Published in final edited form as:

Addict Biol. 2020 November ; 25(6): e12832. doi:10.1111/adb.12832.

STRESS-RELATED SUPPRESSION OF PERIPHERAL CYTOKINES PREDICTS FUTURE RELAPSE IN ALCOHOL DEPENDENT INDIVIDUALS WITH AND WITHOUT SUB-CLINICAL DEPRESSION

Helen C Fox¹, Verica Milivojevic², Alicia MacDougall², Heather LaVallee², Christine Simpson³, Gustavo A Angarita⁴, Rajita Sinha²

¹Department of Psychiatry, School of Medicine, Stony Brook University, 101 Nicolls Road HSC, T-10, room 040B, Stony Brook, NY 11794-8101, USA.

²The Yale Stress Center, Department of Psychiatry, Yale University School of Medicine, 2 Church Street South, Suite 209, New Haven, CT 06519, USA.

³Department of Internal Medicine, Yale University School of Medicine, New Haven, USA.

⁴Clinical Neuroscience Research Unit, The Connecticut Mental Health Center, Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA.

Abstract

Chronic alcohol abuse and depressive symptoms are both associated with peripheral cytokine changes. Despite this, cytokine adaptations have not been assessed in co-morbid populations, or prospectively as predictors of relapse. We examine cytokine responses to stress in alcohol dependent individuals and social drinkers, both with and without sub-clinical depression. We also examine the potential link between cytokine adaptations in response to stress and prospective alcohol relapse risk. Thirty-three, alcohol dependent individuals (21 with and 12 without high depressive symptoms) and 37 controls (16 with and 21 without high depressive symptoms) were exposed to two, 5-minute personalized guided imagery conditions (stress and neutral) across consecutive days in a randomized and counterbalanced order. Alcohol craving and serum measures of tumor necrosis factor alpha (TNF α), tumor necrosis factor receptor 1 (TNFR1), interleukin-6 (IL-6), and interleukin-1 receptor antagonist (IL-1ra), were collected prior to and following imagery exposure. Following treatment discharge, follow up interviews were conducted over 90 days to assess relapse. Dampened IL-1ra and IL-6 in response to stress was observed as a function of alcohol dependence and not moderated by depressive symptoms. Lower levels of IL-6

Helen Fox, PhD, helen.fox@stonybrookmedicine.edu, Phone: +1-631-638 0057.

AUTHORS CONTRIBUTION

HFC and RS were responsible for the study concept and design. HCF drafted the manuscript. VM assisted HCF and RS with data analyses, interpretation of findings and revision of the manuscript for intellectual content. AM and HL were responsible for recruitment and data acquisition under the supervision of VM and HCF. CS performed all cytokine analyses. As study physician, GAA was responsible for advising HCF on the continued health and psychological welfare of all participants during their stay on the Clinical Neuroscience Research Unit (CNRU) of the Connecticut Mental Health Center (CMHC) inpatient facility. All authors critically reviewed content and approved final version for publication.

DISCLOSURE

The authors declare no conflict of interest

following stress also predicted greater drinking days following treatment. Conversely, high depressive symptomatology was associated solely with pro-inflammatory adaptations. Stress-related suppression of TNF α predicted drinking severity only in alcohol dependent individuals with sub-clinical depression and suppressed TNFR1 following stress was only seen in individuals with sub-clinical depression. Stress-induced suppression of pro-inflammatory TNF markers may indicate a risk factor for alcohol dependent individuals with co-occurring depressive symptoms.

Keywords

Alcohol Dependence; Anti-inflammatory; Cytokines; Depressive Symptomatology; Pro-inflammatory; Stress

INTRODUCTION

Extensive epidemiological data indicate high co-morbidity between alcohol use disorder (AUD) and depressive symptoms (1, 2). Additionally, one third of dependent individuals meet diagnostic criteria for major depression at some point in their drinking time-line (3). This may culminate in a reciprocal relationship where alcohol use may augment and prolong the course of depressive symptoms (4, 5) and, in turn, depressive symptoms may elevate compulsive alcohol seeking and risk of relapse (6, 7). Despite this common relationship, specific biomarkers of such co-morbidity and their link to alcohol outcomes have not been studied thus far.

As distinct disorders, both depression (8, 9) and excessive drinking (10) are linked to robust changes in peripheral pro- and anti-inflammatory cytokines that are known to readily cross the blood brain barrier (BBB) (11, 12). For example, both in vivo and in vitro human studies have indicated that acute administration of alcohol at low, moderate and binge doses produce an anti-inflammatory state characterized by increases in mediators such as interleukin-10 (IL-10) and interleukin-1 receptor antagonist (IL-1ra) and attenuation of pro-inflammatory markers including lipopolysaccharide (LPS)-induced tumor necrosis factor- α (TNF α) and interleukin-1 beta (IL-1 β) (13–15). Chronic alcohol abuse has also been associated with tonic blunting of IL-1ra and IL-6 in active drinkers, as well as a dampened interleukin-6 (IL-6), TNF α and tumor necrosis factor receptor-1 (TNFR1) response to stress in abstinent alcohol dependent individuals (16, 17). Notably, these adaptations are also linked to both alcohol cue-induced craving and alcohol intake following stress (16, 17).

In addition, depression and depressive symptoms not only compound poor clinical outcome from alcohol treatment (1, 18), but dysregulate peripheral cytokines differentially dependent upon depression sub-type, time of day and other factors pertaining to individual differences (8). For example, pathological activation of pro-inflammatory marker, TNF α , as well as IL-6 and TNFR1, which mediate the pro-inflammatory effects of TNF α , have been associated with depression and depressive mood in chronically ill patients (19–21). Moreover the treatment of both patients (22, 23) and animals (24, 25) with TNF α can produce negative affective states similar to those associated with the negative reinforcing effects of withdrawal from alcohol. Several studies have also shown IL-6 to be elevated both peripherally (9, 26) and centrally (27) in individuals with major depressive disorder (MDD). More recently,

direct evidence of brain inflammation in the anterior cingulate cortex (ACC), as measured by distribution volume of translocator protein (TSPO) in activated microglia, also supports the role of neuro-inflammation in major depressive episodes (28). These studies help to highlight the potential for peripheral cytokines to represent sensitive markers of clinical outcome in alcohol dependent individuals, by characterizing the link between stress response mechanisms, depressive mood and motivation for alcohol.

Despite this, no previous research has delineated the relative contribution of depressive symptoms and chronic drinking on peripheral cytokine adaptations during protracted abstinence within co-morbid populations, or assessed the effectiveness of these markers in prospectively predicting relapse. Thus, using an experimental approach in an inpatient treatment setting, and a prospective clinical outcome design, we examined whether the co-occurrence of depressive symptomatology would moderate stress-induced immunosuppression of IL-1ra (anti-inflammatory cytokine), TNF α and TNFR1 (pro-inflammatory markers) and IL-6 (pro- and anti-inflammatory properties), in a group of early abstinent alcohol dependent individuals and socially drinking controls, with and without high depressive symptoms. We additionally assessed whether these cytokine adaptations were associated with future alcohol relapse outcomes in alcohol dependent individuals with and without depressive symptomatology.

METHOD

Thirty-three treatment seeking alcohol dependent individuals (n=12 without depressive symptoms; n=21 with depressive symptoms) and 37 socially drinking controls (n=21 without depressive symptoms; n=16 with depressive symptoms), were recruited from around the greater New Haven area. Current alcohol dependence was determined using the structured clinical interview for the diagnostic and statistical manual of mental disorders IV-TR (SCID I -(29), and participants also produced a positive urine toxicology screen upon admission to inpatient treatment. Exclusion criteria for alcohol dependent patients included DSM-IV dependence for any drug other than alcohol or nicotine and any psychiatric illness requiring medication. All social drinkers (25 drinks or less per month) were classified using the Cahalan quantity frequency variability index (30) and were excluded if they met current or lifetime dependence criteria for alcohol or any other drug other than nicotine. All participants underwent electrocardiography and laboratory tests of renal, hepatic, pancreatic, hematopoietic and thyroid function to ensure good physical health. Any participants failing to meet health requirements were ineligible.

Participants were categorized into two depressive symptom groups (High Vs Low), using the Center for Epidemiologic Studies - Depression Scale (CES-D; High: ≥ 13 and Low < 13). While cut-off scores of 16 are traditionally recommended (31, 32), we chose to use a slightly lower score for several reasons. First, a cut-off of 16 on the CES-D has often been documented as producing a high number of false negative rates when compared with Research Diagnostic Criteria (RDC) (33). For example, in a community sample of 525 participants, the CES-D yielded a false-negative rate of 40% compared with a false positive rate of 16% as determined by RDC (34). Similarly, other studies have shown a false-negative rate of 36.4% compared with 6.1 % false positives in another community sample of 515

individuals (35). Second, the emphasis of the current study was to distinguish between individuals with and without depressive symptoms, rather than detect individuals with significant clinical depression. In light of this, in the current sample 13 was calculated as being the median score for the entire sample, representing an appropriate measure of central tendency.

All participants gave written and verbal consent and the Human Investigation Committee of the Yale University School of Medicine approved the study.

General Procedures

Alcohol Dependent Participants—were admitted to the Clinical Neuroscience Research Unit (CNRU) of the Connecticut Mental Health Center (CMHC) for 4 to 5 weeks of standard group counseling for alcohol dependence (36) and study participation. During week 4 of inpatient stay, all participants took part in two laboratory challenge studies, run across consecutive days, where they were presented with two personalized 5-minute imagery conditions (stress and neutral), one per day, in a randomized and counterbalanced order. Personalized imagery scripts were developed and scripted during the second week of inpatient stay, from participants' recent life events (37). Following discharge from the inpatient unit, participants were required to return for face-to-face interviews after 14 days, 30 days and 90 days.

Socially Drinking Controls—were required to spend two consecutive afternoons at the Yale Stress Center, located several blocks away from the inpatient unit. During this time they took part in an identical 2-day laboratory challenge study. Lunch was provided and comprised a similar diet to that provided for the alcohol group in the inpatient unit. Breathalyzer tests were provided to ensure none of the participants had consumed alcohol during the course of the morning. Baseline demographics, psychiatric and substance use assessments as well as imagery scripts were prepared prior to the laboratory challenge procedures.

Imagery Script Development Procedures:

Imagery script development was conducted in a session prior to the laboratory procedures, approximately 14 days following inpatient admission. Procedures are based on methods developed by Lang and colleagues (38) and further adapted in our previous studies (17, 39). The stress imagery script was based on subjects' descriptions of a recent personal stressful event that made them "sad, mad, or upset," and which they were not able to control in the moment, and which was experienced as being "most stressful." "Most stressful" was determined by having the subjects rate their perceived stress on a 10-point Likert scale where 1 = *not at all stressful* and 10 = *the most stress* they felt recently in their life. Only situations rated as 8 or above were accepted as appropriate for script development (e.g., being fired from their job, marital conflict situation). Stressful situations pertaining to alcohol or identified as traumatic and involving the re-experiencing of trauma-related anxiety symptoms were excluded. A relaxing, non-physiologically arousing script was also developed to represent the participants' intra-individual control condition. These comprised

descriptions of a personal, relaxing situation which again, did not include alcohol-related scenarios (e.g., being at the beach; fall afternoon reading at the park).

Subjects verbally expressed the scenarios to an interviewer who documented all details of the scene descriptions. For the stress scenarios, the subject then completed a checklist of interoceptive sensations by selecting specific moods and physiological bodily feelings that were relevant to the situation being described. The checklist included a list of 67 bodily sensations describing changes in heart rate, chest breathing, perspiration, muscle tension, stomach, anxiety/fear, sadness/depression, anger-related feelings and urges, and excitement. These interoceptive behaviors were then included into the 5 minute script of each scenario, and each scenario was subsequently recorded onto tape and played back to the participants during the laboratory procedures (see Laboratory Sessions section).

In addition to the script development, on the day prior to the laboratory sessions, subjects were brought into the testing room to acclimatize themselves to specific study procedures. These included being familiarized with the subjective rating forms, IV insertion without blood draws, as well as training in relaxation and imagery procedures (previously described in (40).

Laboratory Sessions

On each testing day, subjects were brought into the testing room at 1:15PM. All participants were allowed an initial smoke break at 1:00 PM in order to reduce the potential for nicotine withdrawal. After settling into a sitting position on a hospital bed, a heparin-treated catheter was inserted into the antecubital region of the subject's non-preferred arm, to periodically obtain blood samples. This was followed by a 30-minute adaptation period during which the participants were instructed to practice the relaxation techniques that they had learned prior to the laboratory challenge days. At 2:20 PM, participants were provided with headphones and given the following instructions: *"Close your eyes and imagine the situation being described, 'as if' it were happening right now. Let your body and mind get completely involved in the situation, doing what you would do in the real situation"*. The audio tape was then played, and the length of each script was approximately 5-minutes. If participants' anxiety remained above baseline levels following the final time-point, they were taken through another series of relaxation procedures until their ratings returned to baseline levels. After the final assessment, the subjects were disconnected from the apparatus.

Subjective measures of alcohol craving and serum samples were collected 5 -minutes prior to imagery exposure (baseline), immediately following imagery (0 time-point) and periodically at various recovery time-points (+5, +15, +30 minutes post imagery). The study was double blind, with neither the investigator nor the participant aware of which imagery condition was being presented on which day.

Laboratory Assessments:

Serum Cytokine Measures: Following collection, serum samples were inverted then stored at room temperature for approximately 30 minutes and allowed to clot. Samples were

then separated by centrifugation at 4 C for 15 minutes at 1000 X *g*, aliquoted and stored in polypropylene tubes at -70 C until the time of the assay.

Cytokine concentrations were quantitatively determined by enzyme-linked immune-sorbent assays using the DuoSet ELISA Development Kit from R&D systems (Minneapolis, MN, USA). Assaying of serum IL-1ra, IL-6, TNF α and TNFR1 were conducted at the Yale Center for Clinical Investigation (YCCI), core mineral metabolism laboratory under the supervisor of Christine Simpson. For TNF α , the assay kit sensitivity ranged from 0.5 to 5.5 pg/mL with an assay range of 15.6 – 1,000 pg/mL and specificity for natural and recombinant human TNF α . Intra-assay precision from three samples ranged from 4.2% to 5.6%, and inter-assay precision from three samples ranged from 4.6% to 7.4%. For IL-6, the assay kit had a sensitivity of less than 0.7 pg/mL, an assay range of 3.1 – 300 pg/mL, and specificity for natural and recombinant human IL-6. Intra-assay precision from three samples ranged from 1.6% to 4.2%, and inter-assay precision from three samples ranged from 3.3% to 6.4%. For IL-1ra, the assay kit had a sensitivity range of 2.2 to 18.3 pg/mL, an assay range of 31.2 – 2,000 pg/mL, and specificity for natural and recombinant human IL-1ra. Intra-assay precision from three samples ranged from 3.7% to 7.3%, and inter-assay precision from three samples ranged from 6.7% to 11.0%. For TNFR1, the assay kit had a sensitivity of 1.2 pg/mL, an assay range of 7.8 – 500 pg/mL and specificity for natural and recombinant human IL-1ra. Intra-assay precision from three samples ranged from 3.6% to 5.0%, and inter-assay precision from three samples ranged from 3.7% to 8.8%.

Subjective Measures:

Alcohol Craving: The desire for using alcohol was assessed using a 10-point visual analog scale (VAS) in which 1 = ‘not at all’ and 10 = ‘extremely high’.

Prospective Follow-up Procedures:

All alcohol dependent participants were scheduled for face-to-face follow-up interviews at 14, 30, and 90 days post discharge. Breath and urine alcohol assessments were collected as well as daily retrospective reporting of alcohol use using the timeline follow-back method (41). Thirty-one of the 33 alcohol dependent individuals (94%) who completed the laboratory sessions attended at least 1 follow-up session.

Statistical Analysis

Between Group Analyses: Experimental groups were compared on demographics and alcohol use using ANOVA or chi square analysis. Any significant group differences effecting the Dependent Variables were included as covariates in all models. Linear mixed effect (LME) models were performed to analyze all data using SPSS software (version 21). The Between-Subjects factors of Drug Group (alcohol dependent Vs socially drinking controls) and Depressive Group (with Vs without depressive symptoms) as well as the Within-Subjects factors of Imagery Condition (stress Vs neutral) and Time-points (varying levels) represented the fixed effects factors. Participants represented the random effects factor. In order to account for individual baseline variability on each testing day, baseline and change from baseline analyses were performed for all dependent variables. A compound symmetry

covariance structure was applied to all analyses and Bonferroni tests were used as adjustments for multiple comparisons.

Prospective Alcohol Relapse Analyses: Standard regression and proportional hazards models were used to explore whether cytokine response to stress could predict future relapse factors following treatment. Primary relapse outcome factors were: *number of days to relapse, number of days spent drinking, and total alcohol use following treatment discharge* as well as binge measures including: *maximum and average number of drinks consumed on any one occasion, and longest period of time spent drinking across any one occasion.*

RESULTS

Participant Demographics:

Both alcohol dependent groups were significantly older, with less education and greater nicotine smoking history than both socially drinking control groups (Table 1). These factors were entered as covariates into all LME models.

Alcohol Craving:

Change from baseline analyses showed an Alcohol Group X Imagery Condition X Time-point interaction [$F_{3, 378} = 2.7$; $p < .05$, $f = .34$]. Immediately following stress exposure (0 time-point) the alcohol group reported significantly higher ratings of craving compared with the controls ($p = .006$). In addition, the alcohol dependent group also reported significantly higher levels of craving immediately following stress (0 time-point) compared with all other recovery time-points (+5, +15, +30; $p < .0001$, in all cases). An elevation in alcohol craving was also observed immediately following stress imagery compared with neutral imagery exposure in the socially drinking controls ($p = .002$). No significant interactions or main effects of Depressive Group were observed (See Fig. 1).

IL1-ra:

Change from baseline analyses showed an Alcohol Group X Imagery Condition interaction [$F_{1, 370} = 6.5$; $p = .01$, $f = .30$], where all socially drinking controls, irrespective of depressive symptoms, demonstrated an anticipated increase in IL-1ra levels in response to stress relative to neutral imagery ($S > N$, $p = .006$). This was not observed in the alcohol group. No significant interactions or main effects of Depressive Group were observed (See Fig. 2).

IL6:

Change from baseline analyses showed a main effect of Alcohol Group [$F_{1, 51} = 10.8$; $p = .002$], where all alcohol dependent individuals, regardless of depressive symptoms, demonstrated a general suppression of IL-6 response to both imagery conditions compared with the socially drinking controls. No other significant main effects or interactions for Alcohol Group or Depressive Group were seen (See Fig. 3).

TNFa:

Change from baseline analyses showed a significant Alcohol Group X Depressive Group X Imagery Condition interaction following imagery exposure, [$F_{1,368} = 6.8$; $p = .01$, $f = .31$], which indicated that only the non-depressive socially drinking controls demonstrated an elevated TNFa response to stress imagery compared with the neutral condition ($S > N$, $p < .0001$; See Fig. 4a). In the other three groups the TNFa response to stress was not elevated compared with the neutral condition (See Fig. 4b-d).

TNFR1:

All alcohol dependent individuals, regardless of depressive symptoms, demonstrated significantly elevated basal levels of TNFR1 compared with socially drinking controls, [$F_{1, 47} = 6.0$; $p < .02$, $f = .29$] (See Fig. 5a).

Change from baseline analyses indicated a Depressive Group X Imagery interaction trend [$F_{1, 346} = 3.5$; $p = .06$, $f = .22$], where all non-depressive individuals, regardless of drinking status, demonstrated an increased TNFR1 response to stress compared with neutral imagery ($S > N$, $p = .05$; Fig. 5b). In contrast, all depressive individuals, regardless of drinking status, showed no comparative increase in TNFR1 following stress (Fig. 5c). No significant main effects or interactions for Alcohol Group were observed.

Prior to the addition of covariates, the Depressive Group X Imagery Condition interaction was significant [$F_{1, 406} = 5.2$; $p = .02$, $f = .27$]. However, the significant effect of “*number of days spent smoking*” on the model following the addition of covariates ($p = .002$), suggests that smoking may account for some of the variance.

Prospective assessment of relapse (See Fig. 6)

Fifty-eight percent of the alcohol dependent group (18/31) had relapsed by the Day-14 follow-up interview. A further 5 individuals relapsed by Day-30 (74% total) and another 5 by Day-90 (90% total). Only 3 individuals (10%) remained abstinent 90 days after treatment discharge.

TNFR1:

In all alcohol dependent individuals, regardless of depressive symptoms, lower TNFR1 response to stress predicted a greater maximum number of drinks consumed on any one occasion following treatment discharge [$\beta = -.44$, $t = -2.4$, $p = .02$] (Fig. 6a). TNFR1 response to the neutral imagery condition did not predict outcome. Proportional hazards analysis additionally indicated that dampened TNFR1 response to stress was also predictive of time to alcohol relapse [$\chi^2 = 5.17$; $p = .02$; $HR = 2.8$, 95% CI , 1.11–7.19], with lower response following stress conferring a 2.8-fold greater cumulative risk of alcohol relapse (Fig. 6b). Elevated basal levels of TNFR1 in all alcohol dependent individuals also predicted total alcohol use [$\beta = .44$, $t = 2.0$, $p = .05$] and a higher longest period of alcohol use following discharge from treatment [$\beta = .45$, $t = 2.2$, $p < .05$] (not shown).

TNF α :

Lower levels of TNF α in response to stress predicted a binge-like pattern of drinking following discharge in the depressive alcohol dependent individuals, only. In this case, the lower the TNF α response to stress, the greater the average number of drinks consumed on any one occasion [$\beta = -.55$, $t = -2.3$, $p < .04$] (Fig 6c). TNF α response to the neutral imagery condition in this group did not predict outcome.

IL-6:

In all alcohol dependent individuals, a lower IL-6 response to stress predicted a greater number of days spent drinking following discharge from the inpatient unit [$\beta = -.39$, $t = -2.1$, $p = .04$] (Fig.6d) as well as a higher longest period of alcohol use [$\beta = -.43$, $t = -2.4$, $p < .03$] (not shown). IL-6 response to the neutral imagery condition did not predict either outcome measure.

IL-1ra:

No significant associations were observed between IL-1ra response to stress or neutral imagery and drinking outcomes following treatment discharge in either alcohol dependent group.

DISCUSSION

In the current study we used a combined experimental laboratory challenge and clinical outcome design, to characterize the relative contribution of chronic drinking and sub-clinical depression on changes within peripheral immune system cytokines. Findings are the first to show that specific cytokine changes in response to stress may differ between chronic drinkers with and without co-occurring depressive symptomatology. Moreover, that such cytokine adaptations are also predictive of future relapse outcomes.

All alcohol dependent individuals, irrespective of depressive symptoms, demonstrated a suppression of IL-1ra following exposure to stress compared to the neutral condition. By comparison the socially drinking controls demonstrated an anticipated increased IL-1ra response to stress imagery relative to the neutral imagery condition (Rohleder et al., 2006; Maes, 2001; Assaf et al., 2017). Given that prior findings from our laboratory have shown active problem drinkers to demonstrate dampened tonic levels of IL-1ra (16), a lack of tonic suppression in the current sample may suggest potential normalization of these anti-inflammatory systems following 4- weeks of protracted abstinence. While current findings suggest a recovery may be observed in basal circulating levels of IL-1ra, stress-induced phasic suppression of IL-1ra was still apparent alongside elevated alcohol craving, even after 4-weeks of abstinence. Suppression of anti-inflammatory markers as a possible characteristic of the stress-induced craving state also corroborates our prior research in both active problem drinkers as well as abstinent alcohol dependent and cocaine dependent individuals (16, 17, 42). Findings also provide some support for preclinical research showing that injections of the anti-inflammatory marker, recombinant IL-10, into the basolateral amygdala (BLA) during a Drinking in the Dark (DID) paradigm attenuates binge-like ethanol consumption in mice (43).

No significant group variation was seen in relation to basal levels of IL-6, although a generalized suppression of IL-6 in response to both stress and neutral imagery compared with controls, may reflect a baseline trend. Interestingly, the controls also failed to demonstrate an elevated IL-6 response to stress relative to the neutral imagery condition. One possibility may be that compared with other cytokines, IL-6 is not as sensitive to the personalized stress imagery paradigm used in the current study. This is consistent with our prior research showing that moderate social drinkers appear to demonstrate less robust elevations in IL-6 following stress than other markers (16, 17). It is also consistent with a few other research studies demonstrating no IL-6 response to different stress paradigms including stroop and mirror drawing (44, 45) mental arithmetic and public speaking (46). However, many studies have documented stress-related increased in IL-6 (47–49), and another possibility may be related to the temporal response profile of IL-6, where increased levels may only be observed with greater delayed sampling beyond 30 minutes. Meta-analyses studies have indicated that while IL-6 levels are typically elevated during stress in healthy control groups, more robust effects are observed in studies that incorporated delayed sampling from between 30 to 120 minutes post stress exposure (49, 50). In the current study, cytokine sampling did not extend beyond 30 minutes post imagery intervention.

While future research is required to determine exactly whether alcohol dependence moderates IL-6 response to stress, these findings do indicate that the dampened IL-6 response to stress, but not neutral imagery, predict a greater number of days spent drinking following treatment discharge, as well as a binge-like pattern of drinking represented by a longer period of time spent drinking on any one occasion. These findings hold some support for a prior study where decreases in IL-6 levels from day 7 to day 14 of abstinence in male alcohol dependent individuals treated with carbamazepine and clomethiazole, were associated with motivation for alcohol (51).

All alcohol dependent individuals in the current study also demonstrated elevated basal levels of TNFR1 that did not occur as a function of depressive symptoms. This upregulation of TNFR1 may reflect a rebound effect from persistent alcohol-induced suppression (16, 52, 53) that may be slower to normalize compared with other cytokine markers. It may additionally reflect a general health index, as elevated TNFR1 levels have been shown to play a role in alcohol-induced liver injury (54). Note, however, that all participants were deemed in good health as a part of the inclusion criteria. While further research is warranted to fully understand the mechanisms underlying these cytokine adaptations, phasic TNFR1 suppression was observed in depressive individuals, and also associated with greater severity of drinking following treatment discharge. As such, it may reflect a depression-related marker of vulnerability during protracted abstinence from alcohol.

In this study, depressive symptoms only, and not drinking status, were characterized by stress-induced suppression of pro-inflammatory mediators, TNF α and its receptor, TNFR1. While the influence of pro-inflammatory mediators in the pathogenesis of depression and depressive symptoms is well documented (23, 25, 55) (20, 56), the current study further indicates that these markers may represent a potential indicator of risk in alcohol dependent populations with sub-clinical depression. Specifically, dampened levels of TNF α and TNFR1 in the face of stress may signify a particular risk for binge-like patterns of drinking.

For example, lower levels of TNF α predicted a greater average number of drinks consumed on any one occasion in alcohol dependent individuals with sub-clinical depression. Moreover, low levels of TNFR1, seen only in the depressive alcohol dependent group, predicted a greater maximum number of drinks consumed on any one occasion as well as a fewer number of days to relapse.

Although the cytokine adaptations documented in the current study demonstrated statistical significance, the absolute change in cytokine levels from baseline to peak response were small, raising concerns regarding the relevance of such biomarker responses in terms of clinical validity. With this in mind, findings from several prior studies in healthy populations have shown responses to psychological stress within a similar range to those observed in this study. Findings include similar response levels of IL-6 (57–59), TNF α (60, 61), IL-1ra and TNFR1 (62, 63), suggesting that the extent of cytokine change seen in the current controls, may reflect clinically germane stress response mechanisms. The fact that peripheral cytokines are known to exert robust pharmacological effects at minute doses (64), additionally lends support to the possibility that even negligible suppression of the immune system following stress, may be linked to clinically important outcome measures in abstinent drinkers.

The link between immune suppression in response to stress and motivation for alcohol may reflect allostatic load underlying motivation for alcohol, which has been characterized as sensitization of core stress systems (65, 66), suppressed response to stress (67–70) and dampened response to reward via downregulation of D2 receptors in the ventral striatum (71, 72). As the function of both the stress and immune systems are intrinsically meshed, dampened immune response to stress during protracted abstinence from alcohol dependence, may also be the result of persistent acute upregulation of stress and immune markers either due to acute withdrawal and / or chronic alcohol intake. More broadly, cytokine suppression in response to stress may reflect an alternative regulatory index of allostatic load arising as an attempt to preserve reward function stability (73) and reflecting the move from positive to negative reinforcement (74). This is borne out in the current and prior studies by showing that stress-induced cytokine suppression is a predictor of elevated alcohol craving and relapse (16, 17). Recent findings have also shown that acute alcohol intake may serve to elevate stress-induced blunted cortisol levels (75) known to be a marker of relapse vulnerability during early abstinence from alcohol (68, 76). It is therefore possible that binge-like patterns of drinking during relapse may additionally reflect attempts to boost suppression of discrete pro-inflammatory markers during perturbation of the stress systems. This interpretation does, however, remain conditional upon further research.

The peripheral immune system dissociations reported in the present study are observed as a function of high depressive symptomatology on entry to inpatient treatment, rather than following the three week abstinence period. There were several reasons for this including the fact that robust decreases in symptoms are often observed following treatment engagement (77), making it difficult to discern co-morbidity. Additionally, evidence suggests that pretreatment levels of depressive symptoms and depressive disorders have been associated with several highly salient aspects of treatment outcome including elevated drinking rates (78, 79) shorter time to initial drink (80), lower rates of post-treatment abstinence (81) and

treatment drop-out (82) despite symptom attenuation during treatment. As such, in the present study it was considered important to ascertain whether depressive symptoms at treatment entry would impact the course of immune system changes following early protracted abstinence. It is important to note however, that the manner in which depressive symptoms fluctuate during abstinence may also have a significant impact on alcohol-related outcome measures (83) as well as stress and immune system pathways. In view of this, future research ascertaining cytokine adaptations as a function of depressive symptoms during early abstinence may also be warranted, as the current findings may be limited in interpretation.

Other limitations include the fact that the alcohol dependent individuals were also heavier smokers compared with controls, and while smoking was covaried in all analyses co-morbid smoking-related inflammatory changes may still need to be assessed for potential influence. Although gender ratio was statistically balanced across groups in the current study, sex specific inflammatory changes have been reported (84), suggesting the need to consider sex as a biological variable in future replication studies. Future research studies are also encouraged to extend sampling time to at least 90 minutes post stress intervention in order to optimally capture cytokine adaptations (Marsland et al., 2017). While the current participant sample was small, effect sizes for the relapse analyses generated moderate effect sizes ranging from .39 to .55.

Despite these study constraints, study strengths include use of validated experimental methods within a well-controlled inpatient study design to ensure a drug-free abstinent phase that allowed for careful assessment of future relapse. In view of this, the current findings reveal intriguing evidence to suggest that suppression of peripheral cytokines during stress may serve as potential bio-behavioral markers of drinking risk and be a salient target for treatment development in alcohol dependence. Findings additionally indicate that the suppression of selective pro-inflammatory markers may underlie risk for return to binge patterns of drinking specific to alcohol dependent individuals with co-occurring depressive symptoms.

ACKNOWLEDGEMENTS

We would like to thank the staff at the Yale Stress Center, the Yale Center for Clinical Investigation, as well as the staff based at the Substance Abuse Center and the Clinical Neuroscience Research Unit of the Connecticut Mental Health Center for their assistance in completing this study.

FUNDING

This study was supported in part by grants R01:AA20095 (Fox); R03: AA022500 (Fox); R21AA024880 (Fox); Peter F MacManus Charitable Trust (Fox); R01:AA20504 (Sinha); R01:AA013892 (Sinha); and 5T32DA007238-24 (Milivojevic).

REFERENCES

1. Pettinati HM, O'Brien CP, Dundon WD. Current status of co-occurring mood and substance use disorders: a new therapeutic target. *The American journal of psychiatry*. 2013;170(1):23–30. [PubMed: 23223834]

2. Kessler RC, Crum RM, Warner LA, Nelson CB, Schulenberg J, Anthony JC. Lifetime co-occurrence of DSM-III-R alcohol abuse and dependence with other psychiatric disorders in the National Comorbidity Survey. *Archives of general psychiatry*. 1997;54(4):313–21. [PubMed: 9107147]
3. Boden JM, Fergusson DM. Alcohol and depression. *Addiction*. 2011;106(5):906–14. [PubMed: 21382111]
4. Armeli S, Sullivan TP, Tennen H. Drinking to Cope Motivation as a Prospective Predictor of Negative Affect. *J Stud Alcohol Drugs*. 2015;76(4):578–84. [PubMed: 26098033]
5. Lazareck S, Robinson JA, Crum RM, Mojtabai R, Sareen J, Bolton JM. A longitudinal investigation of the role of self-medication in the development of comorbid mood and drug use disorders: findings from the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC). *The Journal of clinical psychiatry*. 2012;73(5):e588–93.
6. Chi FW, Satre DD, Weisner C. Chemical dependency patients with cooccurring psychiatric diagnoses: service patterns and 1-year outcomes. *Alcoholism, clinical and experimental research*. 2006;30(5):851–9.
7. DeMarce JM, Lash SJ, Stephens RS, Grambow SC, Burden JL. Promoting continuing care adherence among substance abusers with co-occurring psychiatric disorders following residential treatment. *Addictive behaviors*. 2008;33(9):1104–12. [PubMed: 18573617]
8. Young JJ, Bruno D, Pomara N. A review of the relationship between proinflammatory cytokines and major depressive disorder. *Journal of affective disorders*. 2014;169:15–20. [PubMed: 25128861]
9. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, et al. A meta-analysis of cytokines in major depression. *Biological psychiatry*. 2010;67(5):446–57. [PubMed: 20015486]
10. Crews FT, Sarkar DK, Qin L, Zou J, Boyadjieva N, Vetreno RP. Neuroimmune Function and the Consequences of Alcohol Exposure. *Alcohol Res*. 2015;37(2):331–41, 44–51. [PubMed: 26695754]
11. Banks WA, Erickson MA. The blood-brain barrier and immune function and dysfunction. *Neurobiology of disease*. 2010;37(1):26–32. [PubMed: 19664708]
12. Pan W, Stone KP, Hsueh H, Manda VK, Zhang Y, Kastin AJ. Cytokine signaling modulates blood-brain barrier function. *Curr Pharm Des*. 2011;17(33):3729–40. [PubMed: 21834767]
13. Mandrekar P, Catalano D, White B, Szabo G. Moderate alcohol intake in humans attenuates monocyte inflammatory responses: inhibition of nuclear regulatory factor kappa B and induction of interleukin 10. *Alcoholism, clinical and experimental research*. 2006;30(1):135–9.
14. Afshar M, Richards S, Mann D, Cross A, Smith GB, Netzer G, et al. Acute immunomodulatory effects of binge alcohol ingestion. *Alcohol*. 2015;49(1):57–64. [PubMed: 25572859]
15. Neupane SP. Neuroimmune Interface in the Comorbidity between Alcohol Use Disorder and Major Depression. *Front Immunol*. 2016;7:655. [PubMed: 28082989]
16. Milivojevic V, Ansell E, Simpson C, Siedlarz KM, Sinha R, Fox HC. Peripheral Immune System Adaptations and Motivation for Alcohol in Non-Dependent Problem Drinkers. *Alcohol Clin Exp Res*. 2017;41(3):585–95. [PubMed: 28147432]
17. Fox HC, Milivojevic V, Angarita GA, Stowe R, Sinha R. Peripheral immune system suppression in early abstinent alcohol-dependent individuals: Links to stress and cue-related craving. *J Psychopharmacol*. 2017;31(7):883–92. [PubMed: 28675117]
18. Prisciandaro JJ, DeSantis SM, Chiuzaan C, Brown DG, Brady KT, Tolliver BK. Impact of depressive symptoms on future alcohol use in patients with co-occurring bipolar disorder and alcohol dependence: a prospective analysis in an 8-week randomized controlled trial of acamprosate. *Alcoholism, clinical and experimental research*. 2012;36(3):490–6.
19. O'Connor JC, Lawson MA, Andre C, Moreau M, Lestage J, Castanon N, et al. Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. *Mol Psychiatry*. 2009;14(5):511–22. [PubMed: 18195714]
20. Maes M, Yirmiya R, Noraberg J, Brene S, Hibbeln J, Perini G, et al. The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. *Metab Brain Dis*. 2009;24(1):27–53. [PubMed: 19085093]
21. Kiraly DD, Horn SR, Van Dam NT, Costi S, Schwartz J, Kim-Schulze S, et al. Altered peripheral immune profiles in treatment-resistant depression: response to ketamine and prediction of treatment outcome. *Translational psychiatry*. 2017;7(3):e1065.

22. Gohier B, Goeb JL, Rannou-Dubas K, Fouchard I, Cales P, Garre JB. Hepatitis C, alpha interferon, anxiety and depression disorders: a prospective study of 71 patients. *World J Biol Psychiatry*. 2003;4(3):115–8. [PubMed: 12872204]
23. Dunn AJ, Swiergiel AH, de Beaurepaire R. Cytokines as mediators of depression: what can we learn from animal studies? *Neurosci Biobehav Rev*. 2005;29(4–5):891–909. [PubMed: 15885777]
24. Silverman MN, Macdougall MG, Hu F, Pace TW, Raison CL, Miller AH. Endogenous glucocorticoids protect against TNF-alpha-induced increases in anxiety-like behavior in virally infected mice. *Mol Psychiatry*. 2007;12(4):408–17. [PubMed: 17389906]
25. Salome N, Tasiemski A, Dutriez I, Wigger A, Landgraf R, Viltart O. Immune challenge induces differential corticosterone and interleukin-6 responsiveness in rats bred for extremes in anxiety-related behavior. *Neuroscience*. 2008;151(4):1112–8. [PubMed: 18207648]
26. Haapakoski R, Ebmeier KP, Alenius H, Kivimaki M. Innate and adaptive immunity in the development of depression: An update on current knowledge and technological advances. *Prog Neuropsychopharmacol Biol Psychiatry*. 2016;66:63–72. [PubMed: 26631274]
27. Kern S, Skoog I, Borjesson-Hanson A, Blennow K, Zetterberg H, Ostling S, et al. Higher CSF interleukin-6 and CSF interleukin-8 in current depression in older women. Results from a population-based sample. *Brain Behav Immun*. 2014;41:55–8. [PubMed: 24858658]
28. Setiawan E, Wilson AA, Mizrahi R, Rusjan PM, Miler L, Rajkowska G, et al. Role of translocator protein density, a marker of neuroinflammation, in the brain during major depressive episodes. *JAMA psychiatry*. 2015;72(3):268–75. [PubMed: 25629589]
29. First M, Spitzer R, Gibbon M, Williams JPE. Structured Clinical Interview for DSM-IV. . Washington DC: American Psychiatric Press Inc.; 1995.
30. Cahalan D, Cisin IH, Crossley HM. American drinking practices: A national study of drinking behaviors and attitudes. . Monographs of the Rutgers Center of Alcohol Studies. 1969;6(260).
31. Weissman MM, Sholomskas D, Pottenger M, Prusoff BA, Locke BZ. Assessing depressive symptoms in five psychiatric populations: a validation study. *Am J Epidemiol*. 1977;106(3):203–14. [PubMed: 900119]
32. Roberts RE, Rhoades HM, Vernon SW. Using the CES-D scale to screen for depression and anxiety: effects of language and ethnic status. *Psychiatry research*. 1990;31(1):69–83. [PubMed: 2315423]
33. Weissman MM, John K, Merikangas KR, Prusoff BA, Wickramaratne P, Gammon GD, et al. Depressed parents and their children. General health, social, and psychiatric problems. *Am J Dis Child*. 1986;140(8):801–5. [PubMed: 3728409]
34. Roberts RE, Vernon SW. The Center for Epidemiologic Studies Depression Scale: its use in a community sample. *The American journal of psychiatry*. 1983;140(1):41–6. [PubMed: 6847983]
35. Myers JK, Weissman MM. Use of a self-report symptom scale to detect depression in a community sample. *The American journal of psychiatry*. 1980;137(9):1081–4. [PubMed: 7425160]
36. Mercer DE, Woody GE. Individual Drug Counseling. Rockville, MD: US Department of Health and Human Services; 1999.
37. Sinha R Manual for imagery script development procedures. Unpublished Manual, . 2001.
38. Lang PJ, Kozak MJ, Miller GA, Levin DN, McLean A, Jr. Emotional imagery: conceptual structure and pattern of somato-visceral response. *Psychophysiology*. 1980;17(2):179–92. [PubMed: 7375619]
39. Sinha R New findings on biological factors predicting addiction relapse vulnerability. *Current psychiatry reports*. 2011;13(5):398–405. [PubMed: 21792580]
40. Sinha R, Talih M, Malison R, Cooney N, Anderson GM, Kreek MJ. Hypothalamic-pituitary-adrenal axis and sympatho-adreno-medullary responses during stress-induced and drug cue-induced cocaine craving states. *Psychopharmacology*. 2003;170(1):62–72. [PubMed: 12845411]
41. Sobell LC, Brown J, Leo GI, Sobell MB. The reliability of the Alcohol Timeline Followback when administered by telephone and by computer. *Drug and alcohol dependence*. 1996;42(1):49–54. [PubMed: 8889403]
42. Fox HC, D'Sa C, Kimmerling A, Siedlarz KM, Tuit KL, Stowe R, et al. Immune system inflammation in cocaine dependent individuals: implications for medications development. *Hum Psychopharmacol*. 2012;27(2):156–66. [PubMed: 22389080]

43. Marshall SA, McKnight KH, Blöse AK, Lysle DT, Thiele TE. Modulation of Binge-like Ethanol Consumption by IL-10 Signaling in the Basolateral Amygdala. *J Neuroimmune Pharmacol.* 2017;12(2):249–59. [PubMed: 27640210]
44. Dugue B, Leppanen EA, Teppo AM, Fyhrquist F, Grasbeck R. Effects of psychological stress on plasma interleukins-1 beta and 6, C-reactive protein, tumour necrosis factor alpha, anti-diuretic hormone and serum cortisol. *Scand J Clin Lab Invest.* 1993;53(6):555–61. [PubMed: 8266000]
45. Owen N, Steptoe A. Natural killer cell and proinflammatory cytokine responses to mental stress: associations with heart rate and heart rate variability. *Biol Psychol.* 2003;63(2):101–15. [PubMed: 12738402]
46. Heesen C, Schulz H, Schmidt M, Gold S, Tessmer W, Schulz KH. Endocrine and cytokine responses to acute psychological stress in multiple sclerosis. *Brain Behav Immun.* 2002;16(3):282–7. [PubMed: 12009688]
47. Bob P, Raboch J, Maes M, Susta M, Pavlat J, Jasova D, et al. Depression, traumatic stress and interleukin-6. *Journal of affective disorders.* 2010;120(1–3):231–4. [PubMed: 19359044]
48. Carpenter LL, Gawuga CE, Tyrka AR, Lee JK, Anderson GM, Price LH. Association between plasma IL-6 response to acute stress and early-life adversity in healthy adults. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology.* 2010;35(13):2617–23. [PubMed: 20881945]
49. Marsland AL, Walsh C, Lockwood K, John-Henderson NA. The effects of acute psychological stress on circulating and stimulated inflammatory markers: A systematic review and meta-analysis. *Brain Behav Immun.* 2017;64:208–19. [PubMed: 28089638]
50. Steptoe A, Hamer M, Chida Y. The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis. *Brain Behav Immun.* 2007;21(7):901–12. [PubMed: 17475444]
51. Heberlein A, Kaser M, Lichtinghagen R, Rhein M, Lenz B, Kornhuber J, et al. TNF-alpha and IL-6 serum levels: neurobiological markers of alcohol consumption in alcohol-dependent patients? *Alcohol.* 2014;48(7):671–6. [PubMed: 25262503]
52. Bhatta M, Jan BL, Tan W, Pruett SB, Nanduri B. Role of acute ethanol exposure and TLR4 in early events of sepsis in a mouse model. *Alcohol.* 2011;45(8):795–803. [PubMed: 21872420]
53. Pruett SB, Zheng Q, Fan R, Matthews K, Schwab C. Ethanol suppresses cytokine responses induced through Toll-like receptors as well as innate resistance to *Escherichia coli* in a mouse model for binge drinking. *Alcohol.* 2004;33(2):147–55. [PubMed: 15528012]
54. Yin M, Wheeler MD, Kono H, Bradford BU, Gallucci RM, Luster MI, et al. Essential role of tumor necrosis factor alpha in alcohol-induced liver injury in mice. *Gastroenterology.* 1999;117(4):942–52. [PubMed: 10500078]
55. Silverman GJ, Khanna S. B cell modulation in rheumatology. *Curr Opin Pharmacol.* 2007;7(4):426–33. [PubMed: 17625968]
56. McNally L, Bhagwagar Z, Hannestad J. Inflammation, glutamate, and glia in depression: a literature review. *CNS Spectr.* 2008;13(6):501–10. [PubMed: 18567974]
57. Koelsch S, Boehlig A, Hohenadel M, Nitsche I, Bauer K, Sack U. The impact of acute stress on hormones and cytokines, and how their recovery is affected by music-evoked positive mood. *Sci Rep.* 2016;6:23008. [PubMed: 27020850]
58. Edwards KM, Burns VE, Ring C, Carroll D. Sex differences in the interleukin-6 response to acute psychological stress. *Biol Psychol.* 2006;71(3):236–9. [PubMed: 16098655]
59. Edwards JP, Zhang X, Frauwirth KA, Mosser DM. Biochemical and functional characterization of three activated macrophage populations. *J Leukoc Biol.* 2006;80(6):1298–307. [PubMed: 16905575]
60. Yamakawa K, Matsunaga M, Isowa T, Kimura K, Kasugai K, Yoneda M, et al. Transient responses of inflammatory cytokines in acute stress. *Biol Psychol.* 2009;82(1):25–32. [PubMed: 19446599]
61. Miller GE, Rohleder N, Stetler C, Kirschbaum C. Clinical depression and regulation of the inflammatory response during acute stress. *Psychosomatic medicine.* 2005;67(5):679–87. [PubMed: 16204423]

62. Rohleder N, Wolf JM, Herpfer I, Fiebich BL, Kirschbaum C, Lieb K. No response of plasma substance P, but delayed increase of interleukin-1 receptor antagonist to acute psychosocial stress. *Life sciences*. 2006;78(26):3082–9. [PubMed: 16414081]
63. Steptoe A, Owen N, Kunz-Ebrecht S, Mohamed-Ali V. Inflammatory cytokines, socioeconomic status, and acute stress responsivity. *Brain Behav Immun*. 2002;16(6):774–84. [PubMed: 12480506]
64. Aziz N Measurement of Circulating Cytokines and Immune-Activation Markers by Multiplex Technology in the Clinical Setting: What Are We Really Measuring? *For Immunopathol Dis Therap*. 2015;6(1–2):19–22.
65. Koob GF, Schulkin J. Addiction and stress: An allostatic view. *Neurosci Biobehav Rev*. 2018.
66. Kwako LE, Koob GF. Neuroclinical Framework for the Role of Stress in Addiction. *Chronic Stress* (Thousand Oaks). 2017;1.
67. Fox HC, Sinha R. Sex differences in drug-related stress-system changes: implications for treatment in substance-abusing women. *Harvard review of psychiatry*. 2009;17(2):103–19. [PubMed: 19373619]
68. Breese GR, Sinha R, Heilig M. Chronic alcohol neuroadaptation and stress contribute to susceptibility for alcohol craving and relapse. *Pharmacology & therapeutics*. 2011;129(2):149–71. [PubMed: 20951730]
69. Fox HC, Bergquist KL, Hong KI, Sinha R. Stress-induced and alcohol cue-induced craving in recently abstinent alcohol-dependent individuals. *Alcoholism, clinical and experimental research*. 2007;31(3):395–403.
70. Sinha R, Fox HC, Hong KI, Hansen J, Tuit K, Kreek MJ. Effects of adrenal sensitivity, stress- and cue-induced craving, and anxiety on subsequent alcohol relapse and treatment outcomes. *Archives of general psychiatry*. 2011;68(9):942–52. [PubMed: 21536969]
71. Jayaram-Lindström N, Ericson M, Steensland P, Jerlhag E. Dopamine and Alcohol Dependence: From Bench to Clinic In: Meil WM, Ruby CL, editors. *Recent Advances in Drug Addiction Research and Clinical Applications*: IntechOpen; 2016.
72. Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Childress AR, et al. Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2006;26(24):6583–8.
73. George O, Le Moal M, Koob GF. Allostasis and addiction: role of the dopamine and corticotropin-releasing factor systems. *Physiol Behav*. 2012;106(1):58–64. [PubMed: 22108506]
74. Koob GF, Le Moal M. Review. Neurobiological mechanisms for opponent motivational processes in addiction. *Philos Trans R Soc Lond B Biol Sci*. 2008;363(1507):3113–23. [PubMed: 18653439]
75. Blaine SK, Nautiyal N, Hart R, Guarnaccia JB, Sinha R. Craving, cortisol and behavioral alcohol motivation responses to stress and alcohol cue contexts and discrete cues in binge and non-binge drinkers. *Addiction biology*. 2018.
76. Lovallo WR, Cohoon AJ, Acheson A, Sorocco KH, Vincent AS. Blunted stress reactivity reveals vulnerability to early life adversity in young adults with a family history of alcoholism. *Addiction*. 2018.
77. Foulds JA, Adamson SJ, Boden JM, Williman JA, Mulder RT. Depression in patients with alcohol use disorders: Systematic review and meta-analysis of outcomes for independent and substance-induced disorders. *Journal of affective disorders*. 2015;185:47–59. [PubMed: 26143404]
78. Hesselbrock V, Bauer LO, Hesselbrock MN, Gillen R. Neuropsychological factors in individuals at high risk for alcoholism. *Recent Dev Alcohol*. 1991;9:21–40. [PubMed: 1758985]
79. Rounsaville BJ, Dolinsky ZS, Babor TF, Meyer RE. Psychopathology as a predictor of treatment outcome in alcoholics. *Archives of general psychiatry*. 1987;44(6):505–13. [PubMed: 3579499]
80. Greenfield SF, Weiss RD, Muenz LR, Vagge LM, Kelly JF, Bello LR, et al. The effect of depression on return to drinking: a prospective study. *Archives of general psychiatry*. 1998;55(3):259–65. [PubMed: 9510220]
81. Ilgen M, Moos R. Deterioration following alcohol-use disorder treatment in project MATCH. *J Stud Alcohol*. 2005;66(4):517–25. [PubMed: 16240559]

82. Curran GM, Kirchner JE, Worley M, Rookey C, Booth BM. Depressive symptomatology and early attrition from intensive outpatient substance use treatment. *J Behav Health Serv Res*. 2002;29(2):138–43. [PubMed: 12032971]
83. Gamble SA, Conner KR, Talbot NL, Yu Q, Tu XM, Connors GJ. Effects of pretreatment and posttreatment depressive symptoms on alcohol consumption following treatment in Project MATCH. *J Stud Alcohol Drugs*. 2010;71(1):71–7. [PubMed: 20105416]
84. Fox HC, Hong KI, Siedlarz KM, Bergquist K, Anderson G, Kreek MJ, et al. Sex-specific dissociations in autonomic and HPA responses to stress and cues in alcohol-dependent patients with cocaine abuse. *Alcohol and alcoholism*. 2009;44(6):575–85. [PubMed: 19793926]

Fig. 1:

Alcohol dependent individuals reported significantly higher craving immediately following stress presentation compared with controls ($p = .006$). In both the controls (Fig 1a) and alcohol dependent group (Fig. 1b), significantly higher elevations in craving were reported immediately following stress imagery compared with neutral imagery exposure. In the alcohol group, craving elevation immediately following stress exposure was also significantly higher than craving at all other recovery time-points ($p < .0001$ in all cases). Y-axis shows change from baseline data.

Fig. 2: Social drinkers demonstrate elevated IL-1ra following exposure to stress imagery (red line) compared with exposure to neutral imagery (black line) (Fig 2a.). This condition variation in IL-1ra was not observed in the alcohol dependent group (Fig. 2b). Y-axis shows change from baseline data.

Fig. 3:

A main effect of Alcohol Dependence was observed ($p=.002$). Alcohol dependent individuals (shown in red) demonstrated suppressed IL-6 response relative to socially drinking controls (shown in black) across both neutral (Fig.3a) and stress (Fig.3b) imagery conditions. Y-axis shows change from baseline data.

Fig. 4:

Socially drinking non-depressive controls show a greater TNF α response to stress (red line) compared with neutral (black line) imagery (Fig. 4a). Neither socially drinking depressive individuals (Fig. 4b), nor non-depressive alcohol dependent individuals (Fig. 4c) nor depressive alcohol dependent individuals (Fig. 4d) show a significant difference in TNF α response to stress (red line) compared with neutral (black line) imagery. Y-axis shows change from baseline data in all cases.

Fig. 5:

Alcohol dependent individuals show greater basal levels of TNFR1 compared with socially drinking controls across laboratory challenge day 1 (white bar, $p=.03$) and day 2 (striped bar, $p<.02$) (Fig. 5a). Following exposure to imagery, non-depressive individuals show a greater TNFR1 response to stress imagery (red line) compared with neutral (black line) imagery ($p=.05$) (Fig.5b). This effect of condition was not observed in the non-depressive individuals (Fig 5c). Y-axes show change from baseline data.

Fig. 6: Stress-related cytokine adaptations and relapse factors

a: Data presented in all alcohol dependent individuals. Low TNFR1 response to stress imagery predicts a greater maximum number of drinks consumed on any one occasion in both non-depressive (red circles) and depressive (black circles) alcohol dependent individuals ($p = .02$).

b: Data presented in all alcohol dependent individuals. Hazard ratios plotted as a function of TNFR1 levels following exposure to stress imagery. After co-varying for depressive symptomatology (CES-D), estimated hazard functions were presented for individuals demonstrating low TNFR1 levels following stress (red line) and high TNFR1 levels following stress (black line). A median split was used to determine low Vs high response groups. Cumulative relapse risk is shown on the Y-axis and the number of days to relapse are shown on the X-axis. Hence the estimated hazard ratio [HR = 2.8, 95% CI, 1.11–7.19] indicates that the cumulative risk of return to drinking from Day 1 of treatment discharge to Day 57, is 2.8 time greater in the low TNFR1 group Vs the high TNFR1 group.

c: Data presented in all alcohol dependent individuals. Low TNF α response to stress imagery predicts a greater average number of drinks consumed on any one occasion in the depressive alcohol dependent individuals ($p < .04$) (black circles). A trend only was observed in the non-depressive alcohol dependent group (red circles).

d: Data presented in all alcohol dependent individuals. Low IL-6 response to stress imagery predicts a greater number of days spent drinking following discharge from inpatient

treatment in both non-depressive (red circles) and depressive (black circles) alcohol dependent individuals ($p=.04$)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1:

Demographics & Substance Use

N = 70	Social Drinkers (n = 37)		Alcohol Dependent (n = 33)		p	SE
	Low Depressive Symptoms (n=21)	High Depressive Symptoms (n=16)	Low Depressive Symptoms (n=12)	High Depressive Symptoms (n=21)		
Depression Score (CES-D)	5.8 ± .21	20.3 ± .60	6.75 ± .25	23.88 ± .54	<.0001	(High Dep) Vs (Low Dep)
Gender (% male)	62%	38%	86%	84%	ns	
Age (years)	31.9 ± 2.2	35.7 ± 2.4	38.7 ± 2.6	39.1 ± 2.4	<.04	
Education (years)	15.6 ± 0.6	14.1 ± 0.7	12.9 ± 0.4	13.3 ± 0.4	.002	(SD) Vs (AD)
Race (%)						
African Am.	47.6%	37.5%	35.7%	47.4%	ns	
Caucasian	38.1%	43.8%	64.3%	52.6%		
Hispanic	4.7%	12.5%	0	0		
Other	9.5%	6.3%	0	0		
Basal IL-6 (pg/mL)	1.98 ± 2.6	1.78 ± 0.9	2.25 ± 1.5	1.88 ± 1.4	ns	
Basal IL-1ra (pg/mL)	445.12 ± 559.4	417.56 ± 238.9	346.49 ± 171.7	440.72 ± 332.6	ns	
Basal TNFα (pg/mL)	1.38 ± 0.4	1.16 ± 0.7	1.27 ± 0.4	1.82 ± 1.8	ns	
Basal TNFR1 (pg/mL)	990.74 ± 209.6	921.41 ± 160.2	1077.2 ± 238.2	1149.8 ± 280.6	<.02	(SD) Vs (AD)
Substance Use (Avg. no. of days used in past 90)						
Alcohol	18.2 ± 2.5	15.4 ± 2.9	38.5 ± 8.3	46.5 ± 6.1	>.0001	(SD) Vs (AD)
Cigarettes	8.9 ± 5.9	17.9 ± 9.2	40.3 ± 11.9	44.6 ± 7.5	.001	(SD) Vs (AD)
Cocaine	0.05 ± 0.05	0.0 ± 0.0	0.07 ± 0.07	2.1 ± 1.8	ns	
Marijuana	0.07 ± 0.07	0.1 ± 0.1	0.9 ± 0.8	8.2 ± 5.1	ns	
Substance Use (Avg. use per day in past 90)						(SD) Vs (AD)
Alcohol (drinks)	0.5 ± 0.1	0.4 ± 0.1	4.0 ± 1.6	5.6 ± 1.6	>.0001	
Cigarettes	3.7 ± 2.5	2.6 ± 1.2	6.5 ± 1.1	5.1 ± 1.1	ns	
Cocaine (grams)	0.02 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	ns	
Marijuana (joints)	0.1 ± 1.1	0.01 ± 0.0	0.9 ± 0.07	0.6 ± 0.3	ns	