Published in final edited form as:

Alcohol. 2015 December ; 49(8): 767–772. doi:10.1016/j.alcohol.2015.09.002.

Summary of the 2014 Alcohol and Immunology Research Interest Group (AIRIG) meeting

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Abstract

On November 21, 2014 the 19th annual Alcohol and Immunology Research Interest Group (AIRIG) meeting was held at Loyola University Chicago Health Sciences Campus in Maywood, Illinois. The meeting focused broadly on inflammatory cell signaling responses in the context of

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alcohol and alcohol use disorders, and was divided into four plenary sessions focusing on the gut and liver, lung infections, general systemic effects of alcohol, and neuro-inflammation. One common theme amongst many talks was the differential roles of macrophages following both chronic and acute alcohol intoxication. Macrophages were shown to play significant roles in regulating inflammation, oxidative stress, and viral infection following alcohol exposure in the liver, lungs, adipose tissue, and brain. Other work examined the role of alcohol on disease progression in a variety of pathologies including psoriasis, advanced stage lung disease, and cancer.

Introduction

The Center for Disease Control recently reported that alcohol-attributed mortality remains one of the leading preventable causes of death in the United States (Stahre, Roeber, Kanny, Brewer, & Zhang, 2014). Clinical and experimental data have strongly correlated alcohol use and abuse with a host of mental and physiological pathologies, including disruptions in the immune system within multiple organs. Recent work has begun to illuminate the effects of alcohol on immune cell populations and signaling in the lung, adipose tissue, and brain (Bird et al., 2010; Naveau et al., 2010; Nixon, Kim, Potts, He, & Crews, 2008; Persidsky et al., 2011). Others are focusing on the role of microRNAs in the gastrointestinal tract, and how the microbiome may be a possible source of alcohol-mediated infection/sepsis (Morris, Li, Earley, & Choudhry, 2015; Mutlu et al., 2012). Finally, groups are starting to understand the underlying mechanisms of alcohol in other pathologies including psoriasis and cancer (Farkas & Kemeny, 2013; Poschl & Seitz, 2004).

The 2014 AIRIG meeting was broken into four sessions, each with a unique focus on alcohol research including gut and liver, lung infections, general systemic effects of alcohol, and neuro-inflammation. Due to the extensive effects of alcohol on human health, the goal of the annual AIRIG meeting is to bring together top researchers in the fields of alcohol and immunology research to share and collaborate on new ideas to expand this important field.

Alcohol, Liver and Gut

Drs. Elizabeth J. Kovacs and M. Katherine Jung opened the 2014 AIRIG meeting with a welcome and an overview of collaborative relationships that have been established amongst alcohol researchers. This was followed by the first plenary session, chaired by Dr. Robert Siggins (Louisiana State University), and Dr. Ping Zhang (Northeast Ohio Medical University). The focus of the first session was on the effects of alcohol in the intestines and liver. Dr. Carol A. Casey, University of Nebraska Medical Center, opened the first session by discussing the hepatic asialoglycoprotein receptor (ASGP-R) and its role in alcoholic liver inflammation. It has been well-documented that alcohol induces the progression of liver disease including steatosis, fibrosis, cirrhosis, cancer, and liver failure (Diehl, 2002). However, the mechanisms by which alcohol induces the progression of liver disease remain unknown. It has been previously shown that alcohol significantly impairs protein and glycoprotein trafficking in hepatocytes (Tuma, Casey, & Sorrell, 1990). The ASGP-R is a key receptor found in high concentrations on hepatocytes that functions to properly process and clear inflammatory proteins including cellular fibronectin (CFn), apoptotic bodies,

desialylated carcinoembryonic antigen (CEA), and alkaline phosphatase (ALP) (Casey, Lee, Aziz-Seible, & McVicker, 2008; Lee, Casey, & McVicker, 2009). Alcohol has been shown to lower ASGP-R expression, internalization capability, and recycling, and therefore may be one mechanism by which alcoholic liver disease progresses (McVicker, Thiele, Casey, Osna, & Tuma, 2013). Dr. Casey used a model of self-feeding alcohol administration for 8 weeks in rats to study ASGP-R in the context of alcoholic liver disease, and showed that Kupffer cells increased secretion of both tumor necrosis factor alpha (TNF- α) and interleukin-6 (IL-6) after exposure of apoptotic bodies, CFn, or CEA, which led to hepatocyte apoptosis (Casey et al., 2008). Hepatocytes also displayed an inability to adequately internalize and process ALP in response to alcohol administration, which also contributed to liver inflammation. Data presented by Dr. Casey demonstrated a significant role for the ASGP-R in alcohol-induced liver inflammation.

Tasha Barr, PhD student in laboratory of Dr. Ilhem Messaoudi at the University of California Riverside, presented her work studying the effects of alcohol intoxication on the intestinal microbiome in Rhesus macaques. The intestinal microbiome has been shown to regulate intestinal immune cell function and barrier maintenance, and is nicely summarized in a review from Round and Mazmanian (Round & Mazmanian, 2009). It has been demonstrated that both acute and chronic alcohol intoxication can significantly alter the resident microbiome population of the intestines (Mutlu et al., 2012). However, region-specific changes of bacteria within the small and large intestines and how they may modulate subsequent barrier disruption are under current investigation. Studies were carried out in a Rhesus macaque model of self-administration where animals were progressively introduced to alcohol over a 4-month period, followed by 12 months of self-administration (Grant et al., 2008). To examine if chronic alcohol intoxication results in significant changes in the intestinal microbiome, 16S ribosomal DNA sequencing was performed from duodenum, jejunum, ileum, and colon of heavy-drinker, moderate-drinker, and non-drinker animals. In all regions of the intestines, a 3-fold decrease of the commensal species *Lactobacillus intestinalis* was observed in heavy drinkers compared to non-drinkers. Results also showed a 2-fold increase of the phylum Bacteroidetes observed in the colon of heavy drinkers, which was correlated with a decrease in the Firmicutes phylum. In the duodenum and colon of heavy-drinkers, a 2-fold increase in *Bifidobacterium* and *Prevotella copri* was observed. Finally, a 47-fold decrease in *Helicobacter macacae* was observed in the colon of heavy-drinkers compared to non-drinkers. The data presented reflect significant regional changes of commensal and pathogenic bacterial species in the gut, and may significantly contribute to altering the intestinal immune system following intoxication.

Niya Morris, PhD student in Dr. Mashkoor A. Choudhry's laboratory at Loyola University Chicago, discussed microRNA regulation of the intestinal barrier following alcohol intoxication and burn injury. Previous studies have shown increases in inflammatory mediators and gut barrier leakiness following combined alcohol and burn injury (Li, Akhtar, Kovacs, Gamelli, & Choudhry, 2011; Rendon, Li, Akhtar, & Choudhry, 2013; Zahr et al., 2012). A recent study demonstrated significant increases in intestinal IL-18 expression following combined alcohol and burn injury, which may contribute to gut permeability (Morris et al., 2015). One mechanism that may regulate gut inflammation and leakiness is

microRNA expression in intestinal epithelial cells (McKenna et al., 2010). A well-established rodent model of single binge intoxication (2.9 g/kg) and burn injury (12.5% total body surface area) was used to study microRNA expression in the intestines one day following the combined insult (Li et al., 2011). In order to examine a broad range of microRNA profiles, microRNA from gut intestinal epithelial cells was processed and analyzed by a miScript miRNA PCR Array. From this panel of 86 different microRNA expression profiles, 19 different microRNAs had altered expression profiles. Due to their roles in cell differentiation, inflammation (specifically IL-18 regulation), and proliferation, three microRNAs, mir-7, -150, and -375 were further validated by qRT-PCR. Results showed that mir-7 and -150 were significantly reduced in mouse intestinal epithelial cells one day following intoxication and burn injury. These results suggest that there are significant changes in microRNA profiles in the gut following alcohol intoxication and traumatic injury.

Melissa Fulham, PhD student in Dr. Pranoti Mandrekar's laboratory at the University of Massachusetts, presented her work on myeloid glycoprotein-96 (gp96) signaling following alcohol-induced adipose tissue inflammation and its role in the pathogenesis of alcoholic liver disease (ALD). Previous studies have drawn correlations between ALD and adipose tissue inflammation in mice and humans (Naveau et al., 2010; Xu et al., 2003), however, the mechanism by which this pathogenesis occurs remains unknown. Macrophages have been shown to play a large role in inflammatory responses in liver and adipose tissue. Previous work showed mice lacking TLR4 expression were protected from both alcohol induced liver inflammation and adipose inflammation induced by a high-fat diet (Hritz et al., 2008; Shi et al., 2006). Glycoprotein-96 is a key regulator of TLR expression in macrophages and may contribute to the progression of ALD. To study whether adipose macrophage function is altered following alcohol intoxication through a gp96 dependent pathway, mice were subjected to the Gao-NIAAA Chronic-Binge Lieber-DeCarli which consisted of a 5% v/v alcohol for 11 days followed by a single 5 g/kg ethanol gavage (Bertola, Mathews, Ki, Wang, & Gao, 2013). Mice receiving the chronic alcohol diet displayed significant increases in serum ALT, triglyceride, and inflammatory IL-6 and MCP-1 levels compared to controls. Additionally, significant increases in innate immune cells were observed within adipose tissues of alcohol-fed mice. Myeloid-specific gp96 knockout mice ($LysMcre^{+/-}Hsp90b1^{-/-}$; gp96KO) receiving the chronic alcohol diet were protected from liver injury, and displayed no increases in innate immune cell infiltrates to adipose tissue compared to control animals. Together, these data suggest that gp96 may mediate macrophage homing and inflammatory responses in adipose tissue.

Alcohol and Lung Infections

The second session was chaired by Dr. Ilhem Messaoudi (University of California, Riverside), and Dr. Samantha Yeligar (Emory University), and focused on the role of alcohol intoxication in lung infections. Dr. Viranuj Sueblinvong, Emory University, opened the second session with a talk on alcohol-induced lung fibroblast-to-myofibroblast transdifferentiation. Previous work in both human samples and animal models of alcohol exposure have shown intoxication results in increased oxidative stress, impaired epithelial barrier integrity, and depressed macrophage function within lung alveoli (Guidot et al.,

2000; Velasquez et al., 2002). Many oxidative stress molecules in the lung are regulated by nuclear factor (erythroid-derived 2)-like 2 (Nrf2), a transcription factor which specifically activates the anti-oxidant response element (ARE) in lung fibroblasts (Cho, Reddy, & Kleeberger, 2006). It has been shown that chronic alcohol intoxication leads to decreases in Nrf2 expression and nuclear binding in lung fibroblasts (Sueblinvong et al., 2014). Recently, thioredoxin-1 (Trx-1), a part of the Nrf2-ARE, was observed to be significantly decreased in the lung *in vivo* and in the nucleus and cytoplasm of lung fibroblasts *in vitro* following 60 mM alcohol exposure for 24–72 hours (unpublished data). It was hypothesized that overexpression of Trx-1 in the nucleus of lung fibroblasts would attenuate alcohol-induced fibroblast-to-myofibroblast transdifferentiation. Nuclear Trx-1 overexpressing transgenic mice and littermate controls were given 20% v/v alcohol in drinking water for eight weeks. To induce acute lung injury, intratracheal bleomycin was administered, and mice were euthanized two weeks after to examine levels of fibroblast-to-myofibroblast transdifferentiation. While Trx-1 transgenic mice showed no differences in Nrf2 expression levels, they did display increased levels of the antioxidant glutathione-S-transferase (GST) and decreased transforming growth factor-beta levels. These findings demonstrated that Trx-1 appears to modulate Nrf2 activity as marked by increased GST levels, and provided a new scope of how alcohol may potentiate oxidative stress in lung fibroblasts after an acute injury.

Dr. Carresse Gerald, a post-doctoral fellow in Dr. Todd A. Wyatt's laboratory at the University of Nebraska, presented her work on the effects of alcohol in the context of the lungs following agricultural dust pollutants in a mouse model. It has been shown that people in rural areas drink alcohol in larger quantities than those in urban areas (Brumby, Kennedy, & Chandrasekara, 2013). Farm workers are also regularly exposed to dust and other air pollutants in the barns that house livestock (Alterman, Steege, Li, Petersen, & Muntaner, 2008). Preliminary work has demonstrated that dust from swine confinement facilities induces inflammatory cytokine production in the lungs including TNF- α , IL-6, and interleukin-8 (IL-8), which are mediated through changes in nitric oxide and PKA inhibition of the TNF- α sheddase, ADAM17 (unpublished observation). Inhibition of the innate immune system could increase susceptibility to disease and/or infection from pathogens in inhaled dust. It was hypothesized that activation of the PKA pathway leads to inhibition of ADAM17 activity, which prevents proper inflammatory responses to hog barn dust. BEAS-2B bronchial epithelial cells were used to study the effects of PKA pathway inhibitors following alcohol and hog barn dust exposure. Pretreatment with alcohol resulted in decreased ADAM17 activity and TNF- α release from BEAS-2B cells following hog barn dust exposure, a response that was further enhanced by addition PDE4 inhibitors, which degrade cAMP. However, administration of the adenylyl cyclase inhibitor KH7, or the nitric oxide synthase inhibitor L-NMMA, significantly improved the TNF- α inflammatory response. Taken together, these data show that alcohol inhibits the release of TNF- α following hog barn dust exposure through both NO and PKA dependent signaling pathways in bronchial epithelial cells.

The final presentation of the second plenary session was given by Dr. Bethany Lussier, a medical fellow in Dr. Daniel G. Remick's laboratory at Boston University. Dr. Lussier

discussed the effects of alcohol consumption on triggering asthmatic attacks. Significant increases in mucin production within the airways have been observed within 30 minutes following alcohol consumption, which is paired with the recruitment of pulmonary eosinophils (Bouchard et al., 2012). Due to the acute mucin response in the airways following alcohol ingestion, it was hypothesized that neuronal stimuli may be a significant contributing factor to asthmatic responses following alcohol consumption. To study this, a mouse asthmatic model was generated by sensitizing female mice to cockroach allergens (Vaickus, Bouchard, Kim, Natarajan, & Remick, 2010). One week after the final dose, mice were pretreated to block the muscarinic response with tiotropium bromide four hours before gavage with a single binge with 300 μ l of 32% alcohol. A second set of experiments generated an alpha-nicotinic receptor blockade by intraperitoneal injection with GTS-21 30 minutes before alcohol gavage. Data from all groups showed that alcohol gavage resulted in an asthmatic response as measured by expiratory time, respiratory rate, and compliance based whole body plethysmography. However, neither muscarinic nor α -nicotinic blockade resulted in decreased mucin production measured by computer aided morphometry. These data suggest that the significant increases in mucin production during an asthma attack following alcohol ingestion cannot be prevented by muscarinic or alpha-nicotinic blockade alone.

Systemic Effects of Alcohol

The third session was chaired by Dr. Majid Afshar (Loyola University Chicago) and Dr. Pegah Abbasnia (Loyola University Chicago), and focused on the systemic effects of alcohol. Dr. Eileen O'Halloran, a medical resident in Dr. Elizabeth J. Kovac's laboratory at Loyola University Chicago, opened the third session by discussing her work examining inflammatory mediator production by alveolar macrophages in patients with alcohol use disorders. It has been well documented that alveolar macrophages play a critical role in pulmonary inflammatory responses and clearance of lung pathogens (Haslett, 1999; Nelson & Summer, 1998). Alveolar macrophage responses are reduced following alcohol intoxication, making patients with alcohol use disorders more susceptible to infections (Standiford & Danforth, 1997). To study the effects of alcohol on alveolar macrophages in a patient population, bronchoalveolar lavage samples were collected from patients with alcohol use disorders. Age-, gender-, and smoking history-matched patient samples were used as controls. Samples were analyzed for cytokine production and growth factor expression by a multiplex assay. Patients with alcohol use disorders had significantly higher pro-inflammatory cytokine profiles including interferon gamma, TNF- α , and interleukin-13. Pro-inflammatory chemokines were also upregulated including IL-8, CXCL10, CCL4, and RANTES. Alcohol use disorder samples also showed elevations in the anti-inflammatory cytokines interleukin-1 receptor A and interleukin-10, as well as several growth factors including granulocyte-colony stimulating factor, platelet derived growth factor, and vascular endothelial growth factor. Pro-inflammatory macrophages marked by CD80 expression were significantly higher in alcohol use disorder patients than in controls. In summary, these data show that chronic alcohol exposure results in the generation of a pro-inflammatory phenotype in alveolar macrophages. Therapeutics targeting these pro-inflammatory alveolar

macrophages in patients with alcohol use disorders may be helpful to prevent pulmonary infection and/or disease.

James O'Brien, a second year medical student working with Dr. Erin M. Lowery Loyola University Chicago, presented his study of how alcohol use affects patients with advanced stage lung disease (Boe, Vandivier, Burnham, & Moss, 2009; Sisson, 2007). The study was performed by obtaining a sample of patients who were new or awaiting transplant from the lung transplant clinic at Loyola University Medical Center. Alcohol use among this cohort of patients was assessed using two different alcohol use tests; the Alcohol Use Disorder Identification Test (AUDIT), and the Short Michigan Alcohol Screening Test (SMAST) (MacKenzie, Langa, & Brown, 1996). Data obtained from these tests were compared to historical alcohol use based on the notes of physicians and/or social workers' assessment. 24 total patients took the AUDIT and SMAST exams, 12 males and 12 females, with a mean age of 59. None of the patients in this cohort had AUDIT or SMAST scores that indicated heavy alcohol use, which was corroborated by their historical alcohol use based on physicians/social workers' notes. While no patients in this cohort were currently heavy drinkers, many were former heavy drinkers, which may be important to take into account in future studies. A larger sample patient population will be required to draw conclusions about alcohol use in patients with advanced stage lung disease, and this work is ongoing.

The next presentation was given by Dr. Rhonda Brand, University of Pittsburgh. Dr. Brand discussed her work examining the effects of alcohol in the context of psoriasis. Psoriasis is an autoimmune disease that affects about 2% of the population, and is correlated with a host of comorbidities including depression and self-destructive habits including excess alcohol consumption (Poikolainen, Karvonen, & Pukkala, 1999). Previous studies have shown that alcohol use can worsen symptoms associated with psoriasis (Farkas & Kemeny, 2013; Gupta, Schork, Gupta, & Ellis, 1993). To study the mechanism of how alcohol use exacerbates the symptoms associated with psoriasis, Dr. Brand utilized a mouse model of psoriasis whereby mice were treated on four consecutive days with Aldara (Imiquimod) to induce psoriasis (Wohn, Pantelyushin, Ober-Blobaum, & Clausen, 2014). Mice were also separated into groups that either received alcohol (Lieber-DeCarli Diet) for 10 days, or regular diet. Results showed that alcohol increases psoriasis severity by a number of factors including increasing skin thickness and cellular infiltrates, as well as increasing the pro-inflammatory mediators IL-6 and TNF- α (unpublished data). Taken together, these results demonstrate that alcohol does increase the severity of psoriasis, and should be taken into consideration for treatment of patients.

The final presentation of the third session was given by Dr. Stephanie Watkins, Loyola University Chicago. Dr. Watkins discussed the role of gender and alcohol use on the ability of dendritic cells to initiate anti-tumor immune responses. While the problems associated with all forms of cancer are well characterized, differences between male and female responses (Naugler et al., 2007), as well as how alcohol contributes to cancer progression (Poschl & Seitz, 2004), have been less studied. Dr. Watkins has previously demonstrated significant immune suppression of dendritic cells in a murine model of prostate cancer (Watkins et al., 2011). To further expand the role of gender and alcohol on cancer progression, Dr. Watkins presented data from three different mouse cancer models including

breast cancer, melanoma, and hepatocellular carcinoma (HCC). Results showed that females have increased tumor-associated macrophages, and lower T-cell and dendritic cell populations. Male mice had a higher number of tumor-associated dendritic cells, and these dendritic cells also had higher MHC expression than those in female mice. While the ability of dendritic cells to activate naïve antigen specific T-cells following alcohol exposure remained unchanged, immune tolerance and immunosuppressive signaling pathways (protein kinase B (AKT) and mitogen activated protein kinase) were significantly upregulated in dendritic cells following alcohol exposure (unpublished data). Together these data suggest that alcohol may play a significant role in generating an anti-tumor response *in vivo*, especially if the patient is receiving immunotherapy treatment for their cancer.

Alcohol and Neuro-inflammation

The final session of the 2014 AIRIG meeting focused on alcohol and neuro-inflammation. This session was chaired by Dr. Sulie Chang (Seton Hall University) and Dr. Brenda Curtis (Loyola University Chicago). The first talk was given by Dr. Yuri Persidsky, Temple University. Dr. Persidsky discussed alcohol abuse and its correlation with the progression of HIV-1 associated neurocognitive disorders (HAND). Inflammation within the central nervous system following HIV infection is caused by low virus replication within macrophages and lasting injury to the blood brain barrier (Persidsky, Zheng, Miller, & Gendelman, 2000). Alcohol abuse is a common co-morbidity observed in people with HIV infections, and has been observed to exacerbate both HIV progression and HAND pathology in both *in vitro* and *in vivo* studies where mice were fed 4% ethanol for 1–2 weeks (Persidsky et al., 2011; Potula et al., 2006). Attempts to reduce inflammation using pharmacologic interventions such as cannabinoid receptor 2 (CB2) agonists have been shown to be extremely effective (Mukhopadhyay et al., 2010). Recent studies have demonstrated that elevated CB2 signaling results in protective anti-inflammatory, barrier strengthening effects in brain endothelium (Ramirez et al., 2012). CB2 agonists have also shown to suppress HIV replication in primary human macrophages (Ramirez et al., 2013). These studies collectively demonstrate the efficacy of CB2 agonist as novel therapeutic treatments for HIV patients that are at risk for HAND.

The last talk of the neuro-inflammation session was given by Dr. Kimberly Nixon of the University of Kentucky. Dr. Nixon shared her research examining how inflammation affects neurobiology in the context of alcohol use disorders. In spite of extensive data reporting detrimental effects of alcohol abuse on the brain (Crews & Nixon, 2009), the mechanisms underlying this phenomenon remain largely unknown. Recent work examined glial cells and their role in neuro-inflammation in animal models of alcohol use disorders and abstinence (Nixon et al., 2008). Dr. Nixon's laboratory has shown that excessive alcohol exposure in a binge model (25% w/v ethanol gavage three times daily for 4 days) leads to significant proliferation of microglia in many regions of the brain, especially the hippocampus (Nixon et al., 2008). As the main component of the brain's innate immune response, glial cells are capable of changing inflammatory responses, which can be detrimental to brain structure and therefore, function (Graeber & Streit, 2009). However, new studies have demonstrated that glial cells are not always pro-inflammatory, and depending upon the environment, can switch phenotypes to be cytotoxic or neuroprotective (Graeber & Streit, 2009). Studies in a

rat model of alcohol use disorders show that microglia are activated, but repeated binge doses of alcohol do not result in classical activation of microglia (Marshall et al., 2013). Further, microglia activation precedes reactive adult neurogenesis, an event that is hypothesized to repopulate the hippocampal dentate gyrus (Nixon et al., 2008). Preliminary observations suggest that binge alcohol appears to result in microglia cell death (unpublished observation), but more work will be required to confirm this observation. Together, these data provide insight into potentially new protective roles for microglia following alcohol exposure and abstinence.

Summary

The 2014 AIRIG meeting focused on the effects of alcohol mediated inflammation and oxidative stress. Many presentations focused on signaling and homing of macrophages to different tissues following intoxication. In liver and adipose tissue, knockout of macrophage glycoprotein-96 expression was shown to be protective following chronic alcohol intoxication. In the lung, alveolar macrophage function is suppressed following alcohol intoxication, which may make patients more susceptible to infection and/or disease. Inflammatory CD80⁺ macrophages were found to be elevated in bronchoalveolar lavage fluid of patients with alcohol use disorders, and may be a useful target to limit pulmonary infection in these patients. Macrophages also appear to play differential roles in the brains of patients with history of alcohol use disorders and through alcohol abstinence. Additional studies in the brain have shown that alcohol exacerbates symptoms associated with HIV-1 infection, which can be attenuated through pharmacologic intervention using CB2 agonists. Other work examining the role of alcohol in the intestines shows that intoxication greatly alters the intestinal microbiome and immune system, and may disrupt the barrier in a microRNA dependent fashion. Together, data presented at this meeting highlighted the global effects of alcohol on human physiology, and how acute, binge, or chronic intoxication is able to influence multiple organ systems and disease progression.

Acknowledgments

The authors and participants would like to thank the NIAAA and the Loyola University Chicago Alcohol Research Program for financial support of the meeting. Support was also provided by the National Institutes of Health (NIH) under award numbers R13 AA020768 (EJK), R01 AA012034 (EJK), R21 AA023193 (EJK), GM 115257 (EJK), AA022566 (JAI), T32 AA013527 (EJK), R01 AA015731 (MAC), R21 AA022324 (MAC). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. The authors would like to thank Tasha Barr, Melissa Fulham, James O'Brien, and Drs. Carresse Gerald, Bethany Lussier, Eileen O'Halloran, Rhonda Brand, and Stephanie Watkins for presenting their work at the 2014 AIRIG meeting, and Paula Soczek for logistical support.

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Article Highlights

- Inflammatory cell signaling responses in the context of alcohol use disorders
- Roles of macrophages following both chronic and acute alcohol intoxication
- Neuroinflammatory responses following alcohol exposure