Review

ETHANOL CONSUMPTION AND INNATE NEUROIMMUNITY

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ABSTRACT

*Emerging researches from human and animal models have shown the role of ethanol in innate immune system modulation, particularly in the central nervous system. The activation of receptors of the innate immunity, Toll-like receptors and nucleotide-binding oligomerization domain-like (NOD-like) receptors, triggers the signaling pathways that bring to the production of pro-inflammatory cytokines and chemokines, which, in turn, provokes neuroinflammation and neural damage. The neuro-immune system response to ethanol intake, in specific brain regions such as amygdala, hippocampus and frontal cortex, is involved in addiction and in behavioural deficits observed in alcoholism. In murine models, the knockout for Toll-like or NOD-like receptors abolishes most of the effects of ethanol on the immune system and preserves these mice from neural damage, neuroinflammation and alcohol dependence. Molecular targeting of immune system pathways is a new and promising area of research for the discovery of new biomarkers for neuroinflammation and for the development of novel pharmacotherapies in order to treat neurological and behavioural consequences of ethanol addiction. **Biomed Rev 2017; 28: 49-61.***

Keywords: ethanol addiction, neuroinflammation, neuroimmunity, Toll-like receptors, NOD-like receptors, inflammasomes

INTRODUCTION

Ethanol modifies the immune inflammatory signals in the central nervous system (CNS) as well as in the rest of the body, so the immune system is strongly modulated by alcohol intake (1, 2). Alcohol consumption may disrupt tight junctions in gut epithelium, allowing the bacterial lipopolysaccharides (LPS), part of the external membrane of the Gram-negative bacteria, to leak from the gut, where they are usually confined, and pass into the blood stream (3, 4). Lipopolysaccharides (also termed endotoxin) activates the immune receptor 4 in liver Kupffer cells promoting an alcohol-induced liver

inflammation enhanced by immune cells; monocytes, macrophages, T lymphocytes and dendritic cells are stimulated to secrete pro-inflammatory cytokines such as interleukins (IL), IL-1b, IL-6 and tumor necrosis factor-alpha (TNF- α) (1). Indeed, this pro-inflammatory cascade (5), induced by alcohol in the periphery, stimulates both the adaptive and the innate immune system (3, 6). Lymphocytic cells that specifically recognize and memorize invading pathogens, producing antibodies, constitute the adaptive immune system that creates the immunological memory to defend the organism from future infections. Instead, in innate immunity, pathogens trigger a non-specific immune response that, secreting

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pro-inflammatory cytokines and chemokines stimulate CNS neuroimmune cells (microglia and astrocytes) to further produce cytokines (7).

Pro-inflammatory cytokines may use different routes to access the brain: *via* afferent vagal fibres (8), through leaky regions of the blood-brain barrier (area postrema), by active cytokine-specific transporter or through second messenger molecules of CNS endothelia (9, 10).

THE ACTIVE IMMUNE DEFENCE IN THE CNS: MICROGLIA AND ASTROCYTES

Microglia, a type of glial cells in the CNS and spinal cord, actively manages immune defence in CNS; along with the resident macrophages and astrocytes, microglia recognizes and destroys infectious agents and prevents the damage to the neural tissue (11–13). Microglia cells also control the overall brain homeostasis, as they continuously scavenge the CNS looking for damaged neuron cells and pathogens (12). The multiplicity of microglia functions are made possible by their exceptional plasticity, these cells can change their morphology and phenotype to respond to local chemical signals (14, 15). In absence of pathogens or dying cells microglia adopt the “resting form” that consists in a small cellular body with long branching processes, that constantly scan the surrounding area to sense even small changes in physiological conditions (16, 17).

When microglia detects an endogenous or exogenous insult, it becomes activated and undergoes changes in morphology and gene expression through a response gradient that goes from a ramified form to a totally active phagocytic form (18, 19). In this state, microglia quickly uptakes the major histocompatibility complex (MHC) class I/II proteins and becomes an efficient antigen presenter for T-cell activation. Also in this form these cells can destroy foreign materials and interact with astrocytes, other components of neuroimmune system able to secrete cytokines after activation (20,21).

THE TOLL-LIKE RECEPTOR SYSTEM

Lipopolysaccharides as well as other microbe-derived evolutionarily conserved molecules are recognized by the innate immune Toll-like receptors (TLR; from 1995 Nobel Prize winner Christiane Nüsslein-Volhard's 1985 exclamation *Das ist ja Toll* - that's great/amazing). The TLRs belong to an evolutionary conserved family of receptors involved in the protection against microbial infections (22). The family of

these receptors includes 11 members (TLR1-11) in humans, the most studied of which is TLR4. This kind of receptors are pattern recognition receptors (PRRs) that identify the pathogen-associated molecular pattern (PAMPs) and molecular signatures of different classes of bacteria (23). The cytoplasmic nucleotide binding domain leucine-rich repeat containing receptors (known as NLRs or nucleotide-binding oligomerization domain-like receptors, in brief NOD-like receptors) can also activate an innate immune response, recognizing circulating pathogens. Both, cytoplasmic NLRs and membrane TLRs react not only to pathogens but also to host-cell derived molecules, called damage-associated molecular patterns (DAMPs, danger signals, or alarmins), which are generic markers for damage (24). Among them, there are the IL-33 and the High-Mobility Group Box 1 (HMGB1) proteins, which, when activated, become hyperacetylated on lysine residues, translocate from nucleus to cytosol where bind to different receptors and finally activate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) (25, 26). The role of alarmins in eliciting the “sterile inflammation” without the presence of pathogens is crucial in the CNS neurodegenerative diseases (27).

INTERACTION BETWEEN ETHANOL AND THE IMMUNE RESPONSE

The first demonstration that ethanol can activate the immune signalling pathway and the synthesis of pro-inflammatory cytokines in the brain was obtained by experimental studies of Valles (28). The up-regulation of NF- κ B and the expression of pro-inflammatory proteins induced by ethanol was observed *in vitro* in rat brain slices (29) and in primary cultured astroglial and microglial cells (30, 31). Ethanol can modulate the signalling of different immunoreceptors, the most studied are TLR4 and NLRP3 (NACHT, LRR and PYD domains-containing protein 3). The link between them is the activation of NF- κ B (32). The receptor signalling associates with its internalization and trafficking (33) and through the activation of MAPK and transcription factors such as NF- κ B or AP-1. The subsequent production of cytokines such as pro-IL-1 β , IL-6, IL-8 and TNF- α , chemokines, like the monocyte chemoattractant protein-1 (MCP-1), and inflammatory mediators such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), leads, finally, to neuroinflammation (30, 31, 34).

The activation of receptor signalling starts with a “priming signal” of TLR4 that generates, via induction of NF- κ B, the synthesis of pro-IL-1 β . This signal, along with reactive

oxygen species (ROS) production, elicited by the action of ethanol on mitochondria, generates a second signal that activates the formation of a big multiprotein complex called inflammasomes that mediates the activation of caspase-1 that, in turn, produces and releases the mature form of IL-1 β (35). The crucial role of TLR4 in inflammatory response and glial activation induced by ethanol was confirmed *in vitro* using siRNA. Interference against TLR4 abolished inflammatory response *in vivo*, in mice models of chronic ethanol intake, and *in vitro*, in cultured glial cells or in glial cells from TLR4 knockout mice (34, 35). In these models, TLR4 knockout mice resulted to be protected from neuroinflammation, myelin disruption and apoptosis in cerebral cortex, mediated by induction of MAPK and NF- κ B (36–40).

Alcohol also impairs the pathways of ubiquitin-proteasome and autophagy-lysosome: ubiquitinated proteins accumulate contributing to brain damage and neurodegeneration (41). The activation of TLR4/NLRP3 inflammasomes promotes leukocyte infiltration, compromises the blood-brain-barrier integrity (40) and inhibits hippocampal neurogenesis (42). The high levels of cytokines induced by ethanol remain in the brain for a long period after alcohol exposure whereas in periphery cytokines return more rapidly to normal levels (29). This long-term effect of ethanol in the neuroimmune system was also observed in animals exposed to alcohol in adolescence (43, 44) and in postmortem alcoholic brain (45, 46), where, in addition, blood-brain-barrier integrity alterations have been found (47). Moreover, ethanol induces activation (through acetylation) and extracellular release of HMGB1, its pro-inflammatory action is mediated by the activation of TLR4. Ethanol promotes also the expression of NLRP3 in neurons and in astrocytes; the increased expression in these cells was also observed in post-mortem alcoholic brains.

ACUTE AND CHRONIC ETHANOL CONSUMPTION EFFECTS

The immunomodulatory regulation of ethanol produces opposite effects depending on acute or chronic alcohol drinking (5), but the exact molecular mechanism behind this effect is still unclear. In animal models exposed to a pathogen, acute alcohol administration decreases the levels of IL-6, TNF- α and IL-1 β (48). LPS stimulated human monocytes or binge drinking animal models have shown that acute alcohol administration induces TLR4/LPS tolerance, which develops through the up-regulation of the nuclear protein Bcl-3 that binds to the p50 subunit of the nuclear factor NF- κ B (32A).

This binding blocks the transcription of NF- κ B regulated genes, that include pro-inflammatory cytokines (49), while stimulates the transcription (50) of apoptotic genes (51). In contrast, chronic ethanol exposure converts the anti-inflammatory response to pro-inflammatory through NF- κ B activation and TNF- α induction (50,52,53).

While the immune response to ethanol is a sex-independent event, the extent of neuroinflammation, the levels of inflammatory markers such as iNOS and COX-2, the neuronal loss and the activation of caspase-1 are much more pronounced in females (38).

HYPOTHALAMIC-PITUITARY-ADRENAL-AXIS (HPA)

Dysregulation of HPA and imbalance of glucocorticoid receptor are commonly observed during alcohol administration; the severity of these effects depends on the stage of alcoholism and on the ethanol dose (54). In chronic ethanol intake, basal adrenocorticotrophic hormone (ACTH) levels are elevated and the stress-induced release of cortisol and corticotrophins is inhibited (55). During withdrawal, the negative behavioural effects and the dysphoric symptoms such as tremor, anxiety and agitation arise from an immune-mediated activation of brain stress circuitry, because of the inflammatory cytokines that are strong inducers of the hypothalamic corticotrophin-releasing factor (CRF) (56, 57).

Glucocorticoids produced by inflammatory cytokines dysregulate the catabolic pathway of tryptophan, the serotonin precursor, activating two different enzymes: the hepatic tryptophan 2,3-dioxygenase (TDO) (58, 59) and indolamine 2,3 dioxygenase (IDO) in the brain. Both of them degrade tryptophan along the neurotoxic kynurenine pathway (60, 61). The activation of this metabolic pathway causes a depletion of serotonin, while the production of metabolites of kynurenine such as anthranilic and quinolinic acid by brain microglia, that acts as NMDA receptor agonists, causes neurotoxic effects (62). Dysregulation of tryptophan metabolism lasts for a long period during abstinence and induces physiological responses with mood disorders and emotional and behavioural lability, traits in common with depressive disorders (63–65).

NEUROIMMUNE SIGNALLING IN ADDICTION AND ALCOHOL DRINKING

Many pieces of evidence have shown that ethanol-induced neuroimmune signalling is involved in the progression of addiction (66). The alcohol-dependent alteration of the limbic system and frontal cortex imbalances long-term po-

tentiation and long-term depression and reduces neuronal plasticity; particularly, binge drinking is followed by a long term default in learning, (67, 68) emotive response and memory, typical of alcoholism (7, 67–70) (Fig. 1).

Zou and Crews, using an *ex vivo* model of organotypic hippocampal-entorhinal cortex brain slice culture, showed that 4 days of treatment with 100mM ethanol decreased neurogenesis, cellular proliferation (73) and caused an im-

balance of neuroimmune/neurotrophic factors. Augmented pro-inflammatory cytokine IL-1 β increased the expression of cAMP response element binding protein (CREB), involved in the activation of many different transcription factors, while brain-derived neurotrophic factor (BDNF) levels were reduced. The blocking of IL-1 β with a neutralizing antibody or administration of inhibitors of NF- κ B reversed the ethanol-induced inhibition of neurogenesis. Neurogen-

Figure 1. Neuroimmune molecular pathway in addiction.

The alcohol-mediated stimulation of microglia and astrocytes in the central nervous system activates the neuroimmune receptors TLRs and RAGE and the alarmin HMGB1. This alcohol-mediated stimulation of microglia and astrocytes downregulates CREB and increases the expression of NF- κ B that in turn activates the transcription of innate immune genes. This pathway produces multiple cycles of neuroimmune gene activation which lead to amplification and spreading of neuroinflammation causing neuronal cell death and up-regulation of the limbic system circuitry. This neuroimmune gene activation elicits also negative effects on anxiety and craving, frontal cortex down-regulation with changes in impulsivity, progressive loss of attention and reduced decision making. All these behavioural modifications culminate in addiction (adapted from 45, 66).

esis and hippocampal stem cells proliferation were also impaired in vivo models of chronic binge drinking (74). Anti-depressive treatment reversed the depression-like behaviour and the inhibition of neurogenesis (71, 73).

Chronic alcohol intake determines a permanent activation of microglia and astrocytes (74) that, at the end, results in neuronal damage and cell death (28). Interestingly, in a murine model, administration of prednisone, a synthetic glucocorticoid, was found to increase anxiety-like behaviour and to induce microglial proliferation in frontal cortex and hippocampus (77). These evidences that represent a link between neuroimmune signalling, endocrine system and behavioural alterations, can be at the basis of novel strategies for the treatment of addictions. Moreover, knockout mice models demonstrated that modulation of alcohol intake relies on TLRs/NLRs signalling. TLR4 knockout mice are preserved from up-regulation of brain cytokines and chemokines, do not show cognitive dysfunction or anxiety during withdrawal (after chronic ethanol administration) and manifest a less marked preference for alcohol intake (33, 78–81). Similar results were obtained in knockout mice models for immunorelated genes coding for proteins produced by microglia and astrocytes (82). Recent studies in murine models, employing the infusion *in vivo* of siRNA vectors targeting TLR4/MCP-1 in specific brain regions such as central nucleus of the amygdala or ventral tegmental area, demonstrated that the block of this signalling blunted binge drinking (79). Moreover, a protein crosstalk between TLR4 and CRF was described: alcohol drinking increases the expression of CRF that, in turn, exert a feedback regulation on TLR4/MCP-1 signalling, this mechanism can potentially contribute to the switch to alcohol dependence (79).

CYTOKINES AS BIOMARKERS

The clinical diagnosis of alcoholism needs the development of reliable biomarkers that can be indicators of alcohol use and abuse. An emerging research area concerns the use of plasma or serum cytokine levels as potential biomarkers of alcoholism and alcohol-induced tissue damage (83). To increase sensibility and specificity, serum and plasma levels of cytokines may be tested together with other markers already in use: ethyl glucuronide (EtG) (84,85), gamma-glutamyltransferase (GGT) (86), alanine aminotransferase (ALT), aspartate aminotransferase (AST) (87), mean corpuscular volume (MCV) (88), carbohydrate-deficient transferrin (CDT)

(89), 5-hydroxytryptophol (5HTOL) (90), phosphatidylethanol (PEth) (89).

In neurological diseases like Alzheimer's (AD) and Parkinson's diseases high levels of inflammatory cytokines were observed. Björkqvist and colleagues identified a panel of plasma proteins, including cytokines, which enabled the accurate diagnosis of AD with an accuracy of 90% (92). The development of biomarkers panels (cytokines) as a signature of neurological disease may provide a strong rationale to extend this strategy to alcohol abuse, considering that alcoholism also causes neuroinflammation and neurodegeneration (75). Indeed, TNF- α IL-1 and IL-6 were found to be elevated in both chronic and acute alcohol-induced liver disease (93) and TNF- α levels were higher in hospitalized alcoholics than in general population (94, 95). These higher levels of circulating cytokines, which correlate with liver dysfunction, were also present in alcoholics without liver disease. A biomarker panel, comprehensive of other circulating proteins such as growth factors, could improve sensibility of alcohol abuse testing (96–107). Studies on pregnant women consuming alcohol have shown a profound effect of ethanol intake on the concentration of circulating epidermal growth factor and placental growth factor (108). Recent studies have also shown the role of small non coding RNAs as modulators of neuroinflammation and as potential bio-markers in addiction and neurological diseases (109, 110): miR-155 in cerebellum, that is TLR4-dependent and up-regulates TNF- α and MCP-1 levels, is modified by chronic alcohol abuse (111); miR339-5p inhibits neuroinflammation and regulates NF- κ B signalling (112).

NEUROIMMUNE SYSTEM AND BINGE DRINKING

Studies in animal models confirm that occasional and repeated episodes of binge drinking, a common way of drinking alcohol in adolescence, induce modifications in the hippocampus and brain cortex (113, 114), dysfunction of neurogenesis (45) and disorganization in synapses and myelin (80). During adolescence, the still growing brain performs structural and functional remodelling of neural pathway in regions such as cortex, amygdala, hippocampus and nucleus accumbens (115, 116). These regions are crucial for a normal brain maturation from childhood to adulthood (117). The synaptic remodelling during the adolescence, coupled to an increased sensitivity to alcohol toxicity, impairs self-control and goal setting behaviours and can bring to cognitive and behavioural deficits (43, 44, 80, 119) and to a later

development of alcohol dependence (118).

Binge drinking activates the neuroimmune system cells microglia and astrocytes. The release of pro-inflammatory cytokines, chemokines and ROS causes neuroinflammation and neuronal death (120-122). Binge-like ethanol administration in adolescent rats up-regulates TLRs, receptor for advanced glycation end products (RAGE), HMGB1, TNF- α and IL-1 β mRNA in the prefrontal cortex and activates microglia; this activation lasts for a long period after alcohol consumption (6, 33, 123). Damaged neurons release HMGB1 that activates TLR4, RAGE and the signalling pathway that produces cytokines (124). Interestingly, chronic ethanol exposure has been proven to promote the neuronal release of HMGB1, presumably through a decrease in HDAC activity, mediating this way the pro-inflammatory effects of ethanol (124). Other works have shown that mitochondrial ROS produced by ethanol activates TLR4/NLRs response in microglia stimulating HMGB1 release (35, 40). The stability and persistence of HMGB1 secretion define its role of an enduring activator of inflammation damage (113).

In a rat model, maternal binge drinking during gestation and lactation caused motor coordination impairments during the rotarod test and Y maze performance disruptions in adult male offspring (125), supposedly linked to up-regulation of pro-inflammatory signalling (TLR4, NF- κ B p65, NLR protein3, caspase 1, and IL-1 β). Moreover, neuronal loss and down-regulation of structural myelin proteins in prefrontal cortex and hippocampus (125) were also observed. The consequent neuroinflammation might be responsible for the worsening of behavioural deficits observed in fetal alcohol spectrum disorders (FASD). In a rat model of adolescent mice wild-type and knockout for TLR4, Montesinos and colleagues showed that binge-like ethanol drinking promoted the up-regulation of BDNF and FosB genes (through epigenetic changes in the promoter regions) in the medial prefrontal cortex. In these young animals, long-term rewarding and anxiogenic effects that increase alcohol preference were observed, while TLR4-knockout mice were protected from these alterations (43).

PHARMACOLOGICAL APPROACHES MODULATING NEUROIMMUNE SYSTEM ACTIVATION

The involvement of neuroimmune system in alcohol consumption and dependence and its role in neurological damage associated with ethanol abuse have encouraged the development of pharmacological treatments in an attempt to normal-

ize the immune signalling. The aims are to control the short and long-term effects of ethanol intake in adulthood and in adolescence and to fine-tune a more effective immune-based pharmacotherapy. Many anti-inflammatory compounds that treat the neural damage induced by alcohol and reduce microglia activation are under observation. Minocyclines, for example, inhibit microglia activation and reduce ethanol intake in a 2-bottle choice voluntary drinking model (126). Anakinra, an antagonist of IL-1 receptor, blocks the activation of NLRP3 and reduces alcohol-induced sedation in mice models (111, 127). Nonsteroidal anti-inflammatory substances, such as indomethacin, an inhibitor of COX-2, reduces neuroinflammation and behavioural deficits in a binge drinking model of rats receiving alcohol in adolescence (121). Antagonists that block the over-expression of HMGB1, such as glycyrrhizin or ethyl pyruvate, avoid the brain damage induced by chronic alcohol consumption (128). Recently, it was shown that oleoylethanolamide, a compound with anti-inflammatory and neuroprotective properties, was able to stop the neuroimmune signalling in the rat frontal cortex and to improve the behavioural deficits induced by ethanol binge administration (129).

CONCLUSIONS AND PERSPECTIVES

The complex pathology of alcoholism strongly depends on genetic, epigenetic and environmental factors. A deeper knowledge is needed to understand the global pattern of cellular and molecular mechanisms related to alcoholism and to unravel the role of other proteins such as growth factors and adipokines (adipose tissue-derived signaling proteins). In addition, the influence of other components such as life style, nutrition, aging and gender differences need to be further investigated. Polyphenols are a family of compounds consistently present in the human diet. There are different types of polyphenols like tannins, flavonoids and resveratrol. Polyphenols can counteract inflammation because of their action on transcription factors and their inhibitory effect on NF- κ B signalling. Many polyphenols have been studied for their anti-inflammatory and/or antioxidant properties and for their role in regulating neurotrophins levels (130). Their potential therapeutic application against inflammation and oxidative imbalance extends to a large number of conditions affecting different organs, brain included (131). A better comprehension of the protective effect provided by polyphenols might be of primary interest for drug discovery and diet-based prevention of the neuroinflammatory insults associated with

chronic alcohol abuse.

CONFLICT OF INTEREST STATEMENT

The authors certify that they have no affiliations with or involvement in any organization with any financial interest in the subject matter discussed in the present review.

REFERENCES

1. Crews FT, Vetreno RP. Mechanisms of neuroimmune gene induction in alcoholism. *Psychopharmacology (Berl)* 2016;233:1543–1557. DOI:10.1007/s00213-015-3906-1
2. Szabo G, Saha B. Alcohol's effect on host defense. *Alcohol Res Curr Rev* 2015;37:159–170.
3. Mandrekar P, Szabo G. Signalling pathways in alcohol-induced liver inflammation. *J Hepatol* 2009;50:1258–1266. DOI:10.1016/j.jhep.2009.03.007
4. Ferrier L, Bérard F, Debrauwer L, Chabo C, Langella P, Buéno L, *et al.* Impairment of the intestinal barrier by ethanol involves enteric microflora and mast cell activation in rodents. *Am J Pathol* 2006;168:1148–1154. DOI:10.2353/ajpath.2006.050617
5. Szabo G, Mandrekar P. A recent perspective on alcohol, immunity, and host defense. *Alcohol Clin Exp Res* 2009;33:220–232. DOI:10.1111/j.1530-0277.2008.00842.x
6. Vetreno RP, Qin L, Crews FT. Increased receptor for advanced glycation end product expression in the human alcoholic prefrontal cortex is linked to adolescent drinking. *Neurobiol Dis* 2013;59:52–62. DOI:10.1016/j.nbd.2013.07.002
7. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci* 2008;9:46–56. DOI:10.1038/nrn2297
8. Watkins LR, Maier SF, Goehler LE. Cytokine-to-brain communication: a review & analysis of alternative mechanisms. *Life Sci* 1995;57:1011–1026. DOI: 10.1016/0024-3205(95)02047-M
9. Banks WA, Erickson MA. The blood-brain barrier and immune function and dysfunction. *Neurobiol Dis* 2010;37:26–32. DOI:10.1016/j.nbd.2009.07.031
10. Qin L, Wu X, Block ML, Liu Y, Breese GR, Hong J-S, *et al.* Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia* 2007;55:453–62. DOI:10.1002/glia.20467
11. Kreutzberg GW. Microglia, the first line of defence in brain pathologies. *Arzneimittelforschung* 1995;45:357–60.
12. Gehrman J, Matsumoto Y, Kreutzberg GW. Microglia: intrinsic immune effector cell of the brain. *Brain Res Brain Res Rev* 1995;20:269–87. [DOI: 10.1016/0165-0173(94)00015-H]
13. Davis EJ, Foster TD, Thomas WE. Cellular forms and functions of brain microglia. *Brain Res Bull* 1994;34:73–78. DOI:10.1016/0361-9230(94)90189-9]
14. Rock RB, Gekker G, Hu S, Sheng WS, Cheeran M, Lockensgard JR, *et al.* Role of microglia in central nervous system infections. *Clin Microbiol Rev* 2004;17:942–964, table of contents. DOI:10.1128/CMR.17.4.942-964.2004
15. Eggen BJL, Raj D, Hanisch U-K, Boddeke HWGM. Microglial phenotype and adaptation. *J Neuroimmune Pharmacol* 2013;8:807–823. DOI:10.1007/s11481-013-9490-4
16. Ferrer I, Bernet E, Soriano E, del Rio T, Fonseca M. Naturally occurring cell death in the cerebral cortex of the rat and removal of dead cells by transitory phagocytes. *Neuroscience* 1990;39:451–458.
17. Aloisi F. Immune function of microglia. *Glia* 2001;36:165–79. [DOI: 10.1002/glia.1106]
18. Graeber MB, Streit WJ. Microglia: biology and pathology. *Acta Neuropathol (Berl)* 2010;119:89–105. DOI:10.1007/s00401-009-0622-0
19. Graeber MB. Changing face of microglia. *Science* 2010;330:783–8. DOI:10.1126/science.1190929
20. Aschner M, Sonnewald U, Tan KH. Astrocyte modulation of neurotoxic injury. *Brain Pathol Zurich Switz* 2002;12:475–481. DOI: 10.1111/j.1750-3639.2002.tb00465.x
21. Aschner M. Astrocytes as mediators of immune and inflammatory responses in the CNS. *Neurotoxicology* 1998;19:269–281.
22. Medzhitov R, Preston-Hurlburt P, Janeway CA. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature* 1997;388:394–397. DOI:10.1038/41131
23. Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol* 2001;2:675–680. DOI:10.1038/90609
24. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. *J Leukoc Biol* 2007;81:1–5. DOI:10.1189/jlb.0306164
25. Ibrahim ZA, Armour CL, Phipps S, Sukkar MB. RAGE

- and TLRs: relatives, friends or neighbours? *Mol Immunol* 2013;56:739-744. DOI:10.1016/j.molimm.2013.07.008
26. El Mezayen R, El Gazzar M, Seeds MC, McCall CE, Dreskin SC, Nicolls MR. Endogenous signals released from necrotic cells augment inflammatory responses to bacterial endotoxin. *Immunol Lett* 2007;111:36-44. DOI:10.1016/j.imlet.2007.04.011
 27. Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. *Cell* 2010;140:918-934. DOI:10.1016/j.cell.2010.02.016
 28. Vallés SL, Blanco AM, Pascual M, Guerri C. Chronic ethanol treatment enhances inflammatory mediators and cell death in the brain and in astrocytes. *Brain Pathol Zurich Switz* 2004;14:365-371. DOI: 10.1111/j.1750-3639.2004.tb00079.x
 29. Qin L, He J, Hanes RN, Pluzarev O, Hong J-S, Crews FT. Increased systemic and brain cytokine production and neuroinflammation by endotoxin following ethanol treatment. *J Neuroinflam* 2008;5:10. DOI:10.1186/1742-2094-5-10
 30. Blanco AM, Vallés SL, Pascual M, Guerri C. Involvement of TLR4/type I IL-1 receptor signaling in the induction of inflammatory mediators and cell death induced by ethanol in cultured astrocytes. *J Immunol* 2005;175:6893-6899. DOI:10.4049/jimmunol.175.10.6893
 31. Blanco AM, Perez-Arago A, Fernandez-Lizarbe S, Guerri C. Ethanol mimics ligand-mediated activation and endocytosis of IL-1RI/TLR4 receptors via lipid rafts caveolae in astroglial cells. *J Neurochem* 2008;106:625-639. DOI:10.1111/j.1471-4159.2008.05425.x
 32. Guo S, Yang C, Diao B, Huang X, Jin M, Chen L, *et al.* The NLRP3 Inflammasome and IL-1 β Accelerate Immunologically Mediated Pathology in Experimental Viral Fulminant Hepatitis. *PLoS* 2015;11:e1005155. DOI:10.1371/journal.ppat.1005155
 33. Pascual-Lucas M, Fernandez-Lizarbe S, Montesinos J, Guerri C. LPS or ethanol triggers clathrin- and rafts/caveolae-dependent endocytosis of TLR4 in cortical astrocytes. *J Neurochem* 2014;129:448-62. DOI:10.1111/jnc.12639
 34. Fernandez-Lizarbe S, Pascual M, Guerri C. Critical role of TLR4 response in the activation of microglia induced by ethanol. *J Immunol Baltim Md* 2009;183:4733-44. DOI:10.4049/jimmunol.0803590
 35. Alfonso-Loeches S, Ureña-Peralta JR, Morillo-Bargues MJ, Oliver-De La Cruz J, Guerri C. Role of mitochondria ROS generation in ethanol-induced NLRP3 inflammasome activation and cell death in astroglial cells. *Front Cell Neurosci* 2014;8:216. DOI:10.3389/fncel.2014.00216
 36. Alfonso-Loeches S, Guerri C. Molecular and behavioral aspects of the actions of alcohol on the adult and developing brain. *Crit Rev Clin Lab Sci* 2011;48:19-47. DOI:10.3109/10408363.2011.580567
 37. Alfonso-Loeches S, Pascual M, Gómez-Pinedo U, Pascual-Lucas M, Renau-Piqueras J, Guerri C. Toll-like receptor 4 participates in the myelin disruptions associated with chronic alcohol abuse. *Glia* 2012;60:948-64. DOI:10.1002/glia.22327
 38. Alfonso-Loeches S, Pascual M, Guerri C. Gender differences in alcohol-induced neurotoxicity and brain damage. *Toxicology* 2013;311:27-34. DOI:10.1016/j.tox.2013.03.001
 39. Alfonso-Loeches S, Pascual-Lucas M, Blanco AM, Sanchez-Vera I, Guerri C. Pivotal role of TLR4 receptors in alcohol-induced neuroinflammation and brain damage. *J Neurosci J Soc Neurosci* 2010;30:8285-95. DOI:10.1523/JNEUROSCI.0976-10.2010
 40. Alfonso-Loeches S, Ureña-Peralta J, Morillo-Bargues MJ, Gómez-Pinedo U, Guerri C. Ethanol-Induced TLR4/NLRP3 Neuroinflammatory Response in Microglial Cells Promotes Leukocyte Infiltration Across the BBB. *Neurochem Res* 2016;41:193-209. DOI:10.1007/s11064-015-1760-5
 41. Pla A, Pascual M, Renau-Piqueras J, Guerri C. TLR4 mediates the impairment of ubiquitin-proteasome and autophagy-lysosome pathways induced by ethanol treatment in brain. *Cell Death Dis* 2014;5:e1066. [doi:10.1038/cddis.2014.46.]
 42. Zou J, Vetreno RP, Crews FT. ATP-P2X7 receptor signaling controls basal and TNF α -stimulated glial cell proliferation. *Glia* 2012;60:661-73. [DOI: 10.1002/glia.22302]
 43. Montesinos J, Alfonso-Loeches S, Guerri C. Impact of the Innate Immune Response in the Actions of Ethanol on the Central Nervous System. *Alcohol Clin Exp Res* 2016;40:2260-70. [DOI:10.1111/acer.13208]
 44. Montesinos J, Pascual M, Rodríguez-Arias M, Miñarro J, Guerri C. Involvement of TLR4 in the long-term epigenetic changes, rewarding and anxiety effects induced by intermittent ethanol treatment in adolescence. *Brain Behav Immun* 2016;53:159-171. DOI:10.1016/j.bbi.2015.12.006
 45. Vetreno RP, Crews FT. Current hypotheses on the mechanisms of alcoholism. *Handb Clin Neurol* 2014;125:477-

97. DOI:10.1016/B978-0-444-62619-6.00027-6
46. Crews FT, Vetreno RP. Neuroimmune basis of alcoholic brain damage. *Int Rev Neurobiol* 2014;118:315–57. DOI:10.1016/B978-0-12-801284-0.00010-5
47. Rubio-Araiz A, Porcu F, Pérez-Hernández M, García-Gutiérrez MS, Aracil-Fernández MA, Gutierrez-López MD, *et al.* Disruption of blood-brain barrier integrity in postmortem alcoholic brain: preclinical evidence of TLR4 involvement from a binge-like drinking model. *Addict Biol* 2017;22:1103–16. DOI:10.1111/adb.12376
48. 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 Fayettev N* 2004;33:147–55. DOI:10.1016/j.alcohol.2004.08.001
49. Kuwata H, Watanabe Y, Miyoshi H, Yamamoto M, Kai-sho T, Takeda K, *et al.* IL-10-inducible Bcl-3 negatively regulates LPS-induced TNF-alpha production in macrophages. *Blood* 2003;102:4123–4129. DOI:10.1182/blood-2003-04-1228
50. Deak T, Okrainets A, Doremus-Fitzwater TL. Mechanisms of stress-dependent neuroinflammation and their implications for understanding consequences of alcohol exposure. *Neural-Immune Interact. Brain Funct. Alcohol Relat. Disord.*, Springer; 2013, p. 133–66.
51. Barr T, Helms C, Grant K, Messaoudi I. Opposing effects of alcohol on the immune system. *Prog Neuropsychopharmacol Biol Psychiatry* 2016;65:242–51. DOI:10.1016/j.pnpbp.2015.09.001
52. Mandrekar P, Bala S, Catalano D, Kodys K, Szabo G. The opposite effects of acute and chronic alcohol on lipopolysaccharide-induced inflammation are linked to IRAK-M in human monocytes. *J Immunol Baltim Md* 1950 2009;183:1320–7. DOI:10.4049/jimmunol.0803206
53. Laso FJ, Vaquero JM, Almeida J, Marcos M, Orfao A. Production of inflammatory cytokines by peripheral blood monocytes in chronic alcoholism: relationship with ethanol intake and liver disease. *Cytometry B Clin Cytom* 2007;72:408–15. DOI:10.1002/cyto.b.20169
54. Burke HM, Davis MC, Otte C, Mohr DC. Depression and cortisol responses to psychological stress: a meta-analysis. *Psychoneuroendocrinology* 2005;30:846–56. DOI:10.1016/j.psyneuen.2005.02.010
55. Sinha R, Fox HC, Hong K-IA, Hansen J, Tuit K, Kreek MJ. Effects of adrenal sensitivity, stress- and cue-induced craving, and anxiety on subsequent alcohol relapse and treatment outcomes. *Arch Gen Psychiatry* 2011;68:942–52. DOI:10.1001/archgenpsychiatry.2011.49
56. Breese GR, Overstreet DH, Knapp DJ, Navarro M. Prior multiple ethanol withdrawals enhance stress-induced anxiety-like behavior: inhibition by CRF1- and benzodiazepine-receptor antagonists and a 5-HT1a-receptor agonist. *Am Coll Neuropsychopharmacol* 2005;30:1662–1669. DOI:10.1038/sj.npp.1300706
57. Alonso R, Griebel G, Pavone G, Stemmelin J, Le Fur G, Soubrié P. Blockade of CRF1 or V(1b) receptors reverses stress-induced suppression of neurogenesis in a mouse model of depression. *Mol Psychiatry* 2004;9:278–286, 224. DOI:10.1038/sj.mp.4001464
58. Badawy AA-B, Dougherty DM, Marsh-Richard DM, Steptoe A. Activation of liver tryptophan pyrrolase mediates the decrease in tryptophan availability to the brain after acute alcohol consumption by normal subjects. *Alcohol Alcohol Oxf* 2009;44:267–271. DOI:10.1093/alcalc/agp005
59. Branchey L, Branchey M, Shaw S, Lieber CS. Relationship between changes in plasma amino acids and depression in alcoholic patients. *Am J Psychiatry* 1984;141:1212–1215. DOI:10.1176/ajp.141.10.1212
60. Dantzer R, O'Connor JC, Lawson MA, Kelley KW. Inflammation-associated depression: from serotonin to kynurenine. *Psychoneuroendocrinology* 2011;36:426–36. DOI:10.1016/j.psyneuen.2010.09.012
61. O'Connor JC, André C, Wang Y, Lawson MA, Szegedi SS, Lestage J, *et al.* Interferon-gamma and tumor necrosis factor-alpha mediate the upregulation of indoleamine 2,3-dioxygenase and the induction of depressive-like behavior in mice in response to bacillus Calmette-Guerin. *J Neurosci Off J Soc Neurosci* 2009;29:4200–9. DOI:10.1523/JNEUROSCI.5032-08.2009
62. Cui C, Grandison L, Noronha A. Neural-immune interactions in brain function and alcohol related disorders. Springer Science & Business Media; 2012.
63. Maes M, Bosmans E, Suy E, Vandervorst C, De Jonckheere C, Raus J. Immune disturbances during major depression: upregulated expression of interleukin-2 receptors. *Neuropsychobiology* 1990;24:115–120.
64. Smith RS. The macrophage theory of depression. *Med Hypotheses* 1991;35:298–306.
65. Maes M, Yirmiya R, Norberg J, Brene S, Hibbeln J, Perini G, *et al.* The inflammatory and neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. *Me-*

- tab Brain Dis* 2009;24:27–53. DOI:10.1007/s11011-008-9118-1
66. Crews FT, Vetreno RP. Addiction, adolescence, and innate immune gene induction. *Front Psychiatry* 2011;2:19. DOI:10.3389/fpsy.2011.00019
 67. Ceccanti M, Coriale G, Hamilton D, Carito V, Coccorello R, Scalese B, *et al.* Virtual Morris task responses in individuals in an abstinence phase from alcohol. *Can J Physiol Pharmacol* 2017. DOI:10.1139/cjpp-2017-0013
 68. Ceccanti M, Hamilton D, Coriale G, Carito V, Aloe L, Chaldakov G, *et al.* Spatial learning in men undergoing alcohol detoxification. *Physiol Behav* 2015;149:324–30. DOI:10.1016/j.physbeh.2015.06.034
 69. Zhao C, Teng EM, Summers RG, Ming G-L, Gage FH. Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. *J Neurosci Off J Soc Neurosci* 2006;26:3–11. [doi:10.1523/JNEUROSCI.3648-05.2006.]
 70. Altman J, Das GD. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J Comp Neurol* 1965;124:319–35.
 71. Stanton PK. LTD, LTP, and the sliding threshold for long-term synaptic plasticity. *Hippocampus* 1996;6:35–42. DOI:10.1002/(SICI)1098-1063(1996)6:1<35::AID-HIPO7>3.0.CO;2-6
 72. Maggio N, Shavit-Stein E, Dori A, Blatt I, Chapman J. Prolonged systemic inflammation persistently modifies synaptic plasticity in the hippocampus: modulation by the stress hormones. *Front Mol Neurosci* 2013;6:46. DOI:10.3389/fnmol.2013.00046
 73. Zou J, Crews F. Induction of innate immune gene expression cascades in brain slice cultures by ethanol: key role of NF- κ B and proinflammatory cytokines. *Alcohol Clin Exp Res* 2010;34:777–789. DOI:10.1111/j.1530-0277.2010.01150.x
 74. Nixon K, Crews FT. Binge ethanol exposure decreases neurogenesis in adult rat hippocampus. *J Neurochem* 2002;83:1087–93. DOI: 10.1046/j.1471-4159.2002.01214.x
 75. Crews FT, Bechara R, Brown LA, Guidot DM, Mandrekar P, Oak S, *et al.* Cytokines and alcohol. *Alcohol Clin Exp Res* 2006;30:720–30. DOI:10.1111/j.1530-0277.2006.00084.x
 76. McClain JA, Morris SA, Deeny MA, Marshall SA, Hayes DM, Kiser ZM, *et al.* Adolescent binge alcohol exposure induces long-lasting partial activation of microglia. *Brain Behav Immun* 2011;25 Suppl 1:S120–128. DOI:10.1016/j.bbi.2011.01.006
 77. Gonzalez-Perez O, Ramos-Remus C, Garcia-Estrada J, Luquin S. Prednisone induces anxiety and glial cerebral changes in rats. *J Rheumatol* 2001;28:2529–2534.
 78. Blednov YA, Bergeson SE, Walker D, Ferreira VMM, Kuziel WA, Harris RA. Perturbation of chemokine networks by gene deletion alters the reinforcing actions of ethanol. *Behav Brain Res* 2005;165:110–125. DOI:10.1016/j.bbr.2005.06.026
 79. June HL, Liu J, Warnock KT, Bell KA, Balan I, Bollino D, *et al.* CRF-amplified neuronal TLR4/MCP-1 signaling regulates alcohol self-administration. *Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol* 2015;40:1549–1559. DOI:10.1038/npp.2015.4
 80. Montesinos J, Pascual M, Pla A, Maldonado C, Rodríguez-Arias M, Miñarro J, *et al.* TLR4 elimination prevents synaptic and myelin alterations and long-term cognitive dysfunctions in adolescent mice with intermittent ethanol treatment. *Brain Behav Immun* 2015;45:233–44. DOI:10.1016/j.bbi.2014.11.015
 81. Pascual M, Baliño P, Alfonso-Loeches S, Aragón CMG, Guerri C. Impact of TLR4 on behavioral and cognitive dysfunctions associated with alcohol-induced neuroinflammatory damage. *Brain Behav Immun* 2011;25(Suppl 1):S80–91. DOI:10.1016/j.bbi.2011.02.012
 82. Blednov YA, Ponomarev I, Geil C, Bergeson S, Koob GF, Harris RA. Neuroimmune regulation of alcohol consumption: behavioral validation of genes obtained from genomic studies. *Addict Biol* 2012;17:108–20. DOI:10.1111/j.1369-1600.2010.00284.x
 83. Achur RN, Freeman WM, Vrana KE. Circulating cytokines as biomarkers of alcohol abuse and alcoholism. *J Neuroimmune Pharmacol Off J Soc NeuroImmune Pharmacol* 2010;5:83–91. DOI:10.1007/s11481-009-9185-z
 84. Kissack JC, Bishop J, Roper AL. Ethylglucuronide as a biomarker for ethanol detection. *Pharmacotherapy* 2008;28:769–781. DOI:10.1592/phco.28.6.769
 85. Ferraguti G, Ciolli P, Carito V, Battagliese G, Mancinelli R, Ciafrè S, *et al.* Ethylglucuronide in the urine as a marker of alcohol consumption during pregnancy: Comparison with four alcohol screening questionnaires. *Toxicol Lett* 2017;275:49–56. DOI:10.1016/j.toxlet.2017.04.016
 86. Taracha E, Habrat B, Woźniak P, Walkowiak J, Szukalski B. The activity of beta-hexosaminidase (uHex) and gamma-glutamyl-transferase (uGGT) in urine as non-invasive markers of chronic alcohol abuse: I. Alcohol-dependent subjects. *World J Biol Psychiatry Off J World Fed Soc*

- Biol Psychiatry* 2001;2:184–9.
DOI: 10.3109/15622970109026807
87. Niemelä O. Biomarker-based approaches for assessing alcohol use disorders. *Int J Environ Res Public Health* 2016;13:166. DOI:10.3390/ijerph13020166
88. Koch H, Meerkerk G-J, Zaat JOM, Ham MF, Scholten RJPM, Assendelft WJJ. Accuracy of carbohydrate-deficient transferrin in the detection of excessive alcohol consumption: a systematic review. *Alcohol Alcohol Oxf Oxf* 2004;39:75–85.
89. Golka K, Wiese A. Carbohydrate-deficient transferrin (CDT)—a biomarker for long-term alcohol consumption. *J Toxicol Environ Health B Crit Rev* 2004;7:319–337. DOI:10.1080/10937400490432400
90. Helander A, Eriksson CJP, WHO/ISBRA Study on State and Trait Markers of Alcohol Use and Dependence Investigators. Laboratory tests for acute alcohol consumption: results of the WHO/ISBRA Study on State and Trait Markers of Alcohol Use and Dependence. *Alcohol Clin Exp Res* 2002;26:1070–1077. DOI: 10.1111/j.1530-0277.2002.tb02641.x
91. Comasco E, Nordquist N, Leppert J, Orelund L, Kronstrand R, Alling C, et al. Adolescent alcohol consumption: biomarkers PEth and FAEE in relation to interview and questionnaire data. *J Stud Alcohol Drugs* 2009;70:797–804. DOI:10.15288/jsad.2009.70.797
92. Björkqvist M, Ohlsson M, Minthon L, Hansson O. Evaluation of a previously suggested plasma biomarker panel to identify Alzheimer's disease. *PloS One* 2012;7:e29868. DOI:10.1371/journal.pone.0029868
93. An L, Wang X, Cederbaum AI. Cytokines in alcoholic liver disease. *Arch Toxicol* 2012;86:1337–1348. DOI:10.1007/s00204-012-0814-6
94. Gonzalez-Quintela A, Alende R, Gude F, Campos J, Rey J, Meijide LM, et al. Serum levels of immunoglobulins (IgG, IgA, IgM) in a general adult population and their relationship with alcohol consumption, smoking and common metabolic abnormalities. *Clin Exp Immunol* 2008;151:42–50. DOI:10.1111/j.1365-2249.2007.03545.x
95. Gonzalez-Quintela A, Garrido M, Gude F, Campos J, Linneberg A, Lojo S, et al. Sensitization to cross-reactive carbohydrate determinants in relation to alcohol consumption. *Clin Exp Allergy* 2008;38:152–60. DOI:10.1111/j.1365-2222.2007.02863.x
96. Carito V, Ceccanti M, Ferraguti G, Coccurello R, Ciafrè S, Tirassa P, et al. NGF and BDNF alterations by prenatal alcohol exposure. *Curr Neuropharmacol* 2017. DOI:10.2174/1570159X15666170825101308
97. Ceccanti M, De Nicolo S, Mancinelli R, Chaldakov G, Carito V, Ceccanti M, et al. NGF and BDNF long-term variations in the thyroid, testis and adrenal glands of a mouse model of fetal alcohol spectrum disorders. *Ann Ist Super Sanita* 2013;49:383–90. DOI: 10.4415/ann_13_04_11
98. Ceccanti M, Mancinelli R, Tirassa P, Laviola G, Rossi S, Romeo M, et al. Early exposure to ethanol or red wine and long-lasting effects in aged mice. A study on nerve growth factor, brain-derived neurotrophic factor, hepatocyte growth factor, and vascular endothelial growth factor. *Neurobiol Aging* 2012;33:359–367. DOI: 10.1016/j.neurobiolaging.2010.03.005
99. Ceccanti M, Carito V, Vitali M, Iannuzzi S, Tarani L, De Nicolò S, et al. Serum BDNF and NGF modulation by olive polyphenols in alcoholics during withdrawal. *J Alcohol Drug Depend* 2015;3:1–6. DOI:10.4172/2329-6488.1000214]
100. Ceccanti M, Coccurello R, Carito V, Ciafrè S, Ferraguti G, Giacobazzo G, et al. Paternal alcohol exposure in mice alters brain NGF and BDNF and increases ethanol-elicited preference in male offspring. *Addict Biol* 2016;21:776–787. DOI:10.1111/adb.12255
101. De Nicolo S, Tarani L, Ceccanti M, Maldini M, Natella F, Vania A, et al. Effects of olive polyphenols administration on nerve growth factor and brain-derived neurotrophic factor in the mouse brain. *Nutrition* 2013;29:681–687. DOI: 10.1016/j.nut.2012.11.007
102. Fiore M, Mancinelli R, Aloe L, Laviola G, Sornelli F, Vitali M, et al. Hepatocyte growth factor, vascular endothelial growth factor, glial cell-derived neurotrophic factor and nerve growth factor are differentially affected by early chronic ethanol or red wine intake. *Toxicol Lett* 2009;188:208–13. DOI: 10.1016/j.toxlet.2009.04.013
103. Aloe L. Alcohol intake during prenatal life affects neuro-immune mediators and brain neurogenesis. *Ann Ist Super Sanita* 2006;42:17–21.
104. De Simone R, Aloe L. Influence of ethanol consumption on brain nerve growth factor and its target cells in developing and adult rodents. *Ann Ist Super Sanita* 1993;29:179–183.
105. Aloe L, Bracci-Laudiero L, Tirassa P. The effect of chronic ethanol intake on brain NGF level and on NGF-target tissues of adult mice. *Drug Alcohol Depend* 1993;31:159–167. DOI:10.1016/0376-8716(93)90068-2

106. Aloe L, Tirassa P. The effect of long-term alcohol intake on brain NGF-target cells of aged rats. *Alcohol* 1992;9:299–304. DOI:10.1016/0741-8329(92)90070-Q
107. Aloe L, Tuveri MA, Guerra G, Pinna L, Tirassa P, Micera A, *et al.* Changes in human plasma nerve growth factor level after chronic alcohol consumption and withdrawal. *Alcohol Clin Exp Res* 1996;20:462–465. DOI: 10.1111/j.1530-0277.1996.tb01076.x
108. Vuorela P, Sarkola T, Alfthan H, Halmesmaki E. Hepatocyte growth factor, epidermal growth factor, and placenta growth factor concentrations in peripheral blood of pregnant women with alcohol abuse. *Alcohol Clin Exp Res* 2002;26:682–687. DOI: 10.1111/j.1530-0277.2002.tb02591.x
109. Kocerha J, Faghihi MA, Lopez-Toledano MA, Huang J, Ramsey AJ, Caron MG, *et al.* MicroRNA-219 modulates NMDA receptor-mediated neurobehavioral dysfunction. *Proc Natl Acad Sci USA* 2009;106:3507–3512. DOI:10.1073/pnas.0805854106
110. Kocerha J, Kauppinen S, Wahlestedt C. microRNAs in CNS disorders. *Neuromolecular Med* 2009;11:162–172. DOI:10.1007/s12017-009-8066-1
111. Lippai D, Bala S, Petrasek J, Csak T, Levin I, Kurt-Jones EA, *et al.* Alcohol-induced IL-1 β in the brain is mediated by NLRP3/ASC inflammasome activation that amplifies neuroinflammation. *J Leukoc Biol* 2013;94:171–82. DOI:10.1189/jlb.1212659
112. Zhang Y, Wei G, Di Z, Zhao Q. miR-339-5p inhibits alcohol-induced brain inflammation through regulating NF- κ B pathway. *Biochem Biophys Res Commun* 2014;452:450–456. DOI:10.1016/j.bbrc.2014.08.092
113. Vetreno RP, Yaxley R, Paniagua B, Johnson GA, Crews FT. Adult rat cortical thickness changes across age and following adolescent intermittent ethanol treatment. *Addict Biol* 2017;22:712–723. DOI: 10.1111/adb
114. Risher ML, Sexton HG, Risher WC, Wilson WA, Fleming RL, Madison RD, *et al.* Adolescent intermittent alcohol exposure: dysregulation of thrombospondins and synapse formation are associated with decreased neuronal density in the adult hippocampus. *Alcohol Clin Exp Res* 2015;39:2403–2413. DOI: 10.1111/acer.12913
115. Spear LP. The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev* 2000;24:417–463.403–2413. [doi.org/10.1016/S0149-7634(00)00014-2
116. Spear LP. Adolescents and alcohol. *Curr Dir Psychol Sci* 2013;22:152–157. DOI: 10.1177/0963721412472192
117. Luna B, Marek S, Larsen B, Tervo-Clemmens B, Chahal R. An integrative model of the maturation of cognitive control. *Annu Rev Neurosci* 2015;38:151–170. DOI: 10.1146/annurev-neuro-071714-034054
118. Grant BF. The impact of a family history of alcoholism on the relationship between age at onset of alcohol use and DSM-IV alcohol dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. *Alcohol Health Res World* 1998;22: 144–147.
119. Gao F, Liu Z, Ren W, Jiang W. Acute lipopolysaccharide exposure facilitates epileptiform activity via enhanced excitatory synaptic transmission and neuronal excitability in vitro. *Neuropsychiatr Dis Treat* 2014;10:1489–1495. DOI: 10.2147/NDT.S65695
120. Guerri C, Pascual M. Mechanisms involved in the neurotoxic, cognitive, and neurobehavioral effects of alcohol consumption during adolescence. *Alcohol* 2010;44:15–26. DOI: 10.1016/j.alcohol.2009.10.003
121. Pascual M, Blanco AM, Cauli O, Minarro J, Guerri C. Intermittent ethanol exposure induces inflammatory brain damage and causes long-term behavioural alterations in adolescent rats. *Eur J Neurosci* 2007;25:541–550. DOI: 10.1111/j.1460-9568.2006.05298.x
122. Ward RJ, Colivicchi MA, Allen R, Schol F, Lallemand F, de Witte P, Ballini C, Corte LD, Dexter D. Neuro-inflammation induced in the hippocampus of ‘binge drinking’ rats may be mediated by elevated extracellular glutamate content. *J Neurochem* 2009;111:1119–1128. DOI: 10.1111/j.1471-4159.2009.06389.x
123. Vetreno RP, Crews FT. Adolescent binge drinking increases expression of the danger signal receptor agonist HMGB1 and Toll-like receptors in the adult prefrontal cortex. *Neuroscience* 2012;226:475–488. [doi: 10.1016/j.neuroscience.2012.08.046]
124. Zou JY, Crews FT. Release of neuronal HMGB1 by ethanol through decreased HDAC activity activates brain neuroimmune signaling. *PLoS One* 2014;9:e87915. DOI: 10.1371/journal.pone.0087915
125. Cantacorps L, Alfonso-Loeches S, Moscoso-Castro M, Cuitavi J, Gracia-Rubio I, López-Arnau R, *et al.* Maternal alcohol binge drinking induces persistent neuroinflammation associated with myelin damage and behavioural dysfunctions in offspring mice. *Neuropharmacology* 2017;123:368–384. DOI: 10.1016/j.neuropharm.2017.05.034
126. Agrawal RG, Hewetson A, George CM, Syapin PJ, Bergeson SE. Minocycline reduces ethanol drinking.

- Brain Behav Immun* 2011;25(Suppl 1): S165–S169. DOI: 10.1016/j.bbi.2011.03.002
127. Wu Y, Lousberg EL, Moldenhauer LM, Hayball JD, Robertson SA, Collier JK, Watkins LR, Somogyi AA, Hutchinson MR. Attenuation of microglial and IL-1 signaling protects mice from acute alcohol-induced sedation and/or motor impairment. *Brain Behav Immun* 2011;25(Suppl 1):S155–S164. DOI: 10.1016/j.bbi.2011.01.012
 128. Whitman BA, Knapp DJ, Werner DF, Crews FT, Breese GR. The cytokine mRNA increase induced by withdrawal from chronic ethanol in the sterile environment of brain is mediated by CRF and HMGB1 release. *Alcohol Clin Exp Res* 2012;37:2086–2097. DOI: 10.1111/acer.12189
 129. Anton M, Alen F, Gomez de Heras R, Serrano A, Pavon FJ, Leza JC, *et al.* Oleylethanolamide prevents neuro-immune HMGB1/TLR4/NF- κ B danger signaling in rat frontal cortex and depressive-like behavior induced by ethanol binge administration. *Addict Biol* 2017;22:724–741. DOI: 10.1111/adb.12365.
 130. Carito V, Ceccanti M, Tarani L, Ferraguti G, Chaldakov GN, Fiore M. Neurotrophins' modulation by olive polyphenols. *Curr Med Chem* 2016;23:3189–3197. DOI: 10.2174/0929867323666160627104022
 131. Carito V, Ceccanti M, Cestari V, Natella F, Bello C, Cocurello R, Mancinelli R, Fiore M. Olive polyphenol effects in a mouse model of chronic ethanol addiction. *Nutrition* 2017;33:65–69. DOI: 10.1016/j.nut.2016.08.014.

ABBREVIATIONS

5HTOL	5-hydroxytryptophol
ACTH	Adrenocorticotrophic hormone
AD	Alzheimer's disease
ALT	Alanine aminotransferase
AP-1	Activator protein 1
AST	Aspartate aminotransferase
BBB	Blood–brain barrier
BDNF	Brain-derived neurotrophic factor
CDT	Carbohydrate-deficient transferrin
CeA	Central nucleus of the amygdala
CNS	Central nervous system
COX2	Cytochrome c oxidase subunit 2
CREB	cAMP response element-binding protein
CRF	Corticotropin-releasing factor

DAMPs	Damage-associated molecular patterns
EGF	Epidermal growth factor
EtG	Ethylglucuronide
FASD	Fetal alcohol spectrum disorders
FosB	FBJ murine osteosarcoma viral oncogene homolog B
GGT	Gamma-glutamyltransferase
HMGB1	High mobility group box 1
HPA	Hypothalamic–pituitary–adrenal axis
IDO	Indoleamine-pyrrole 2,3-dioxygenase
IL	Interleukins
iNOS	Inducible Nitric oxide synthases
LPS	Lipopolysaccharide endotoxin
LTP	Long-term potentiation
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemoattractant protein 1
MCV	Mean corpuscular volume
MHC	Major histocompatibility complex
miR	microRNA
mPFC	Medial prefrontal cortex
mROS	Mitochondrial Reactive oxygen species
NAc	Nucleus accumbens
NF- κ B	Nuclear factor kappa-light-chain-enhancer of activated B cells
NLR	Nucleotide-binding oligomerization domain-like receptors
NLRP	NLR family pyrin domain containing 1
NMDA	N-Methyl-D-aspartic acid
PAMPs	Pathogen-associated molecular patterns
PD	Parkinson's disease
PEth	Phosphatidylethanol
PGF	Placental growth factor
Pro-IL	Interleukin precursor
PRRs	Pattern recognition receptors
RAGE	Receptor for advanced glycation endproduct
siRNA	Small interfering RNA
TDO	Tryptophan 2,3-dioxygenase
TLR	Toll-like receptors
TNF- α	Tumor necrosis factor alpha
VTA	Ventral tegmental area