Liver-brain inflammation axis

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D’Mello C, Swain MG. Liver-brain inflammation axis. Am J Physiol Gastrointest Liver Physiol 301: G749–G761, 2011. First published August 25, 2011; doi:10.1152/ajpgi.00184.2011.—It is becoming increasingly evident that peripheral organ-centered inflammatory diseases, including chronic inflammatory liver diseases, are associated with changes in central neural transmission that result in alterations in behavior. These behavioral changes include sickness behaviors, such as fatigue, cognitive dysfunction, mood disorders, and sleep disturbances. While such behaviors have a significant impact on quality of life, the changes within the brain and the communication pathways between the liver and the brain that give rise to changes in central neural activity are not fully understood. Traditionally, neural and humoral communication pathways have been described, with the three cytokines TNFα, IL-1β, and IL-6 receiving the most attention in mediating communication between the periphery and the brain, in the setting of peripheral inflammation. However, more recently, we described an immune-mediated communication pathway in experimentally induced liver inflammation whereby, in response to activation of resident immune cells in the brain (i.e., the microglia), peripheral circulating monocytes transmigrate into the brain, leading to development of sickness behaviors. These signaling pathways drive changes in behavior by altering central neurotransmitter systems. Specifically, changes in serotonergic and corticotropin-releasing hormone neurotransmission have been demonstrated and implicated in liver inflammation-associated sickness behaviors. Understanding how the liver communicates with the brain in the setting of chronic inflammatory liver diseases will help delineate novel therapeutic targets that can reduce the burden of symptoms in patients with liver disease.

hepatic inflammation; cytokines; sickness behaviors; mood disorders

IT IS BECOMING INCREASINGLY evident that chronic liver diseases are more than simply purely liver-centered diseases. Animal models and clinical observations have documented that chronic liver inflammation is associated with changes in the central nervous system (CNS) that manifest as behavioral changes (23, 72, 88, 91). Changes in behavior, such as fatigue, loss of motivation, malaise, and loss of social interest, are collectively termed sickness behaviors. While the presence of sickness behaviors can serve an adaptive purpose during acute systemic infections, in the setting of chronic inflammation they can greatly affect quality of life. Although sickness behaviors and altered mood states are highly prevalent in patients with chronic inflammatory liver diseases, as well as nonliver systemic diseases (e.g., rheumatoid arthritis and inflammatory bowel disease), they have received little attention because of a limited understanding of how these changes within the CNS develop in the setting of peripheral organ-centered inflammation (14, 45, 49, 88). Furthermore, hepatic encephalopathy, which is normally associated with advanced liver disease, is not a requirement for the development of changes within the CNS in patients with chronic inflammatory liver disease (32, 34, 71). Changes in neurotransmission that give rise to behavioral alterations can occur in the absence of any pathological CNS tissue damage (32, 34, 78). Furthermore, changes in central neural activity are often observed in patients with early-stage liver disease as well (61, 71). This review focuses on how the inflamed liver can communicate with the brain and outlines the associated changes in brain function that have been observed to occur as a result of this communication pathway. Although sickness behaviors and mood disorders have been documented in the setting of liver diseases of varied etiologies, including cholestatic liver diseases, autoimmune hepatitis, and viral hepatitis, their occurrence in the cholestatic liver disease primary biliary cirrhosis (PBC) and in hepatitis C (Hep C) have received the most attention and are therefore mainly discussed in this review.

How Does the Liver Communicate With the Brain?

While changes within the brain have been largely overlooked in the setting of peripheral organ-centered inflammatory diseases, much of what we know of the communication pathway between the periphery and the brain comes mainly from experimental studies examining central changes induced by systemic administration of the gram-negative bacterial cell wall component LPS. LPS is a potent inducer of three cytokines that have received the most attention in mediating com-
munication between the periphery and the CNS, namely, TNFα, IL-1β, and IL-6 (14, 24, 59, 100). Elevated plasma endotoxin levels have been documented in patients with chronic liver disease (88, 108). Administration of endotoxin or cytokines to healthy volunteers or rodents results in the development of sickness behaviors and depressed mood (7, 29, 41, 59). Furthermore, peripheral administration of cytokine antagonists results in significant improvement in such behaviors, thereby highlighting the importance of peripheral cytokines in communicating with the brain (47, 60, 99).

The liver is home to the largest resident macrophage population, the Kupffer cell. Liver inflammation is typically associated with activation of Kupffer cells and, thereby, the production of cytokines, including TNFα, IL-1β, and IL-6 (5). In patients with chronic inflammatory liver diseases, circulating levels of TNFα, IL-1β, and IL-6 are elevated (4, 61). Cytokine polymorphisms are known to influence disease susceptibility and severity, and, interestingly, genes involved in TNFα signaling and NF-κB activation have recently been implicated in the pathogenesis of PBC (66). In addition, TNFα polymorphisms have also been linked with sleep abnormalities, fatigue, and mood disorders (1, 20).

The CNS is protected by a blood-brain barrier, which consists of nonfenestrated endothelial cells with tight junctions between them (83). Cytokines are large, hydrophilic molecules that cannot readily cross the blood-brain barrier. Four main potential peripheral communication pathways to the brain have been described in the setting of systemic inflammation (Fig. 1): 1) the neural pathway, 2) signaling via cerebral endothelial cells (CECs), 3) signaling via the circumventricular organs (CVOs), and 4) immune cells.

The neural pathway. The liver is innervated by vagal afferents. Cytokines can directly activate vagal nerve afferents, since they have been described to express cytokine receptors, such as the IL-1 receptor (IL-1R) (30). In addition, macrophages interspersed between vagal fibers within the nerve could also respond to cytokines (35). In LPS-induced peritoneal inflammation, vagal afferent signaling is activated, rapidly relaying information to the brain, as demonstrated by increased Fos (protein expressed by the immediate early gene c-fos, used as an indicator of neuronal activation) expression in the primary cerebral projection area, the nucleus tractus solitarius, and in the secondary cerebral projection areas, including the parabrachial and hypothalamic paraventricular nuclei (PVN) (Fig. 1) (35, 105). In rodents injected intraperitoneally with IL-1β, subdiaphragmatic vagotomy was associated with a reduction in Fos expression in the nucleus tractus solitarius, as well as a decrease in social exploratory behavior (see below) (9). However, in vagotomized rats, administration of IL-1β via alternate routes (i.e., intravenously or centrally) did not have an inhibitory effect on social exploratory behavior (9). Observations of communication of peripheral inflammation to the brain via the neural route in rodents were also confirmed in humans, whereby increased activity in the insula cortex, where afferent vagus and spinal interoceptive neural pathways converge, was observed during performance of the Stroop Task during acute peripheral inflammation induced by typhoid vaccination (39). Interestingly, subjective fatigue in subjects who received typhoid vaccination correlated with activity changes within the insula and the anterior cingulate regions of the brain.

In chronic inflammatory diseases associated with prolonged increases in circulating cytokines, it is likely that the neural communication route plays a less important role in mediating development of sickness behaviors. Patients with PBC who have undergone a liver transplant report little change in their fatigue in the absence of any clinical features indicative of recurrent PBC (65). Liver transplant patients reported no change in fatigue severity even at a 2-yr follow-up (101). Furthermore, neurological dysfunction was still observed in PBC patients after liver transplantation, suggesting that the central neural changes in these patients could be long-lasting or permanent (65).

Signaling via CECs: role of TNFα, IL-1β and IL-6. TNFα is produced by macrophages, as well as T cells, natural killer cells, and natural killer T cells. There are two known biological receptors for TNFα: TNFR1 and TNFR2. TNFα signaling via TNFRs leads to activation of the transcription factors NF-κB and activator protein-1 (75). IL-1β is produced primarily by macrophages. Signaling by IL-1β is mainly through IL-1R1, whereas IL-1RII has no signaling capability (8). However, IL-1RII can still bind to IL-1β and, thereby, serves to decrease IL-1β biological activity by preventing its interaction with IL-1R1. Receptors for TNFα and IL-1β have been documented on CECs, as well as neuronal and glial cells (Fig. 2) (69, 85). NF-κB is normally found bound to its inhibitor IκB in the cell cytoplasm. In response to TNFα and IL-1β signaling, IκB is
and IL-1β receptor signaling pathways) can promote increased expression of NF-κB (e.g., Toll-like receptor signaling cascades that lead to the activation of NF-κB activity (85). Systemic administration of LPS, TNFα, and IL-6 in rodents results in upregulation of IκB mRNA in CECs (58, 69). Since the TNFα and IL-1β genes have an NF-κB binding site in their promoter region, signaling cascades that lead to the activation of NF-κB (e.g., Toll-like receptor signaling pathways) promote increased expression of these respective cytokines.

TNFα and IL-1β can interact with their receptors on CECs to induce the production of secondary messengers [e.g., PGs, nitric oxide (NO)], which can subsequently promote changes in neural activity within the brain (25, 78, 85). NO is produced by NO synthase (NOS). Constitutive NOS isoforms are present in endothelial cells (endothelial NOS) and neuronal cells (neuronal NOS). However, during inflammation, the inducible NOS isoform is upregulated in immune cells (e.g., macrophages), as well as in CECs in response to proinflammatory cytokines such as TNFα (53). PGs are lipophilic molecules produced by the cyclooxygenase (COX) enzymes. COX-1 is constitutively present in CECs, while COX-2 is upregulated in response to inflammatory stimuli. Inducible NOS and COX-2 are target genes for NF-κB (75, 85). COX-2 mediates production of PGs from arachidonic acid. The PG subtype that has received the most attention for its ability to mediate central effects of PGs is derived from arachidonic acid. The PG subtype that has received the most attention for its ability to mediate central effects of PGs is prostaglandin E2 (PGE2).

PGE2 causes rapid and transient expression of c-fos mRNA within multiple regions of the brain (56). Furthermore, inhibition of PGE2 synthesis with COX inhibitors, such as indomethacin, can attenuate the reduction in locomotor activity and the increase in time spent immobile in the forced swim test (FST) generally seen with peripheral LPS administration in rodents (25).

In the liver, hepatocytes and leukocytes (including Kupffer cells and neutrophils) express the IL-6R and also serve as a source of IL-6 (5). On activation, IL-6R can be shed from the surface of cells. LPS stimulation upregulates the expression of IL-6R in CECs and neuronal cells (100). IL-6 can bind to the membrane or soluble form of the IL-6R, and the IL-6-IL-6R complex then interacts with the transmembrane component glycoprotein (gp) 130, which is expressed on CECs, to induce its signaling cascade (85, 100). Conformational changes in gp130 result in activation of the associated enzymes Janus kinases, which then mediate the phosphorylation and activation of the STAT family of transcription factors (Fig. 2). Target genes for the STAT transcription factors include acute-phase proteins and adhesion molecules, such as ICAM-1 (85). Unlike TNFα and IL-1β, systemic administration of IL-6 to rodents does not lead to upregulation of IκB (58).

**Signaling via the CVOs.** The CVOs are regions of the brain that lack an intact blood-brain barrier. These areas include the area postrema, median eminence, and organum vasculare of the lamina terminalis. The capillaries in these regions are fenestrated, thereby allowing molecules within the circulation to have direct access to the CVO parenchyma (83). In response to intravenous administration of IL-1β or TNFα, increased monocyte chemoattractant protein 1 (MCP-1) and TNFR2 mRNA expression, respectively, has been documented within the CVOs (69, 97). Induction of c-fos mRNA (suggestive of neuronal activation) has also been observed in the CVOs with systemic administration of TNFα to rodents (Fig. 1) (69).

**Role of immune cells.** The brain has traditionally been considered an immune-privileged organ because of the presence of a blood-brain barrier, which prevents the cerebral entry of immune cells (83). Microglia, the resident immune cells of the brain, are highly dynamic cells that are constantly surveying their environment (84). In CNS inflammatory diseases, activation of resident microglia and infiltration of immune cells are cardinal pathological features (38, 52, 64). Much of our understanding of the central changes that occur in the setting of peripheral inflammation has come from studies that examined this in rodents that were treated systemically with LPS (41, 42). Such models of LPS-induced peripheral inflammation have demonstrated that microglia can be activated in the setting of systemic inflammation. This can have significant implications for promoting changes within the brain in the setting of systemic inflammation, since activated microglia can produce a wide array of cytokines with neuromodulating effects, including TNFα, IL-1β, and IL-6 (75, 84). In models of CNS inflammatory diseases, such as experimental autoimmune encephalomyelitis (an animal model of multiple sclerosis), that involve cerebral infiltration of T cells, microglia can also serve as antigen-presenting cells and can thereby mediate activation of T cells, which, in turn, can serve as a source of cytokines within the brain. Recently, in experimental cholestasis, microglia within the brain were found to be activated (23) (Fig. 3). Resting microglia have a “ramified” morphology with long filopodia that extend into the extracellular space, while activated microglia adopt a “ramified” morphology with long, spiny processes that extend into the extracellular space.
Fig. 3. Immune cell-mediated liver-to-brain communication pathway. 1) Experimental cholestasis is associated with activation of CECs to express adhesion molecules such as P-selectin and VCAM-1. 2) Circulating chemokine (C-C motif) receptor 2 (CCR2)-expressing inflammatory monocytes are increased in cholestatic rodents. 3) Microglia are activated to express a potent monocyte chemoattractant, MCP-1. 4) CCR2-expressing monocytes roll along CECs and then adhere to and subsequently transmigrate into brains of mice with experimental cholestasis. Monocyte infiltration into the brain can be inhibited by peripheral administration of anti-P-selectin and anti-α4-integrin antibodies. 5) Infiltrating monocytes also express MCP-1 and could further promote monocyte recruitment into the brain. 6) Microglia and cerebral infiltrating monocytes serve as a source of cytokines such as TNFα, a neuromodulator that has been implicated in the genesis of sickness behaviors and mood disorders.

Extended processes, whereas microglia in cholestatic mice were generally more rounded with retracted processes, a morphology typical of activated microglia. Furthermore, activated microglia in the brain were found predominantly in areas around the ventricles and blood vessels, potential routes of entry for infiltrating immune cells (23, 83).

Circulating monocytes in rodents are of two major subtypes: 1) a noninflammatory subtype, which can give rise to tissue resident macrophages, and 2) an inflammatory subtype, which expresses high levels of chemokine (C-C motif) receptor 2 (CCR2) and responds to MCP-1, a potent chemoattractant for monocytes. Similar monocyte subtypes have been characterized in humans (37). Experimental cholestasis was accompanied by an increase in circulating inflammatory monocytes, and a large proportion of them were activated and expressed TNFα (23). Systemic inflammation is associated with activation of circulating immune cells and CECs, likely resulting in increased interactions between them. The leukocyte recruitment paradigm has been well characterized in a number of tissues, whereby activated leukocytes first tether, then roll, and, on encountering an activating stimulus, firmly adhere to vascular endothelium (76). Rolling is primarily mediated by molecules called selectins, while adhesion is mediated by integrins. All three known selectins, P-, E-, and L-selectin, can bind to P-selectin glycoprotein ligand-1. Integrins are heterodimers comprising an α- and a β-subunit and are categorized on the basis of a common β-subunit (76). In cholestatic rodents, increased expression of VCAM-1 was documented on cerebral endothelium (51). VCAM-1, via its interaction with α4-integrin expressed on monocytes, can mediate adhesion of monocytes to CECs. Intravital microscopy of the cerebral vasculature demonstrated increased rolling of leukocytes along cerebral endothelium in cholestatic mice (51). In addition, in response to microglia activation and production of MCP-1, there was an eightfold increase in monocyte transmigration into the brains of mice with experimental cholestasis (23, 51) (Fig. 3). The MCP-1-CCR2 axis was important in mediating recruitment of monocytes into the brains of cholestatic mice, since cerebral monocyte infiltration was inhibited in cholestatic mice that were deficient in MCP-1 or CCR2. Monocytes that infiltrated the brains of cholestatic mice were located in the area around blood vessels and were also present in the brain parenchyma (23). Despite the redundancy in the chemokine system, the MCP-1-CCR2 axis plays a key role in mediating monocyte recruitment in other models of chronic inflammatory diseases, such as human immunodeficiency virus (HIV) encephalopathy and experimental autoimmune encephalomyelitis (38, 46). TNFα signaling via TNFR1 was important in mediating activation of microglia to express MCP-1 and the subsequent cerebral recruitment of monocytes in cholestatic mice, since these events were prevented in cholestatic mice that were deficient in TNFR1.

Endogenous production of cytokines within the brain. While circulating cytokines can mediate their effects on central neural activity though the production of secondary mediators such as PGE2 and NO, they are also capable of inducing de novo synthesis of cytokines within the brain. For instance, peripheral TNFα administration in rodents upregulates IL-1β and TNFα expression within the brain (80). Glial cells and neurons are capable of producing cytokines, and they also express functional cytokine receptors (75, 85). Peripheral LPS administration in rodents results in increased TNFα, IL-1β, and IL-6 expression in the brain (41, 42, 59, 80). Centrally produced cytokines appear to play an important role in mediating the behavioral effects of acutely administered peripheral cytokines. IL-1β production within the brain was demonstrated to be significant in mediating the effects of peripheral LPS, since central administration of IL-1 receptor antagonist, an endogenous inhibitor of IL-1β signaling, reduced the expression of IL-1β, IL-6, and TNFα in the hypothalamus and hippocampus.
of mice and decreased the food intake observed with systemic LPS administration (59).

Summary of potential peripheral signaling pathways to the brain. In the setting of peripheral organ-centered inflammation, communication between the periphery and the brain can take place via neural, immune cell, or cytokine-driven routes. As a result of these communication pathways, alterations in neurotransmission within the brain can manifest as sickness behaviors (e.g., fatigue), altered mood, cognitive defects, or sleep disturbances.

Fatigue and Liver Disease

Fatigue is a common complaint in patients with liver diseases of varied etiologies, including PBC, primary sclerosing cholangitis, autoimmune hepatitis, and chronic viral infection (88). In fact, in the cholestatic liver disease PBC, fatigue can be the presenting symptom and is present in up to 50–80% of patients with PBC (43, 49, 70). Fatigue is a complex symptom that has no association with markers of liver disease severity and can encompass a range of complaints, including lethargy, lack of motivation, malaise, and exhaustion. It has not received the attention it warrants in the clinical setting, in part because of its subjective nature, which also makes it difficult to quantify in humans and in animal models. Furthermore, fatigue has been associated with a poor prognosis, since in a 4-yr follow-up study of fatigue in patients with PBC, fatigue severity was found to be stable over the 4-yr period, and patients with comparatively higher fatigue scores at the beginning of the study period had a significantly reduced survival rate (49).

Fatigue has been described as the “difficulty in initiation of or sustaining voluntary activities” (19). Fatigue can be central or peripheral in origin. Peripheral fatigue is a result of neuromuscular dysfunction and is associated with physical, but not mental, fatigue. It results in a reduced ability to sustain physical activity and can involve metabolic changes, such as increased peripheral muscle acidosis on exercise. Central fatigue is due to changes in central neurotransmitter systems, which result in a reduced ability to perform tasks that require self-motivation or the perception that a task requires more effort (18). Central fatigue can also be perceived as reduced neural drive to muscle, which can result in reduced ability to sustain physical activity. While central and peripheral fatigue can coexist, fatigue in chronic liver disease is generally believed to be central in origin (88). Some of the studies that have examined fatigue in patients with PBC have used transcranial magnetic stimulation during and after a standardized fatiguing exercise as a tool to study central fatigue. In healthy controls, transcranial magnetic stimulation-induced motor evoked potentials increase during a fatiguing exercise (as a result of increased excitability of the primary motor cortex) and, subsequently, decrease. In contrast, in PBC patients with high fatigue levels, the later decrease in motor evoked potentials fails to occur (16). This finding further supports a central mechanism of fatigue in patients with PBC. Furthermore, fatigue is often linked with other neuropsychiatric symptoms, such as anxiety and depression. Moreover, in the setting of chronic inflammatory diseases, changes in neurotransmitter systems within the brain have been documented that likely contribute to the pathogenesis of fatigue (14, 18, 88).

The basal ganglia comprise six nuclei that project to the limbic system and frontal cortex. They are involved in motor coordination, motivation, and emotional control (18). Alterations in neural activity in the basal ganglia have been linked with fatigue. Positron emission tomography scans in patients with multiple sclerosis have demonstrated reduced glucose metabolism in the frontal cortex and basal ganglia in fatigued patients compared with nonfatigued patients (86). Exposure to toxic levels of the heavy metal manganese results in development of severe fatigue. In patients with PBC, elevated plasma concentrations of manganese were associated with increased fatigue levels. In addition, MRI studies conducted in the same group of patients demonstrated increased signal intensity in the basal ganglia area in patients with high fatigue levels, possibly associated with increased manganese deposition (33).

In CNS pathologies, infiltrating immune cells are a significant source of cytokines, and inhibition of immune cell infiltration has significant potential implications for associated behavioral alterations (38, 64). In cholestatic mice, monocytes that infiltrate the brain were found predominantly in the motor cortex, hippocampus, and basal ganglia areas (Fig. 4) (23). Moreover, the infiltrating monocytes in cholestatic mice expressed MCP-1, which could serve to further promote monocyte recruitment into the brain (23). The adhesion molecules P-selectin and α4-integrin were important in mediating the rolling and adhesion of leukocytes along the cerebral vasculature in cholestatic mice, since peripheral administration of a combination of anti-P-selectin and α4-integrin antibodies resulted in inhibition of these respective leukocyte interactions with CECs, and this in turn prevented cerebral infiltration of monocytes in cholestatic mice (23). The time spent by an adult mouse in social investigative behavior toward a juvenile mouse introduced into the home cage of the adult mouse has been used as a behavior paradigm that examines the motivation,

Fig. 4. Region-specific cerebral infiltration of monocytes in cholestatic mice. Monocytes that infiltrate the brains of cholestatic mice were found predominantly in the hippocampus, basal ganglia, and motor cortex areas. These are regions of the brain that have been implicated in the development of sickness behaviors, including fatigue. Inhibition of cerebral monocyte recruitment in experimental cholestasis results in improvement in sickness behaviors. Increased signal intensity (with magnetic resonance imaging) and alterations in cerebral metabolites (as observed using proton magnetic resonance spectroscopy) in the basal ganglia area have been linked with fatigue and cognitive impairment, respectively.
interest, and ability of the adult mouse to engage in social behaviors (7, 8, 23). Cholestatic mice spend less time in social investigative behavior (i.e., interacting with the juvenile mouse) and more time in noninvestigative behaviors (e.g., time spent grooming and immobile). Inhibition of cerebral monocyte infiltration in cholestatic mice prevented this decrease in social investigative behavior (23). Furthermore, a potential role for monocytes in mediating behavior alterations has been suggested by observations made in two different studies. In a study of rheumatoid arthritis patients, treatment with the anti-inflammatory agent methotrexate was associated with an improvement in disease severity, as well as a reduction in CCR2 expression on circulating peripheral blood monocytes (31). Babatin et al. (2), in a study of patients with PBC treated with methotrexate, showed an improvement in fatigue levels.

TNFα is important in relaying information about systemic inflammation to the brain. Administration of TNFα systemically to rodents results in activation of CECs and cerebral microglia (23, 58). The development of sickness behaviors, as well as cerebral inflammation, in response to peripheral LPS in rodents is markedly reduced in the absence of TNFα signaling (47, 80). Circulating monocytes in cholestatic mice are activated and express TNFα (51). A role for peripheral TNFα signaling in mediating changes in central neural activity has been highlighted in cholestatic mice, whereby the inhibition of peripheral TNFα signaling in cholestatic mice was associated with a reduction in central microglial activation, as well as the prevention of monocyte infiltration into the brain (23). Locomotor activity has been used as a surrogate marker of “fatigue-like” behavior in several experimental paradigms (11, 22, 25, 47). Moreover, recording of locomotor activity by a motion-sensing device that was worn on the leg was found to correlate closely with fatigue in patients with chronic fatigue syndrome, as well as in patients with PBC (70, 103). Cholestatic rodents show reduced locomotor activity when placed in an infrared beam activity monitor. Cholestatic mice deficient in TNFα or treated with an anti-TNFα serum peripherally demonstrate an increase in locomotor activity and in social investigative behavior (unpublished observations). Furthermore, cholestatic mice deficient in IL-6 also showed improvement in social investigative behavior (Nguyen and Swain, unpublished observations). In a study of rheumatoid arthritis patients, treatment with the anti-IL6R antagonist tocilizumab reduced fatigue levels (12). In addition, reductions in locomotor activity and social investigative behavior in cholestatic mice have been documented at a time when microglia in the brain are activated but no monocyte infiltration in the brain could be detected (unpublished observations). Delineating the pathways that lead to microglial activation in the setting of peripheral inflammatory diseases can have broad implications for changes in central neural activity. The tetracycline antibiotic minocycline has been used to inhibit microglial activation in animal models and in CNS pathologies (13, 41, 52). Administration of minocycline to rodents was associated with a reduction in LPS-induced microglial expression of proinflammatory cytokines, as well as an improvement in observed sickness behaviors (41). Furthermore, minocycline treatment, which was associated with reduced cerebral neuronal injury in a model of simian immunodeficiency virus encephalitis, has also been linked with a reduction in CCR2-expressing circulating monocytes (13).

Serotonergic cell bodies are primarily located in the dorsal raphe nuclei in the midbrain and project to several regions in the brain, including the hippocampus, hypothalamus, and amygdala. Serotonin [5-hydroxytryptamine (5-HT)] neurotransmission is involved in a wide variety of behaviors, including arousal, the sleep-wake cycle, locomotor activity, and mood (Fig. 5) (62, 68, 88). 5-HT can mediate its effects via several receptor subtypes. The two 5-HT receptors that have received the most attention in relation to fatigue in liver disease are the 5-HT1A and 5-HT3 receptors. The 5-HT1A receptor is present on the cell bodies of serotonergic neurons (i.e., presynaptic 5-HT1A receptors) in the dorsal raphe nucleus, as well as on postsynaptic neurons. Stimulation of the midbrain presynaptic 5-HT1A receptors results in reduced firing of serotonergic neurons and, thereby, reduced release of 5-HT into the synapse. In cholestatic rodents, increased expression of the 5-HT1A receptor in the midbrain has been observed (10). Repeated stimulation of the 5-HT1A receptor with a 5-HT1A agonist (i.e., desensitizes the receptor), which would result in increased 5-HT release into the synapse, was associated with a reduction in the time spent immobile in the FST (see below) (93). The 5-HT3 receptor is structurally different from the 5-HT1A receptor, since it is a ligand-gated ion channel. In experimental cholestasis, decreased hypothalamic 5-HT3 receptors have been documented (72). Administration of the 5-HT3 receptor antagonist tropisetron to cholestatic rodents results in improvement in fatigue-like behavior, as demonstrated by an increase in locomotor activity (72). In the clinical setting, serotonin receptor antagonists have been linked with improvements in fatigue. Specifically, in patients with Hep C who were treated with the 5-HT3 receptor antagonist ondansetron, an improvement in fatigue was observed (77). In patients with PBC, the effect of the 5-HT3 receptor antagonist ondansetron on fatigue was controversial, in part due to patient unblinding during the study as a result of increased constipation associated with the drug (96).

![Fig. 5. Serotonergic neurotransmission within the brain. 5-HT neuronal cell bodies are located in the dorsal raphe nucleus in the midbrain. Stimulation of presynaptic 5-HT1A receptors reduces 5-HT release into the synapse. 5-HT neurons project to various brain regions and can modulate a wide spectrum of behaviors via their action on different receptor subtypes. 5-HT1A and 5-HT3 receptors have received the most attention in relation to fatigue in chronic inflammatory liver diseases. The presynaptic serotonin transporter 5-HTT facilitates the reuptake of 5-HT released into the synapse. 5-HT neurotransmission has been linked to fatigue, mood disorders, cognitive impairment, and sleep disturbance.](http://ajpgi.physiology.org/).
Hypothalamic-Pituitary-Adrenal Axis and Liver Disease

The hypothalamic-pituitary-adrenal (HPA) axis plays a major role in the stress response. External or internal stressors result in activation of the HPA axis (36, 98). Corticotropin-releasing hormone (CRH) is produced by neurons in the PVN. CRH stimulates the pituitary to release ACTH, which promotes glucocorticoid (cortisol in humans and corticosterone in rodents) release from the adrenal cortex. Glucocorticoids can inhibit the release of CRH at the level of the hypothalamus, and at the level of immune cells, glucocorticoids can inhibit the release of proinflammatory cytokines (Fig. 6) (36, 98). Cytokines are capable of activating the HPA axis (28, 69, 89). Receptors for TNFα and PGs have also been found on neurons in the PVN (69, 112). However, in the context of chronic inflammation, cytokine-HPA axis interactions are not completely understood. Generally, in chronic inflammatory diseases that are associated with elevated proinflammatory cytokines (e.g., rheumatoid arthritis), hypoactivation of the HPA axis has been observed (36, 98). Similar observations have also been made in experimental cholestasis (91, 92, 94).

In addition to the PVN, CRH nerve fibers and receptors have been described in other regions of the brain and have been implicated in the modulation of a wide spectrum of behaviors, including anxiety, fatigue, and appetite control (3, 22, 36). In cholestatic rodents, decreased hypothalamic CRH levels have been documented, suggesting that CRH activation of the HPA axis may be altered (11, 91). In addition, reduced Fos expression in the hypothalamic PVN region in response to central administration of IL-1β has been demonstrated in cholestatic rodents, further supporting reduced neuronal activation in this region (92). Consistent with this observation, the increase in ACTH release typically observed in response to central IL-1β administration was markedly reduced in cholestatic rodents (94). CRH has been shown to have behavioral activating effects. Infusion of CRH centrally into cholestatic rats results in increased locomotor activity at a concentration without effect in control rats, suggesting that cholestasis is associated with increased sensitivity to central CRH (11). Alterations in the HPA axis have also been observed in patients with PBC who demonstrate increased ACTH secretion in response to intravenous CRH administration (95). This observation would be consistent with an upregulation of pituitary CRH receptors in these patients, possibly due to reduced central CRH release.

Furthermore, it is likely that cytokines such as TNFα can stimulate corticosterone secretion from the adrenal cortex, findings supported by the observation that cholestatic rodents that were treated with an anti-TNFα serum peripherally showed a reduction in inflammation-induced circulating corticosterone levels with no effect on plasma ACTH levels (89).

CRH can mediate its biological effects via CRH receptors (CRHR1 and CRHR2). CRHR1 has been implicated in the control of locomotor activity, since mice deficient in CRHR1 are resistant to the locomotor-inducing effects of central CRH infusion (22). On the other hand, CRHR2 is associated with appetite control. In cholestatic rodents, increased expression of CRHR1 within the hypothalamus has been documented (11). The increase in hypothalamic CRHR1 expression may be due to decreased CRH levels in this region (11, 91). Furthermore, the behavioral activating effects of CRH in cholestatic rodents were mediated by CRHR1, since pretreatment of cholestatic rats with a specific CRHR1 antagonist attenuated the increase in locomotor activity seen with central infusion of CRH (11). CRHR1 is also thought to be involved in mood disorders, since mice deficient in CRHR2 spent more time immobile in the FST (see below), and this behavior was inhibited in the presence of a CRHR1 antagonist (3).

Mood Disorders and Liver Disease

A role for cytokines in the pathogenesis of mood disorders has become increasingly evident (14, 24, 68). Elevations in circulating cytokines such as TNFα and IL-6 have been observed in patients with depression (61, 79, 109). Furthermore, administration of endotoxin or cytokines (including TNFα and IL-6) to healthy volunteers results in transient development of depressed mood and anxiety (29). Strong clinical support for a role for cytokines in depression also comes from patients with chronic inflammatory conditions that are commonly associated with elevations in inflammatory cytokines (e.g., rheumatoid arthritis, inflammatory bowel disease, and chronic liver disease) or patients treated with cytokine therapy, such as cancer patients and patients with Hep C, all of which are associated with a higher incidence of depression (40, 43, 74, 79). Severe neurological impairment is not necessary for the development of altered mood states, as was observed in the CNS inflammatory disease multiple sclerosis, where depression was not related to severity of neurological impairment and could occur in early stages of the disease (67). Furthermore, administration of anti-cytokine therapies, such as anti-TNFα agents, in a

Fig. 6. Hypothalamic-pituitary-adrenal axis. Hypothalamic paraventricular nucleus neurons release corticotropin-releasing hormone (CRH), which is a potent stimulator of ACTH release from the pituitary. Hypothalamic CRH levels are decreased in experimental cholestasis. ACTH stimulates release of glucocorticoids from the adrenal cortex. Glucocorticoids can act on receptors at the level of the hypothalamus and the pituitary to inhibit release of CRH and ACTH, respectively, and can also act at the level of immune cells to inhibit release of proinflammatory cytokines such as TNFα, IL-1β, and IL-6. In addition to the hypothalamus, CRH nerve fibers and receptors have been described in other regions of the brain and can thereby modulate a wide array of behavior.

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Review
number of organ-centered inflammatory diseases is associated with an improvement in depressive symptoms (60, 99).

Anhedonia, defined as an inability to experience pleasure in things once found enjoyable, is a feature of depression. Assessment of anhedonia is a commonly used experimental paradigm to examine “depressive-like” behavior in animals, and anhedonia is sensitive to antidepressants used to treat human depression (24, 41, 90, 110). Such animal models have been useful in delineating the mechanisms that can lead to altered mood states. Since rodents have a preference for saccharin-sweetened drinking water, a reduced consumption of sweetened drinking water would be reflective of anhedonia. Administration of LPS to rodents results in development of anhedonia (24, 110). When cholestatic rodents were exposed to two water bottles, one containing tap water and the other containing a saccharin solution, a reduced consumption of sweetened drinking water was documented (90). Another behavioral paradigm commonly used to assess “depressive” behavior is the FST (24–26, 62). For the FST, rodents are placed in an inescapable cylinder of water, and the time spent in active swimming vs. time spent immobile is recorded. Immobility in the FST is believed to represent a failure to persist in survival behavior. The FST has been used to examine the effects of antidepressants, since the time spent immobile is markedly reduced in rodents treated with antidepressants (26). In cholestatic rodents, time spent immobile in the FST was increased and was significantly reduced by the repeated administration of a 5-HT1A agonist (93).

Depression has been reported in patients with Hep C, even before they undergo IFN-α therapy. Circulating TNFα was elevated in patients with Hep C prior to treatment with IFN-α, and the increase in plasma TNFα, as well as plasma IL-1β, levels was associated with higher depressive scores (61). Hep C patients treated with IFN-α showed a higher prevalence of mood disorders. In an examination of the effects of IFN-α therapy on plasma IL-6 levels and levels of depression in patients with Hep C, Prather et al. (79) observed that IFN-α treatment resulted in increased circulating levels of IL-6, as well as an increase in depressive scores. Furthermore, in a study of changes within the plasma and the brain in response to IFN-α treatment, levels of IL-6 and MCP-1 were increased in the cerebrospinal fluid of Hep C patients after 12 wk of IFN-α administration compared with control Hep C patients not treated with IFN-α (81). Interestingly, a study that examined associations between gene polymorphisms and depression reported a higher risk of IFN-α-induced depression in patients with Hep C with specific polymorphisms in the phospholipase A2 and COX-2 genes (87).

The 5-HT neurotransmitter system has received the most attention in relation to mood disorders (24, 68). Low 5-HT levels have been commonly linked with depression. In patients with Hep C, administration of therapies that increase cerebral 5-HT levels, such as selective serotonin reuptake inhibitor-type drugs (e.g., citalopram), has been demonstrated to improve IFN-α-induced depression (54). Tryptophan is an essential amino acid that is required for the synthesis of 5-HT. Patients with depression generally show low circulating levels of tryptophan (15, 24, 62). Indoleamine 2,3-dioxygenase (IDO) is an enzyme that is produced by macrophages and, on activation, can convert tryptophan to kynurenine. Cytokines such as TNFα and IFN-γ can promote activation of IDO, which results in less tryptophan being available for 5-HT synthesis (62, 68). Furthermore, administration of 1-methyltryptophan, a competitive inhibitor of IDO, can inhibit LPS-induced depressive-like behavior in rodents (24). Cancer patients who were treated with IFN-α showed reduced levels of tryptophan within 1 mo of therapy, and the decrease in serum tryptophan correlated positively with the severity of depressive symptoms (15). In a study of Hep C patients treated with IFN-α, a decrease in plasma tryptophan and an increase in kynurenine plasma levels were observed, and this in turn was associated with an increase in levels of depression in these patients (21). An alternate role for IDO in the pathogenesis of depression, independent of 5-HT synthesis, has also been postulated. Tryptophan can be converted by IDO to kynurenine, which can be further converted to kynurenic acid and quinolinic acid. Quinolinic acid could alter central glutamatergic neurotransmission, which is also linked with the development of mood disorders (24, 68). Increased levels of kynurenic acid and quinolinic acid have been observed in the cerebrospinal fluid of Hep C patients treated with IFN-α and could potentially contribute to the increased incidence of depression in these patients (82). Limited studies of this aspect of liver disease present a potentially interesting area for future investigation.

There are several reports of comorbidity between depression and sickness behaviors such as fatigue, and this is to be expected, since the neurotransmitter systems that have been implicated in the pathogenesis of these behavioral disorders are similar. A strong correlation between fatigue and depression has been reported in patients with PBC and Hep C. An assessment of fatigue and depression demonstrated that 44.8% of 103 patients with PBC could be classified as having depression as assessed using the Beck Depression Inventory (BDI), and a significant correlation was found between their levels of fatigue and depression (43). A high incidence of fatigue and depression was documented in patients with chronic viral hepatitis as well (50). It should also be mentioned that more caution is needed in making the diagnosis of depression or fatigue. Fatigue is often assumed to be caused by depression, and the patient is then classified as being “depressed,” rather than “fatigued.” While the diagnosis of depression is generally made using questionnaires, there is a stronger need for the diagnosis of depression by psychiatric evaluation. For instance, in a study of 92 PBC and primary sclerosing cholangitis patients, 42% were classified as having “depressive” symptoms according to the Beck Depression Inventory, whereas assessment by a psychiatric interview classified only 3.7% as being “depressed” according to Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria (102). The need for a proper diagnosis of depression also stems from the fact that mood disorders comprise a wide spectrum of depressive disorders. In patients with chronic inflammatory diseases, a higher incidence of the “atypical” depression subtype has been observed (36, 98). Typical depression is linked with features such as decreased appetite, insomnia, and increased anxiety, while atypical depression includes characteristics such as hyperphagia, hypersomnia, and increased sensitivity to social rejection. While the etiology of depression is complex, an important distinguishing factor between typical and atypical depression from a biological perspective is that typical depression is generally associated with increased CRH levels and behavioral activation and, conversely, atypical de-
pression is linked with reduced CRH levels and fatigue (36, 98). Hence, this further strengthens the concern for a proper diagnosis of typical vs. atypical depression, since these diagnoses often require different therapeutic approaches.

**Cognitive Impairment and Liver Disease**

Administration of LPS or cytokines to healthy volunteers results in mild impairments in memory and concentration and has thereby highlighted a role for cytokines in cognitive dysfunction (14, 24). While cognitive impairment has been typically associated with advanced liver disease and often linked with the development of hepatic encephalopathy, it has been observed in patients with PBC or Hep C without evidence of hepatic encephalopathy, and the levels of cognitive dysfunction did not associate with biochemical markers of liver disease severity. In addition, the cognitive impairments were mostly related to problems with memory and concentration (34, 71).

Cerebral proton magnetic resonance spectroscopy (MRS) can be used to obtain information about cerebral biochemical metabolites. The most common metabolites measured are N-acetylaspartate, a neuronal marker, choline-containing compounds, which are components of the cell membrane, and myo-inositol, which is an intracellular metabolite involved in the synthesis of phosphoinositides and is mainly found in glial cells (1a, 6, 17a, 34). Elevations in myo-inositol are postulated to be indicative of glial activation and proliferation, while elevations in choline are thought to be indicative of cellular infiltration or glial proliferation (6). Cerebral MRS studies conducted in patients with PBC or in patients with Hep C infection have indicated elevations in myo-inositol and choline levels. In patients with Hep C infection or PBC, increased choline levels in the cerebral basal ganglia region were associated with cognitive dysfunction (71). In fact, patients with Hep C who had an impairment in two or more tasks during cognitive testing had a higher mean cerebral choline-to-creatine ratio than patients who scored better on these tasks (34).

While the Hep C virus was once considered strictly hepatic in nature, Hep C viral RNA has been detected in peripheral blood mononuclear cells (106). In HIV patients, cerebral infiltrating monocytes serve as a route of entry into the brain for the HIV virus (38). In postmortem brain tissue from six patients infected with Hep C, Hep C viral RNA, while absent in neurons and oligodendrocytes, was found primarily in macrophages in the brain (107). Recently, observations suggestive of cerebral macrophage activation in patients with Hep C have been documented. Specifically, examination of postmortem brain tissue from seven patients with Hep C demonstrated an increased mRNA expression of proinflammatory cytokines such as TNFα and IL-1β in Hep C RNA-positive macrophages compared with Hep C RNA-negative macrophages (106). In addition, cerebral MRS studies in 25 patients with Hep C with mild liver inflammation showed an increased myo-inositol-to-creatine ratio in all 25 patients. In the patients with Hep C who underwent cognitive testing, the increase in the cerebral myo-inositol-to-creatine ratio was associated with prolonged working memory reaction times (32). These findings suggest that glial activation could take place in the setting of viral liver inflammation and has significant implications for mediating alterations in central neural activity.

**Sleep Disturbances and Liver Disease**

Sleep abnormality, specifically, increased daytime sleepiness, is a complaint documented in many liver disease patients. In a study of 48 patients with PBC, daytime sleepiness as measured by the Epworth Sleepiness Scale, was increased in patients with PBC compared with healthy controls, with >50% of patients with PBC having a score >10 (established threshold indicative of significant daytime hypersomnolence). In the patients with PBC, the amount of daytime sleepiness had no association with biochemical markers of liver disease severity (70). In Hep C patients treated with IFN-α and followed over a 4-mo period, reduced quality of sleep over the study period was reported (79).

TNFα, IL-1β, and IL-6 play a role in physiological control of the sleep-wake cycle and likely contribute to the daytime sleepiness observed in patients with liver disease (44). Administration of these cytokines to rodents results in increased time spent asleep, and, conversely, cytokine antagonists have the opposite effects (44, 73). Elevated levels of TNFα and IL-6 have also been reported in patients with sleep disorders such as narcolepsy and sleep apnea, which are commonly associated with increased daytime sleepiness (104). In addition, in Hep C patients treated with IFN-α, elevated levels of plasma IL-6 were associated with poor sleep quality, as observed over a 4-mo treatment period (79). The CRH and 5-HT neurotransmitter systems have been linked with sleep abnormalities. Central infusion of CRH into rodents results in increased time in wakefulness (less time sleeping), and administration of a CRH receptor antagonist, astressin, reduces the time in wakefulness (17). The role of the serotonergic neurotransmitter system in sleep abnormality is complex, and it has been implicated in promoting wakefulness, as well as increased sleep times (44). Furthermore, a strong association between the degree of daytime sleepiness and fatigue has been reported, suggesting the existence of overlap in the neurotransmitter systems and mechanisms that give rise to these respective symptoms (70). Modafinil, a therapeutic agent associated with amelioration of daytime sleepiness, was also found to reduce fatigue severity in a small cohort of patients with PBC (48).

**Conclusions and Future Directions**

Although there are caveats in extrapolating animal data to humans, findings from animal models and clinical studies have contributed significantly to furthering our understanding of some of the mechanisms that can contribute to the symptomology seen in patients with chronic inflammatory liver diseases. While the predominance of these symptoms may vary across liver diseases of varying etiologies, the alterations in underlying neurotransmitter systems that have been implicated in their pathogenesis appear to be quite similar. It is evident that TNFα, IL-1β, and IL-6, the three cytokines that have received the most attention in mediating changes in central neural activity in the setting of peripheral inflammation, are likely key promoters of central neural changes in chronic liver diseases. Moreover, although TNFα, IL-1β, and IL-6 are redundant in some of their effects, the benefit from antagonizing only one of them (e.g., inhibiting TNFα or IL-6) suggests that some cytokines may be more predominant in mediating behavioral changes in the setting of different inflammatory diseases (12, 60, 99). Targeting the 5-HT neurotransmitter...
system has also proven to be advantageous in relation to fatigue and/or depression in patients with liver disease (54, 77).

Recently, cerebral microglial activation was found in post-mortem brain tissue from patients with Hep C infection and patients with cirrhosis and hepatic encephalopathy (106, 111). However, evidence for microglial activation in the setting of other chronic liver diseases is still lacking. In patients with chronic inflammatory liver diseases, this could be investigated using noninvasive methods such as cerebral proton MRS (see above). In experimental cholestasis, microglia are activated and subsequently lead to cerebral monocyte infiltration (23, 51). Importantly, inhibition of both of these events is associated with a significant improvement in sickness behaviors (23). CNS inflammatory diseases that are associated with microglial activation show a marked reduction in neuroinflammation with administration of minocycline (52). In addition, anti-adhesion therapies that have targeted P-selectin and α4-integrin have also been effective in improving sickness behaviors in experimental cholestasis, as well as in reducing cerebral inflammation in CNS pathologies (23, 64). Such therapeutic interventions have not been explored in the setting of chronic inflammatory liver diseases and may be beneficial in improving sickness behaviors in patients with chronic liver diseases. Furthermore, MRI studies in patients in which circulating monocytes have been labeled with small paramagnetic iron oxide particles have been used to track the infiltration of monocytes into the brains of patients with CNS inflammatory diseases, including multiple sclerosis, in which monocyte recruitment into the brain is a pathological feature (27). Similar studies in patients with chronic inflammatory liver diseases would be very useful in demonstrating whether monocytes infiltrate the brains of these patients, thereby presenting a novel therapeutic target in relation to amelioration of sickness behaviors in patients.

While the 5-HT neurotransmitter system has received the most attention in the setting of liver disease, other neurotransmitter systems, such as the GABA, dopamine, and norpinephrine neurotransmitter systems, have been implicated in mediating behavioral alterations (14, 18, 62). These behavioral changes include cognitive dysfunction and fatigue, and, therefore, alterations in these neurotransmitter systems may play a role in promoting behavioral changes in the setting of inflammatory liver diseases. In addition, changes in the CRH neurotransmitter system have been described in the cholestatic liver disease PBC and warrant investigation in other chronic liver inflammatory diseases as well (11, 91, 92, 95). It has become increasingly evident that peripheral organ-centered inflammatory diseases, including chronic liver diseases, are not purely organ-limited diseases but are associated with changes in central neural activity. Continuing to delineate how the diseased liver communicates with the brain to subsequently mediate the changes that occur within the brain will enable us to identify novel therapeutic targets that can serve to decrease the burden of debilitating symptoms such as fatigue in patients with chronic inflammatory liver disease.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


LIVER-BRAIN INFLAMMATION AXIS


