Role of Peripheral Inflammation in Hepatic Encephalopathy

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A growing body of evidence now highlights a key role for systemic inflammation in altering behavior and mood in patients with liver disease. How inflammation occurring in the periphery in the context of liver disease, communicates with the brain to mediate changes in neurotransmission and thereby behavior is incompletely understood. Traditional routes of communication between the periphery and the brain involve neural (i.e. vagal afferent nerves) and humoral (blood-borne) pathways, with increased circulating levels of endotoxin and cytokines (especially Tumor Necrosis Factor α , TNF α) that occur during systemic inflammatory responses, as being primarily implicated in mediating signaling via these pathways. However, in recent years communication via peripheral immune-cell-to-brain and the gut-microbiota-to-brain routes have received increasing attention in the context of liver disease for their ability to modulate brain function, and generate a spectrum of symptoms ranging from fatigue and altered mood to overt Hepatic Encephalopathy (HE). In this review, we discuss periphery-to-brain communication pathways and their potential role in mediating systemic inflammation-associated alterations in behavior, that are in turn ultimately part of a spectrum of brain changes linked to the development of clinically apparent HE. (J CLIN EXP HEPATOL 2018;8:281–285)

Hepatic Encephalopathy (HE) is a serious complication of acute or chronic liver failure and includes a spectrum of neuropsychiatric disturbances. The pathogenesis of HE remains a topic of discussion in the scientific community with several theories proposed, including the central concept that hyperammonemia is a key driving factor.¹ However, serum concentrations of ammonia often correlate poorly with the severity of HE in cirrhotic patients. Other proposed mechanisms involve disruption of the blood-brain barrier, changes in neurotransmission, neuroinflammation, oxidative stress, Small Intestinal Bacterial Overgrowth (SIBO), and brain blood flow abnormalities.¹ More recently an important contribution of peripheral inflammation as a significant contributor to HE has been suggested.^{2,3} Moreover, it is increasingly recognized that a synergistic mechanism exists between ammonia and

peripheral inflammation in regulating the onset and severity of HE.⁴ This review outlines how systemic inflammation may contribute to HE and the changes in behavior associated with this condition.

SIGNALING PATHWAYS LINKING THE PERIPHERAL IMMUNE SYSTEM AND THE BRAIN

Patients with chronic liver disease commonly exhibit peripheral inflammation and experience altered brain function giving rise to symptoms that adversely affect their Quality of Life (QoL).^{5,6} How liver disease-related systemic inflammation and immune activation leads to remote changes in brain function remains unclear. A number of general signaling pathways have been described that link systemic inflammation to changes occurring in the brain, which in turn give rise to altered behavior.⁷ These signaling mechanisms have been divided into three main pathways; namely neural, humoral and immune.^{5,6,8}

- (1) Neural pathway: Vagal afferent nerves innervate the liver and can be activated through cytokine receptors expressed on vagal nerve endings by proinflammatory cytokines released during peripheral immune responses. After activation, vagal afferents carry stimuli to the brain which in turn activate primary and secondary cerebral projection areas, leading to changes in brain function and behavior.
- (2) Humoral pathway: Circulating proinflammatory cytokine levels (e.g. Tumor Necrosis Factor α , TNF α , Interleukin-6, IL-6) are often increased in the setting of peripheral immune activation, and in patients with liver disease.^{5,9} Cerebral Endothelial Cells (CECs) express receptors for TNF α and IL-1 β , and CEC activation by cytokines has been implicated as an important step in periphery to brain

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Abbreviations: CECs: Cerebral Endothelial Cells; HE: Hepatic Encephalopathy; IL: Interleukin; LPS: Lipopolysaccharide; MPA: Monocyte Platelet Aggregate; PSGL-1: Anti-P-Selectin Glycoprotein 1; QoL: Quality of Life; SIBO: Small Intestinal Bacterial Overgrowth; TNF- α : Tumor Necrosis Factor-Alpha

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signaling,⁶⁻⁸ and in the development of sickness behaviors in rodents.^{6,7} Proinflammatory cytokines, including TNF α , can induce the production of secondary messengers (such as prostaglandins and nitric oxide) in CECs, which can be released into the brain and subsequently lead to changes within the brain.⁵⁻⁷ Importantly, cytokines within the circulation can also gain access to brain tissue through areas of the brain devoid of an intact blood-brain barrier (called the circumventricular organs).^{6,8}

- (3) Immune pathway: Peripheral inflammation is commonly associated with increased numbers of circulating activated immune cells. These immune cells can traffic to the brain and adhere to activated CECs.^{6,8} Adherence of immune cells to CECs can lead to stimulation of secondary messenger production by CECs, which in turn are released within the brain parenchyma and activate resident immune cells in the brain (e.g. astrocytes, microglia).⁶⁻⁸ Resident brain immune cells activated in this way, can themselves release inflammatory mediators (e.g. cytokines) that alter neurotransmission and behavior.¹⁰ Alternatively, immune cells may traffic from the blood vessel lumen into the brain parenchyma and subsequently release proinflammatory mediators that elicit these neural changes within the brain.¹¹

IMMUNE SYSTEM-TO-BRAIN SIGNALING IN LIVER DISEASE IN THE ABSENCE OF LIVER FAILURE AND HE

Changes in behavior, including decreased cognition ("brain fog"), fatigue, anorexia and altered mood (depression, anxiety) are commonly experienced by patients with liver disease, regardless of liver disease severity.¹² These symptoms can overlap with symptoms reported by patients with HE,¹ suggesting that the peripheral and central drivers of these two symptom complexes are intimately related and therefore must share similarities in their pathogenesis. Systemic inflammation and associated immune system activation occur in both cirrhotic and non-cirrhotic liver disease.^{2,3,5} Therefore, a spectrum of differential activation of peripheral signaling pathways that drive central changes in neurotransmission and behavior, may underlie differences in the clinical expression of symptoms in liver disease patients, ranging from common-liver disease associated symptoms to those more typical of HE. An improved understanding of the link between peripheral inflammation-driven changes in the three main signaling pathways, and changes in brain function, may provide novel therapeutic approaches to improve symptoms, QoL, and reduce the severity of HE.

In the setting of liver disease, the importance of neural pathways linking the liver and the brain, resulting in altered brain function, is likely of lesser importance compared to the other signaling pathways. Specifically, liver transplantation which denervates the liver, does not typically improve fatigue severity or neurological dysfunction in patients with primary biliary cirrhosis.¹³ Similarly, recurrent HCV infection in a transplanted liver induces

behavioral changes that are similar to infection in a non-denervated liver.¹⁴

An active role for a humoral liver-to-brain communication pathway in the setting of liver disease is possible, as increased circulating proinflammatory cytokine levels have been documented in patients with chronic liver disease.^{5,9} However, these circulating cytokine elevations are often low grade, intermittent and often not reproducible.^{15,16} Moreover, elevations in circulating cytokine levels typically do not correlate with behavioral changes documented in patients with liver disease.^{5,17} In contrast, activated cytokine producing immune cells, including monocytes, have been identified within the peripheral circulation in animal models of liver injury and in patients with liver disease.^{11,18} Monocytes have the capacity to produce large amounts of cytokines, including TNF α .¹⁸ Moreover, activated monocytes can roll along and adhere to CECs that express relevant adhesion molecules on their surface.^{19,20} Through this mechanism high concentrations of cytokines can be delivered in close proximity to endothelium, driving the subsequent generation of secondary signaling molecules such as nitric oxide from the endothelium.^{5,20} The secondary signaling molecules generated in this fashion, can in turn be released within the brain parenchyma where they activate microglia and facilitate changes in neurotransmission that alter behavior.^{6-8,10} Importantly, we have previously shown in an experimental model of liver disease that this process occurs, is regulated by TNF α produced by monocytes adherent to cerebral endothelium, and critically drives microglia activation within the brain and the subsequent generation of adverse liver disease-related behaviors.^{11,19-21}

Although activation of monocytes within the circulation can be associated with liver injury, the mechanism linking liver damage and monocyte activation to produce TNF α remain unclear. The gut microbiome has been increasingly implicated in the regulation of behavior.²² Moreover, a healthy gut microbiome appears to provide overall health benefits which has led to the broad societal intake of supplements, including probiotics, to beneficially alter the gut microbiome. Furthermore, probiotic consumption has been shown to alter brain function and behavior in healthy humans. Specifically, probiotic ingestion can have beneficial effects on mood and cognition,²³ and is associated with changes in neural activity in brain regions involved in emotional processing.²⁴ The mechanism whereby probiotic ingestion leads to changes in brain function and behavior remain unclear, but have been linked to changes in gut flora (although not routinely observed), changes in gut permeability, and shifts in systemic immunity with decreased production of proinflammatory cytokines, including TNF α . Gut microbiome changes have been identified in patients with chronic liver disease, and these changes have been implicated in altered behavior and HE through a gut-liver-brain axis.²⁵

VSL#3 is a probiotic mixture that is widely used clinically. Furthermore, VSL#3 intake has recently been linked to an effect on brain function in that VSL#3 ingestion altered gene expression within the brain.²⁶ Based on these observations, we hypothesized that VSL#3 treatment would alter systemic immunity and improve adverse behaviors in the setting of liver disease. To examine this we administered VSL#3 to mice with liver inflammation and determined its effects on liver disease-associated alterations in behavior. In addition, we delineated the mechanism whereby oral VSL#3 administration leads to changes within the brain. Our findings highlighted the existence of a novel pathway whereby probiotic administration mediated improvements in liver disease-associated adverse behaviors by altering systemic immunity, decreasing microglial activation within the brain, and reducing recruitment of TNF α -secreting monocytes to the brain²¹; effects that were independent of changes in overall gut microbiome composition or gut permeability.²¹ Although the exact mechanism through which gut microbiota influences the brain and behavior remains unclear, these experiments demonstrate a novel pathway where probiotic ingestion affects systemic immune activation and circulating TNF α levels, microglial activation, monocyte-CEC interaction, and leukocyte infiltration of the brain ultimately resulting in improvements in behavior.²¹

The gut-liver-brain axis appears to be broadly regulated by bacterial byproducts (e.g. ammonia) or degradation products (endotoxin; LPS) released from the gut into the circulation.^{1,3,4,9} Importantly, levels of endotoxin (LPS) are increased within the blood and liver in patients with liver disease,^{9,27} and may activate circulating immune cells via Toll-Like Receptors-4 (e.g. TLR4) to induce cytokine production. Platelets secrete cytokines and chemokines, and also express adhesion molecules such as P-selectin that enable leukocyte adhesion to vascular endothelium, greatly facilitating leukocyte-endothelial cell adhesion in vascular beds such as the brain where P-selectin expression is low.^{19,20} Platelets can also interact with immune cells and modulate their function.¹⁹ For example, peripheral blood monocytes when bound to platelets promote a proinflammatory monocyte phenotype; an effect prevented by blocking monocyte-platelet interactions with an Anti-P-Selectin Glycoprotein 1 (PSGL-1) (counter-receptor for P-selectin) antibody.¹⁹ Monocyte Platelet Aggregate (MPA) formation within the circulation is increased in a range of inflammatory diseases, including liver disease.²⁸ Importantly, stimulation of TLR4 by ligands including LPS, can induce platelet activation.¹⁹ Therefore, we hypothesized that platelet-monocyte interactions within the circulation would be important for monocyte activation, an effect driven by TLR4 activation, and that formation of MPAs would lead to the induction of changes within the CNS that alter behavior in liver disease. Indeed, we found that in

experimental liver disease MPA formation was important for circulating monocyte activation and TNF α production, as well as monocyte recruitment to the brain and associated adverse liver disease-associated behavior development.¹⁹ Moreover, these effects were regulated by platelet activation via TLR4.¹⁹ These observations link the gut microbiome in liver disease to altered systemic immunity and development of adverse liver disease-associated behavior.¹⁹

THE GUT-LIVER-BRAIN AXIS, SYSTEMIC INFLAMMATION AND HE

Liver disease is commonly associated with gut microbiota dysbiosis, which becomes even more prominent in cirrhotic patients and in the setting of hepatic decompensation.²⁵ In addition, liver disease is commonly associated with the appearance of a systemic inflammatory response, including increased circulating cytokine levels (especially TNF α) and immune cell activation, which can be of variable severity and linked to increased gut permeability and elevated systemic levels of gut-related products, such as endotoxin.^{1-3,9,15,27} This systemic inflammatory response is an important driver of liver disease-related neuroinflammatory changes within the brain, changes in neurotransmission and subsequent adverse changes in behavior.^{3,5,7,29} In the context of cirrhosis, these changes can be further amplified, with more pronounced increases in gut permeability, enhanced circulating levels of endotoxin, proinflammatory cytokines and ammonia, and the formation of MPAs within the peripheral circulation.^{1-3,9,15,27,29} As liver function declines, a point is ultimately reached where HE becomes clinically apparent.¹ Importantly, a strong link between the gut microbiome, systemic inflammation, endotoxemia, neuroinflammation and altered brain function have been clearly defined in patients with liver failure and HE.^{1,25,29} The changes occurring within the brain in this setting are further exacerbated by the increased availability of gut derived ammonia within the circulation.^{1,2,4} Microglia activation within the brain is an important component of the neuroinflammatory response during HE, is linked to systemic inflammation, and occurs widely throughout the brain in the context of HE.^{1,29} Consistent with this observation, HE is commonly precipitated in cirrhotic patients by superimposed systemic inflammation driven by infection.^{3,27,30}

Therapeutics targeted at altering the gut microbiome (and presumably the generation of gut microbiome-derived byproducts such as endotoxin and ammonia) are the mainstay of treatment for HE, and include the antibiotic rifaximin and the oral oligosaccharide lactulose.¹ Rifaximin treatment reduces endotoxemia in cirrhotic patients with HE, in parallel to improvements in cognitive performance.³¹ Probiotics are also beneficial in cirrhotic patients with HE, and the probiotic VSL#3

decreases plasma levels of proinflammatory cytokines (e.g., TNF α), improves adverse behavioral changes associated with advanced liver disease and QoL, and reduces hospitalizations for HE.³² The mechanism of this effect is unclear, however it has been linked to changes in the gut flora, changes in gut permeability, and shifts in systemic immunity and the production of cytokines such as TNF α .^{32,33}

Therefore, systemic inflammation, linked to changes in the gut microbiome (i.e. dysbiosis), occurs commonly in patients with liver disease. Immune activation, which is closely associated with systemic inflammation in liver disease, is an important driver of subsequent microglia activation within the brain and associated neuroinflammation.^{1,29} These liver-disease associated neuroinflammatory changes alter neurotransmission pathways in the brain, and the behaviors that are linked to normal functioning of those pathways.⁵⁻⁸ Importantly, these changes in brain function exist as a continuum in patients with advanced liver disease, and can augment the clinical expression of HE (Figure 1). Therefore, alterations in brain function can manifest clinically as symptoms ranging from mild cognitive changes, altered mood (e.g. depression, anxiety) and fatigue in patients with cirrhosis but

without HE, to behavioral changes that are more classically associated with HE, including slowed mentation and confusion that can progress ultimately to unresponsiveness and coma.^{1,5-7,29}

SUMMARY

Systemic inflammation in patients with liver disease drives changes within the brain that are linked to a wide spectrum of behavioral alterations ranging from mild changes in cognition and mood, to overt confusion and coma observed in the setting of advanced HE. An enhanced understanding of the pathways that link the gut, liver and the brain in patients with liver disease, to generate altered brain function and behavior, will allow for the development of targeted therapies to better manage the neurological and behavioral complications of liver failure.

CONFLICTS OF INTEREST

The authors have no conflicts of interest relevant to this manuscript.

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Figure 1 Schematic showing potential link between liver disease progression, systemic inflammation, neuroinflammation and development of clinically apparent HE. Liver disease can progress over time (x-axis) and is linked to variable degrees of systemic inflammation, which in turn are associated with microglia activation within the brain and evidence of neuroinflammation (y-axis); which can progress as liver disease progresses (green arrow). With variable systemic insults (e.g. infection, increased gut permeability leading to enhanced systemic availability of gut-derived microbial products like LPS) acute exacerbations of neuroinflammatory responses can be precipitated (blue arrows) that can in turn lead to clinically apparent HE (red dotted line). As liver disease further progresses the systemic inflammatory insults necessary to precipitate HE can be of lesser severity, and the clinical expression of HE can be more severe. In addition, after removal of the specific peripheral inflammatory insult, in more advanced liver disease neuroinflammatory changes may be established to the point where baseline behavior is clinically consistent with chronic overt HE (right side of graph). (For interpretation of the references to color in this legend, the reader is referred to the web version of the article.)

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