Gut-brain axis in traumatic brain injury: impact on neuroinflammation

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The gastrointestinal track is inhabited by tens of trillions of microorganisms. The gut microbiota is involved in gut motility, nutrient absorption and synthesis of metabolites that influence homeostasis, metabolism and immune function. Given the influence gut microbiota has on health, there is a growing body of literature describing the gut microbiota's impact on brain and behavior. The bidirectional nature of the gut-brain axis involves neurological, immunological and hormonal mechanisms that can induce perturbations in gut or brain homeostasis. Studies using different but complementary approaches, such as germ free mice, antibiotics, probiotics, gastrointestinal infection, and fecal microbiota transplant, have shown that the gut microbiota acting via the gut-brain axis contribute to the regulation of brain and behavior, impacting depression, stress and cognition. Moreover, gut microbiota disruption has been associated with neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and implicated in modulating disease severity in stroke. Traumatic brain injury (TBI) is a complex, acute neurological insult that can lead to chronic neurodegeneration. Understanding the influence of the gut-brain axis in the setting of TBI may create new avenues of therapeutic approaches for TBI survivors.

Preclinical investigations provide evidence that TBI can impact intestinal function and the gut microbiota. In a moderate TBI rat model utilizing 16S rRNA sequencing of stool, changes in gut microbiota were detected as early as 2 days after injury (Nicholson et al., 2019). TBI's impact on gut microbiota may be long-lasting with significant changes in gut bacterial populations for up to 28 days after injury (Opeyemi et al., 2021). Larger lesion volume was associated with alterations of gut microbiota that may influence functional outcome. In another preclinical study of diffuse brain injury, TBI-induced microbial dysbiosis with gastrointestinal dysfunction and alteration of bile acid was an important mediator of gut microbiome-host interactions (You et al., 2021). Gut microbial dysbiosis was associated with decreased levels of bile acid in feces and plasma. This study supported the hypothesis that TBI-induced gut microbial dysbiosis contributed to intestinal inflammation by decreasing bile acid (You et al., 2021). Fecal microbiota transplant for 7 days after TBI in rats restored gut microbiota, improving neurological outcomes and exerting an anti-oxidative effect via decreasing TBI-induced trimethylamine N-oxide, a gut microbiota metabolite, and increasing the antioxidant enzyme methionine sulfoxide reductase A expression in the hippocampus (Du et al., 2021). This data supports the concept that the gut-brain axis is bidirectional and TBI can induce alterations in the gut microbiome.

The composition of the gut microbiota is shaped significantly by the immune system and resident microbes provide signals for normal immune system development. Disruption of the gut-immune axis induces profound consequences in host health. However, there are still major gaps in our understanding of how the immune system can regulate microbiota and how the microbiota shape host immunity. Utilizing germ free mice and transient alteration of gut microbiota by antibiotic treatment, Erny et al. (2015) demonstrated that gut microbiota control microglia maturation and homeostasis. Microglia dysfunction in germ free mice was rescued by partial recolonization of the gut with complex microbiota or supplementation with short-chain fatty acids (SCFAs), known gut bacterial metabolites (Erny et al., 2015). Monocytes have also been implicated as an important mechanistic link in the gut microbiota's influence on brain homeostasis (Mohle et al., 2016). Hippocampal neurogenesis was reduced in antibiotic-treated mice but was rescued by exercise, probiotics and adoptive transfer of Ly6C^{high} monocytes (Mohle et al., 2016). Gut microbiota have been also described as influencing the brain's response to injury. In an ischemic stroke model, antibiotic-induced alteration of the gut microbiota modulated the trafficking of effector T cells from the gut to the leptomeninges impacting stroke severity (Benakis et al., 2016). They demonstrated that intestinal dysbiosis alters immune homeostasis in the small intestine, leading to an increase in regulatory T cells and a reduction in interleukin IL-17-positive T cells inducing a neuroprotective effect after stroke (Benakis et al., 2016). Hence, a deeper understanding of the gut microbiome's influence on neuroinflammation in the setting of TBI may provide new approaches to neuroprotection (**Figure 1**).

The gut microbiota's influence on TBI is of paramount clinical significance as TBI patients are highly susceptible to developing gut microbial dysbiosis due to frequent antibiotic administration, prolonged hospitalization, and autonomic dysfunction. In our most recent published preclinical study (Celorrio et al., 2021), we hypothesized that microbial dysbiosis leads to worsened outcomes after TBI, and this effect is mediated by microbial-host immune intersections, which are dependent on changes induced by the peripheral or resident immune cells such as microglia. To test this hypothesis, adult male C57Bl/6J mice experienced moderate controlled cortical impact (2 mm depth) or sham surgery and were randomized to a combination of broad-spectrum antibiotics (vancomycin, neomycin, ampicillin, and metronidazole) or Kool-Aid as a control in drinking water for 7 days after injury. All mice resumed regular drinking water after 7 days. We performed flow cytometry, histological assessments, and behavior testing at various time points after injury.

16S rRNA sequencing of stool samples confirmed dramatically altered gut bacterial populations after 7 days of antibiotic exposure, with persistent changes still detected at 1 month after injury. Microglia showed time-dependent lasting changes after TBI that were impacted by microbial dysbiosis up to 3 months post-injury. After 7 days of antibiotic exposure after TBI, we found increased microglia activation surface markers (toll-like receptor 4, and major histocompatibility complex II) that were associated with microglial morphology changes toward amoeboid microglia. At 3 months, we found evidence of chronic microglial activation and increased hippocampal neuronal loss in antibiotic-exposed injured mice. Microglia play an important role in TBI-induced neurogenesis (Willis et al., 2020). Utilizing 5-bromodeoxyuridine injections on post-injury days 3–6, we observed reduced neurogenesis in the dentate gyrus of antibiotic-exposed injured mice. We also found that gut microbial dysbiosis induced an acute suppression of monocyte circulation in the peripheral blood and infiltration into the brain parenchyma with no associated changes in T cell infiltration 3 days after injury. However, by 7 days after injury, TBI-induced recruitment of lymphocytes (CD4$^+$, CD8$^+$, and CD4$^+$CD25$^+$ cells) and monocytes (Ly6C^{high}) into the hippocampus was suppressed in antibiotic-injured mice, an effect that persisted for up to 1 month. Our initial characterizations of reduced T cell infiltration associated with changes in microglial morphology and neurodegeneration in antibiotic-injured mice suggest that T cell-microglia crosstalk may be an important mechanistic link of gut microbiota modulation of TBI.

These changes in neuroinflammation following antibiotic-induced gut microbial dysbiosis after TBI impacted long-term neuropathology. Neuronal degeneration in the CA3 region of the injured hippocampus was exacerbated in antibiotic-treated animals despite discontinuation of antibiotics 1 week after injury and normalization of gut microbiota by 3 months. Chronic neuronal degeneration was accompanied by altered fear memory response in antibiotic-injured mice. Nevertheless, other studies have shown a neuroprotective effect of broad-spectrum antibiotics prior to brain injury in TBI model (Simon et al., 2020). Simon et al. (2020) demonstrated antibiotic exposure immediately after TBI reduced hippocampal cell loss, decreased microglia density, and increased fear conditioning response. The opposing results of our data could be related to the timing of antibiotic exposure (pre-injury versus post-injury), injury severity, timing, and region of histologic and behavioral assessments. In summary, induction of gut microbial dysbiosis immediately after injury altered microglia homeostasis, suppressed monocyte and lymphocyte infiltration and was associated with reduced neurogenesis, increased hippocampal neurodegeneration, and fear memory changes long after antibiotic exposure.

Bacteria metabolites, specifically SCFAs, have been shown to reverse gut microbial dysbiosis induced microglia dysfunction (Erny et al., 2015). In addition, supplementation of soluble SCFAs prior to and after TBI improved spatial learning in mice (Opeyemi et al., 2021). However, how these mediators overcome the blood-brain barrier and dilutional challenges is currently unknown. Recently, it has been shown that brain resident CD4$^+$ T cells are required for microglia maturation, and their absence results in defective microglial synaptic pruning and behavior deficits (Pasciuto et al., 2020). Furthermore, brain-resident T cells derived from activated circulatory T cells were influenced by the gut microbiota, suggesting a possible mechanistic link for gut microbiota control of microglia maturation and homeostasis. This link is further supported by

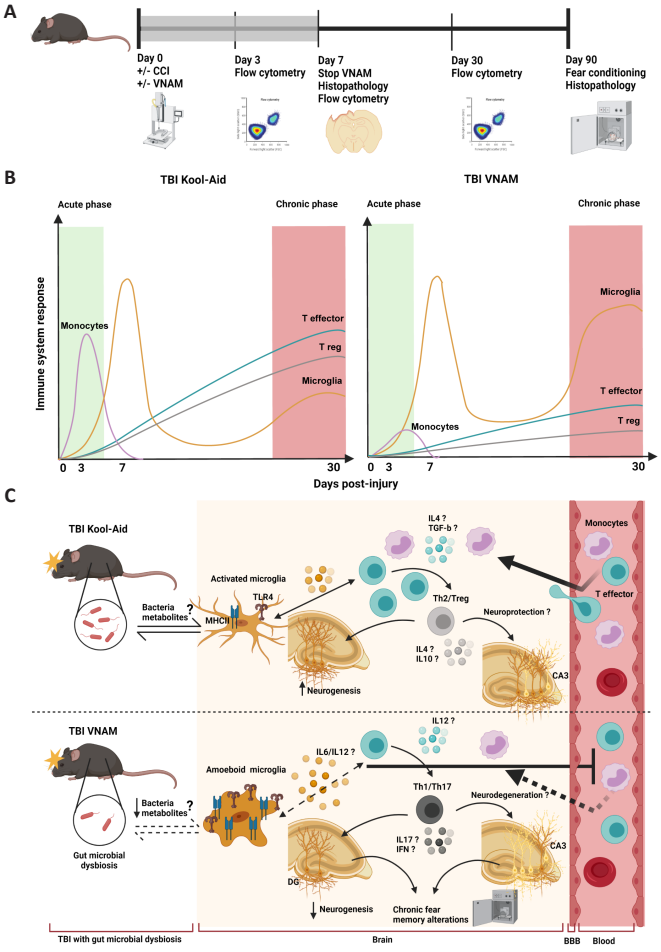

Figure 1 | **Gut microbial dysbiosis after traumatic brain injury modulates the immune response and impairs neurogenesis.**
(A) Experimental design for the murine model of microbial dysbiosis after traumatic brain injury (TBI). Adult male C57BL/6J mice underwent controlled cortical impact (CCI) or sham surgery, and were immediately randomized to broad-spectrum antibiotics in the drinking water (vancomycin, neomycin-sulfate, ampicillin, metronidazole (VNAM)) and Kool-Aid or Kool-Aid alone. After 1 week, all mice resumed normal drinking water. Mice were sacrificed at 3 days for flow cytometry, 7 days for flow cytometry and histopathological analysis, 1 month for flow cytometry and at 3 months post-injury for fear conditioning testing and histopathological analysis. (B) Temporal innate and adaptive immune response in TBI with and without microbial dysbiosis. In injured mice with a normal microbiota (TBI Kool-Aid), monocytes infiltrated the brain constituting the innate immune response (3 days post-injury) followed by activation of microglia with a peak at 7 days post-injury. The innate immune response makes a crucial contribution to the activation of adaptive immunity with T cell (T effector and T reg) infiltration into damaged tissue. In the case of injured mice with microbial dysbiosis, infiltration of the monocytes and T cells was suppressed with increased microglial activation at 3 months. (C) Summary of major findings from Celorrio et al. (2021) and potential mechanistic links in TBI Kool-Aid (top) and TBI VNAM (bottom) mice. The number of microglia in the injured hippocampus did not differ between TBI Kool-Aid and TBI VNAM mice; however, microbial dysbiosis induced an increase of pro-inflammatory microglial surface markers (TLR4 and MHCII) with changes in microglial morphology toward an amoeboid shape. Reduction in bacterial metabolites produced by gut bacteria after VNAM exposure may be responsible for the modulation in microglial morphology and activation which may alter cytokine signaling and T cells populations that are recruited to the injury site. Reduced monocyte infiltration associated with gut microbial dysbiosis may also play a role in microglial activation and T cell infiltration. Amoeboid microglia may liberate pro-inflammatory cytokines such as IL-12 and IL-6, which may modulate the T effector cell response toward Th1/Th17 and away from a Th2/T reg neuroprotective phenotype. These neuroinflammatory changes were associated with reduced neurogenesis in the dentate gyrus (DG), increased neuronal degeneration in the CA3 region of the hippocampus, and altered fear memory response in TBI VNAM mice. BBB: Blood-brain barrier; IL: interleukin; MHCII: MHC class II; TLR4: Toll-like receptor 4.

a recent study highlighting the requirement of peripheral lymphocytes for SCFA modulation of microglia after experimental stroke (Sadler et al., 2020). Further research is needed to determine the underlying mechanistic links by which TBI-associated neuroinflammation is modified by the enteric microbiome before developing rationally-based strategies to modify the gut microbiota and neuroinflammation to benefit neuroprotection.

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