Behavioural Brain Research 312 (2016) 1–8

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Alcohol intake alters immune responses and promotes CNS viral persistence in mice

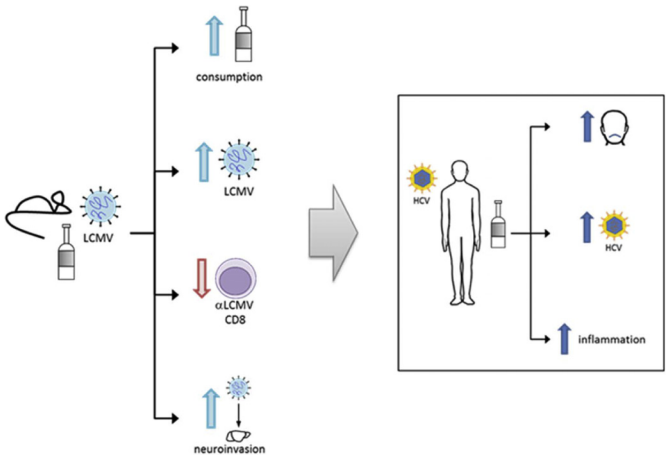
Jennifer M. Loftis[a,b,*], Jonathan Taylor[a,b], Hans-Peter Raué[c], Mark K. Slifka[c], Elaine Huang[a,b]

[a] Research & Development Service, Veterans Affairs Portland Health Care System, 3710 SW U.S. Veterans Hospital Rd., Portland, OR 97239, USA
[b] Department of Psychiatry, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd., Portland, OR 97239, USA
[c] Division of Neuroscience, Department of Molecular Microbiology and Immunology, Oregon National Primate Research Center, Oregon Health & Science University, 505 NW 185th Avenue, Beaverton, OR 97006, USA

HIGHLIGHTS

- LCMV clone 13 infection is associated with increased alcohol intake.
- Alcohol consumption is associated with delayed viral clearance.
- LCMV-specific CD8$^+$ T cell frequencies are reduced in mice with alcohol exposure.
- Alcohol intake may contribute to CNS viral persistence in the context of a chronic viral infection.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 April 2016
Received in revised form 1 June 2016
Accepted 3 June 2016
Available online 4 June 2016

Keywords:
Blood brain barrier
Ethanol
Hepatitis C virus
Lymphocytic choriomeningitis virus
Neurotoxicity
T-cells

ABSTRACT

Chronic hepatitis C virus (HCV) infection leads to progressive liver disease and is associated with a variety of extrahepatic effects, including central nervous system (CNS) damage and neuropsychiatric impairments. Alcohol abuse can exacerbate these adverse effects on brain and behavior, but the molecular mechanisms are not well understood. This study investigated the role of alcohol in regulating viral persistence and CNS immunopathology in mice infected with lymphocytic choriomeningitis virus (LCMV), a model for HCV infections in humans. Female and male BALB/c mice (n = 94) were exposed to alcohol (ethanol; EtOH) and water (or water only) using a two-bottle choice paradigm, followed one week later by infection with either LCMV clone 13 (causes chronic infection similar to chronic HCV), LCMV Armstrong (causes acute infection), or vehicle. Mice were monitored for 60 days post-infection and continued to receive 24-h access to EtOH and water. Animals infected with LCMV clone 13 drank more EtOH, as compared to those with an acute or no viral infection. Six weeks after infection with LCMV clone 13, mice with EtOH exposure evidenced higher serum viral titers, as compared to mice without EtOH exposure.

Abbreviations: AST, aspartate aminotransferase; BBB, blood brain barrier; CNS, central nervous system; EtOH, ethanol; HCV, hepatitis C virus; LCMV, lymphocytic choriomeningitis virus; PFU, plaque forming units.

* Corresponding author at: VA Portland Health Care System (R&D 16), 3710 SW US Veterans Hospital Rd Portland, OR 97239, USA.
E-mail addresses: jennifer.loftis2@va.gov, loftisj@ohsu.edu (J.M. Loftis), taylorjo@ohsu.edu (J. Taylor), raueh@ohsu.edu (H.-P. Raué), slifkam@ohsu.edu (M.K. Slifka), huange@ohsu.edu (E. Huang).

http://dx.doi.org/10.1016/j.bbr.2016.06.006
0166-4328/Published by Elsevier B.V.

EtOH intake was also associated with reductions in virus-specific $CD8^+$ T cell frequencies (particularly $CD11a^{hi}$ subsets) and evidence of persistent CNS viremia in chronically infected mice. These findings support the hypothesis that EtOH use and chronic viral infection can result in combined toxic effects accelerating CNS damage and neuropsychiatric dysfunction and suggest that examining the role of EtOH in regulating viral persistence and CNS immunopathology in mice infected with LCMV can lead to a more comprehensive understanding of comorbid alcohol use disorder and chronic viral infection.

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1. Introduction

In addition to being hepatotropic, HCV is also lymphotrophic, and it is the virus' ability to infect lymphocytes that appears to contribute to the etiology of HCV's extrahepatic symptoms, including central nervous system (CNS) abnormalities and neuropsychiatric impairments [1,2]. Fatigue, mood disturbances, and cognitive dysfunction are frequent in patients with chronic HCV infection, including those who have mild liver disease [3–6]. These findings raise the possibility that HCV infection of the brain could be directly related to psychiatric and cognitive impairments, as has been demonstrated with other chronic viral infections [*e.g.*, human immunodeficiency virus (HIV) [7]]. Increasingly, reports of HCV infection of the CNS [8–11] are allowing investigators to test the theory that HCV neuroinvasion may induce CNS inflammation, brain dysfunction, and behavioral deficits (*e.g.*, [12]).

Alcohol and HCV are hypothesized to act synergistically to accelerate extrahepatic consequences, including neuropsychiatric sequelae—symptoms that adversely affect quality of life, potentially alter the courses of disease, and make the addiction and viral infection more challenging to treat [13]. Shared molecular mechanisms underlying the pathological interactions between alcohol and HCV involve: 1) altered T cell responses, 2) compromised blood brain barrier (BBB) function, 3) modulation of glial cell activation, cytokine production and immune signaling, 4) neuronal loss, 5) myelin damage, and 6) brain region-specific metabolic abnormalities [2,6,14–18]. Recent studies from our and other laboratories show that either chronic HCV or prolonged use of alcohol and other drugs of abuse augment inflammatory responses (*e.g.*, via T cell mediated mechanisms) and result in increased pro-inflammatory activation and impaired neuropsychiatric function, which has resulted in a paradigm shift linking immune response to addictive behavior (*e.g.*, [19,20]). The potential synergistic effects of a chronic viral infection and alcohol exposure on CNS immunopathology and behavior are not well defined. Given that the prevalence of HCV infection is up to 30-fold higher in alcoholics compared with the general population [21,22], a greater understanding of the mechanisms through which alcohol abuse alters immune and CNS response to chronic infection is needed.

Lymphocytic choriomeningitis virus (LCMV) clone 13 has been postulated as a murine model for chronic viral infections such as HCV in humans [23]. The LCMV clone 13 model has led to the development of experimental therapies to treat persistent infections, including HCV [24]. Similar to HCV and HIV, LCMV infection also has effects on brain and behavior. In mice, LCMV injected (either systemically or centrally) at birth or as adults results in long-lasting CNS and behavioral abnormalities, including cognitive deficits [25–27]; however, to date, there have been no studies investigating the effects of alcohol on viral persistence and CNS immunopathology in LCMV-infected mice. Despite considerable prevalence of alcohol abuse and concurrent HCV infection [28], few studies have examined mechanisms that potentially contribute to heightened burden of disease co-morbidity *in vivo*. Through human studies [29,30] and the establishment of an animal model of co-morbid viral infection and alcohol abuse, our goal is to identify specific mechanisms by which chronic viral infection and alcohol induce abnormalities in T cell function and contribute to persistent neuropsychiatric impairments.

2. Materials and methods

2.1. Animals and experimental design

The house mouse (*Mus musculus*) represents the natural host for LCMV and provides one of the best-characterized models for studying antiviral T cell responses [31,32]. In three experiments, female (n = 24) and male (n = 70) BALB/c mice [purchased from the Jackson Laboratories (Bar Harbor, ME); average baseline body weights of 18.78 g and 22.39 g, respectively] were singly-housed (with environmental enrichment) and exposed to alcohol (ethanol; EtOH) and water (or water only) using a two-bottle choice paradigm, followed one week later by infection with either LCMV clone 13 or LCMV Armstrong (an LCMV strain that results in acute infection). Unlike LCMV Armstrong, which causes an acute infection that is cleared within 8-10 days, LCMV clone 13 establishes a chronic infection that persists for one to three months [33]. Control mice were sham infected with vehicle. Sample sizes consisted of 3–6 mice per treatment group, per experiment. Mice were monitored for 60 days post-infection and continued to receive 24-h access to EtOH and water. Blood samples were collected weekly post-infection to assess changes in viral load liver and liver function over time. Mice were humanely euthanized at the end of the experiments for the collection of splenocytes, blood, and brain samples. All experimental procedures were approved by the Oregon Health & Science University and Veterans Affairs Portland Health Care System Institutional Animal Care and Use Committees. Experiments complied with the ARRIVE guidelines [34] and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. EtOH intake during 24-h free access, two-bottle choice paradigm

After habituation (water only in both tubes), mice were offered 3% EtOH (v/v) versus water for 4 days. Immediately following 3% EtOH, a choice between 6% (v/v) EtOH and water was offered for 4 days, then 9% (v/v) EtOH for 4 days, then 12% (v/v) EtOH for 4 days, then 15% (v/v) EtOH for 4 days, and then 20% (v/v) EtOH for the duration of the experiment (40 days of 20% EtOH). To facilitate 20% EtOH consumption, mice were exposed to a modified sucrose-fading procedure [35,36]. During the sucrose-fading procedure, mice were offered sucrose in EtOH drinking tubes according to the following schedule: 10% sucrose for 4 days, 5% sucrose for 4 days, and 2% sucrose for the remainder of the experiment. Control animals (drinking water only) received sucrose in one of their water tubes according to the same schedule. Water and EtOH consumption volumes were recorded daily.