight loss was the greatest in the group given DSS + alcohol, and that this group lost significantly more weight than the DSS + vehicle group. Moreover, the colons of the mice given DSS + alcohol were shorter than those given DSS alone, and there was more IL-1 β and IL-18 in the colonic tissue of the DSS + alcohol mice versus the DSS + vehicle group. UC is a cyclical disease, with some periods of intestinal repair mediated by increased IL-22 production (X. Sun, Chalmers, Fu, & Zhao, 2012). Thus, Ms. Cannon hypothesized that if any IL-22 mediated intestinal repair mechanisms are impaired during an UC remission period, then an UC flare period could persist. Using the same mouse model as before, she showed a significant reduction in the upregulation of IL-22 in the intestine when the mice were given DSS + alcohol compared to those given DSS + vehicle. Thus, she had two major conclusions based on her data: 1) binge

alcohol consumption could exacerbate clinical symptoms associated with UC, and 2) a potential mechanism for this exacerbation is that alcohol prevents the upregulation of the IL-22 mediated repair response needed for entrance into UC remission. Future experiments involve characterizing the T cell subsets present in the intestine with and without alcohol in the DSS model, and using recombinant IL-22 as a putative therapeutic to help induce the remission phase of UC in mice given DSS + alcohol.

The next talk of the session was given by Dr. Gail Cresci formerly from Dr. Laura Nagy's laboratory at the Cleveland Clinic, titled "Protective role of butyrate during chronic-binge ethanol exposure." Dr. Cresci's work is focused on host-microbiome interactions and how they can influence intestinal immunity via interactions with mucosal epithelial cells and immune cells resident in the gut. Multiple factors impact the gut microbiome, including stress, diet, pharmaceuticals, geography, life style changes, the birthing process, and infant feeding (Cresci & Bawden, 2015). Importantly, dysbiosis of the gut microbiome is associated with many clinical disorders, including: gastrointestinal diseases (IBD, irritable bowel syndrome, and colon cancer), metabolic syndrome, allergic diseases, asthma, antibiotic therapy, autism, and alcohol use disorder (Carding, Verbeke, Vipond, Corfe, & Owen, 2015). Those with an alcohol use disorder, which is over 18 million people in the United States alone (National Institute of Alcohol Abuse and Alcoholism, 2017), are predisposed to alcoholic liver disease. The pathogenesis of alcoholic liver disease is poorly understood, and there have been few advances in disease management (as discussed in prior talks). Thus, Dr. Cresci emphasized the need for novel and specific targets to normalize inflammation in alcoholic liver disease. She noted an important study that corroborated her research in which rats were given chronic alcohol and the metabolic alterations in contents of gastrointestinal tract were characterized. This group of researchers had many interesting findings, but the one that stood out to Dr. Cresci was a reduction in the production of short chain fatty acids in the gut of alcohol-treated versus vehicle-treated rats (Xie et al., 2013). One key short chain fatty acid produced in the gut is butyrate, as it serves as an important fuel source for colonocytes and help maintain gut barrier integrity and modulate immune function. Moreover, decreases in intestinal butyrate levels are associated with increased apoptosis, increased inflammation, and enhanced muscle atrophy in the gut. Finally, it has been shown that therapy with butyrate improves clinical and pathological status in IBD patients (Thangaraju et al., 2009; Thibault et al., 2010). Given this information, Dr. Cresci hypothesized that chronic alcohol use leads to reduced butyrate production in the gut, causing dysbiosis of the gut microbiome and subsequent liver damage. Using a mouse model of chronic-binge alcohol consumption, she showed that supplementing tributyrin (which is broken down to butyric acid) in the diet of mice given alcohol led to the maintenance of gut barrier integrity, whereas those treated with glycerol (vehicle control) had increased barrier permeability due to the chronic alcohol consumption. She replicated these findings *in vitro* using Caco-2 cells, showing that treatment with butyrate rescued the increased barrier permeability imparted by alcohol on these cells. In addition, tributyrin mitigated liver injury and induced a differential liver injury pattern in mice exposed to alcohol compared to alcohol exposed mice given glycerol control. When inflammation was examined in the livers of the alcohol treated mice, those that were given tributyrin did not have the increase in TLR2, 4, or 9, or TNF α expression that occurred in alcohol treated mice given glycerol

control. Thus, she demonstrated that oral supplementation of tributyrin is protective against chronic-binge alcohol-induced gut injury due to a lessened hepatic inflammatory response.

The final talk of the AIRIG 2016 meeting was given by Dr. Waddah A. Alrefai from the Jesse Brown VA Medical Center and University of Illinois at Chicago, entitled "Potential role for cholesterol absorption in alcoholic liver disease." Dr. Alrefai began his talk with the concept that factors responsible for inter-individual variations regarding susceptibility to alcoholic liver disease remain elusive, but he hypothesizes dietary cholesterol can impact the development of the disease. His rationale for this hypothesis is that cholesterol absorption promotes hepatic inflammation and oxidative stress and is an independent risk factor for nonalcoholic steatohepatitis (NASH), and that removal of dietary cholesterol prevented NASH in a mouse model (Ferolla et al., 2015). Moreover, the efficiency of cholesterol absorption is highly variable between individuals, like susceptibility to alcoholic liver disease, but it is known to positively correlate with plasma low-density lipoprotein (LDL) cholesterol (Griffin & Lichtenstein, 2013). For his initial studies, he used a mouse model of mild hypercholesterolemia, in which mice overexpress Sterol Response Element Binding Protein 2 (SREBP2, or ISR2) specifically in the intestine. SREBP2 is a transcription factor that is a master regulator of cholesterol homeostasis (Eberle, Hegarty, Bossard, Ferre, & Foufelle, 2004). Dr. Alrefai showed that in the ISR2 transgenic mice, hepatic triglycerides and cholesterol content is increased, and that these mice exhibit mild steatosis. Thus, he believes that these mice represent a model for accelerated NASH. To test his original hypothesis that altered cholesterol absorption leads to increased alcoholic liver disease susceptibility he analyzed gene expression in the intestine of after chronic alcohol feeding. The results showed that intestinal ATP binding cassette subfamily G member 5 (ABCG5), which limits intestinal absorption and promotes cholesterol excretion (Li, Gu, & Zhang, 2013), is decreased by chronic alcohol feeding in mice and rats. Furthermore, intestinal ATP-binding cassette transporter (ABCA1), a master regulator of cholesterol efflux (Li et al., 2013), is increased by chronic alcohol feeding in mice. Therefore, Dr. Alrefai concluded that chronic alcohol consumption enhances cholesterol absorption leading to higher cholesterol levels that could potentially promote alcoholic liver disease. His future work will focus on following up on these preliminary results to determine if changes in cholesterol absorption lead to susceptibility to alcoholic liver disease.

Summary

The 2016 AIRIG meeting allowed for the sharing of novel preliminary data regarding the influence of alcohol on immunity and epigenetics. Data shared at the meeting suggests that alcohol changes the microbiome, which may lead to worsened diseases like pneumonia or alcoholic liver disease. Moreover, the impact of alcohol on inflammation and immunity was underscored in many studies examining immune cells in circulation and in target tissues, impacting circulating biomarkers of modulated immunity. Alcohol can impact the development of many different diseases, including anxiety-like behaviors, liver disease, colitis, and lung diseases. Collectively, the data presented at this meeting tie together the ability of alcohol to modulate epigenetics in many cell types, and change the microbial composition and location in the body, leading to profound immunological changes for the host.

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Highlights

- The 2016 AIRIG meeting covered new preliminary data regarding the influence of alcohol consumption on epigenetics and the immune system.
- Alcohol changes the microbiome, which has been implicated in worsened pneumonia or alcoholic liver disease.
- Alcohol negatively effects the development of anxiety-like behaviors, liver disease, colitis, and lung diseases.
- Alcohol consumption impacts circulating biomarkers of modulated immunity and inflammation in multiple organs and tissues.
- This meeting tied together the ability of alcohol to modulate epigenetics in many cell types and changes in the microbiome, together leading to profound immunological changes for the host.