Gut microbiota modulates oligodendrocyte lineage cell response after traumatic brain injury

Kirill Shumilov, Stuart Friess*

Traumatic brain injury (TBI) is a significant public health issue, affecting approximately 1.7 million people annually in the United States alone, with over 5 million experiencing long-term disabilities (Roozenbeek et al., 2013). A major sequela of TBI is long-lasting white matter injury (WMI) which includes traumatic axonal injury and loss of myelination, resulting in cognitive, behavioral, and psychiatric deficits in survivors. To date, there are no effective therapies for traumatic WMI, and novel therapeutic approaches are urgently needed. Oligodendrocytes, which provide metabolic support to axons and are the producers of myelin in the central nervous system (CNS), undergo apoptosis after TBI, triggered by direct injury or in response to axonal degeneration (Flygt et al., 2016, 2017). Mature oligodendrocytes present in the brain at the time of injury have limited, if any, capability to contribute to remyelination. Therefore, the bulk of CNS remyelination is attributed to the differentiation of oligodendrocyte progenitor cells (Franklin and Ffrench-Constant, 2017), which can be adversely impacted by inflammation after TBI. This highlights that initial WMI from TBI is amplified by a failure of post-injury reparative mechanisms. Understanding the molecular and cellular mechanisms by which inflammation impairs oligodendrocyte progenitor cell proliferation and remyelination is thus essential to identify targetable post-injury processes to improve recovery.

Recent investigations of the gut–brain axis have focused on the impact of TBI on gut function (dysmotility and increased mucosal permeability) and the gut microbiome (changes in richness and diversity) (Hanscom et al., 2021). However, our understanding of the mechanisms and impact of gut communication to the brain in the setting of TBI is limited in part because the communication is bidirectional (e.g., TBI itself results in rapid and sustained changes in the composition of the gut microbiota). Using mouse models of antibiotic depletion of the gut microbiota and fecal microbiota transplantation (FMT), we have recently demonstrated the gut microbiota's critical role in regulating peripheral and CNS immune response post-TBI and the impact on neurogenesis, neurodegeneration, and behavior (Celorrio et al., 2021, 2023). Emerging evidence links the gut–microbiota–brain axis to myelination, and regulation of myelin-related genes for cortical myelination (Hoban et al., 2016). However, the potential modulation of oligodendrocyte lineage cell (OLC)-mediated remyelination by the gut microbiota following TBI remains unexplored.

We hypothesized that the gut microbiota significantly influences OLC responses to traumatic WMI through the modulation of T cell differentiation and activation. To test this, we investigated the impact of early post-injury gut microbiota depletion on OLC proliferation and remyelination (Shumilov et al., 2024). We further explored the necessity of T cells for gut microbiota-mediated OLC responses and subsequent remyelination (Shumilov et al., 2024). Finally, *in vitro* studies were conducted to elucidate OLC/T-cell interactions within the context of gut microbiome depletion.

To investigate the role of gut microbiota in white matter remyelination, we depleted gut microbiota using a combination of broad-spectrum antibiotics in the drinking water (vancomycin, neomycin, ampicillin, and metronidazole) or a Kool-Aid control for 1 week immediately after moderate controlled cortical impact in adult male C57Bl/6J mice. Three months post-TBI, white matter remyelination was assessed in the peri-contusional corpus callosum (CC) via myelin black gold II staining and observed reduced myelination in antibiotic-treated mice compared to controls. To explore the acute effects of gut microbiota depletion, we assessed myelin debris accumulation (a known inhibitor of oligodendrocyte progenitor cell migration and proliferation) in CC with degraded myelin basic protein immunohistochemistry and OLC proliferation with 5-bromo-2′-deoxyuridine (BrdU) injections on post-injury days 2–6. We observed increased myelin debris and suppressed OLC proliferation in antibiotic-treated mice 7 days after injury compared with injured control mice. To differentiate between direct antibiotic effects and microbiota-mediated influences on remyelination, we employed the FMT approach in germ-free mice (Celorrio et al., 2023). We performed two FMTs of the gut microbiota from VNAM- or Kool-Aid-treated uninjured animals into germ-free 17 and 10 days prior to injury. Consistent with our antibiotic depletion experiments, we observed increased myelin debris staining in the CC of animals that underwent FMT from mice with depleted gut microbiota. These data support our hypothesis that gut microbiota play an important role in modulating post-traumatic myelin debris clearance and OLC proliferation.

Previously we demonstrated that gut microbiota depletion after TBI impairs hippocampal neurogenesis, promotes a pro-inflammatory phenotype in microglia, and surprisingly reduces T cell infiltration into the brain up to one month after injury (Celorrio et al., 2021). Furthermore, microglia maturation and function can be regulated by host microbiota (Erny et al., 2015), and modulate the immune response against myelinating OLCs through a T cell-mediated response (Kedia et al., 2024). Building upon these findings, we further investigated the influence of T cell depletion or absence in the setting of early gut microbiota depletion after TBI. To achieve the depletion of T cells, we employed pharmacological treatment with anti-CD3 IgG or control IgG starting one week before injury and continuing for one month before assessing myelin black gold II staining and OLC proliferation (BrdU injections were given only on post-injury days 2–6). Effective T cell depletion was confirmed with flow cytometry of the peripheral blood. Surprisingly, in the absence of T cells, we did not observe reduced myelination or OLC proliferation in the injured CC of gut microbiota-depleted mice. As an orthogonal approach we employed $TCR\beta^{-/-}TCR\delta^{-/-}$ mice (absence of mature T cells) and found similar results with injured antibiotic-depleted mice having similar density of proliferating OLCs, and myelin debris accumulation compared with control injured mice. These data suggest an essential role for T cells in gut microbiota-OLC communication after TBI.

The precise role of resident immune cells in the CNS is well-known, nonetheless, the influence of non-immune glial cells like astrocytes and OLCs in neuroinflammation and myelin repair has only recently emerged as a critical area of research. Under the context of CNS disorders, disease-specific OLCs are characterized by the expression of immune-specific genes allowing a bidirectional cross-talk between immune cells and modulating their response (Falcão et al., 2018). To further explore the interaction of OLC and T cells we employed an *in vitro* co-culture combining both cell types. T cells isolated from spleens of injured animals with and without microbiota depletion 7 days after injury, were co-cultured with OLCs extracted from P5 mouse cortex. We found that when OLCs were co-cultured with T cells from injured mice without antibiotic depletion, there was an increase in OLC proliferation. However, when T cells isolated from injured gut microbiota-depleted mice were co-cultured for 24 hours with OLCs, we found decreased OLC proliferation. Next, we characterized the T cell differentiation from the *in vitro* co-culture with flow cytometry analysis. No differences in T cell populations between the groups (injured antibiotic-depleted and control injured mice) were observed when T cells were cultured alone. However, in co-culture conditions, the percentage of $CD4^+$ T cells expressing the IL17 cytokine was increased in lymphocytes derived from injured antibiotic-depleted mice compared with injured controls. We then used a trans-well co-culture approach to determine if direct T cell-OLC contact was required. Interestingly, in the trans-well system differences in OLC proliferation between the two groups of T cells were no longer statistically significant however T cells from injured antibiotic-depleted mice continued to have increased IL17 expression. These data suggest that, while modulation of OLC proliferation could have a greater impact when contact with T cells is direct, $CD4^+$ lymphocyte differentiation occurs indirectly through extracellular signaling. Collectively, our findings provide evidence that differentiation of T cells towards a pro-inflammatory phenotype impairs OLC proliferation and could be a plausible explanation for the reduced myelination we observed in our *in vivo* model.

A study presented evidence suggesting that OLCs could express immunomodulatory factors such as cytokines/chemokines, also, major histocompatibility complex (MHC) class I and II was found in OLCs when exposed to interferon (Falcão et al., 2018). To further explore the mechanism

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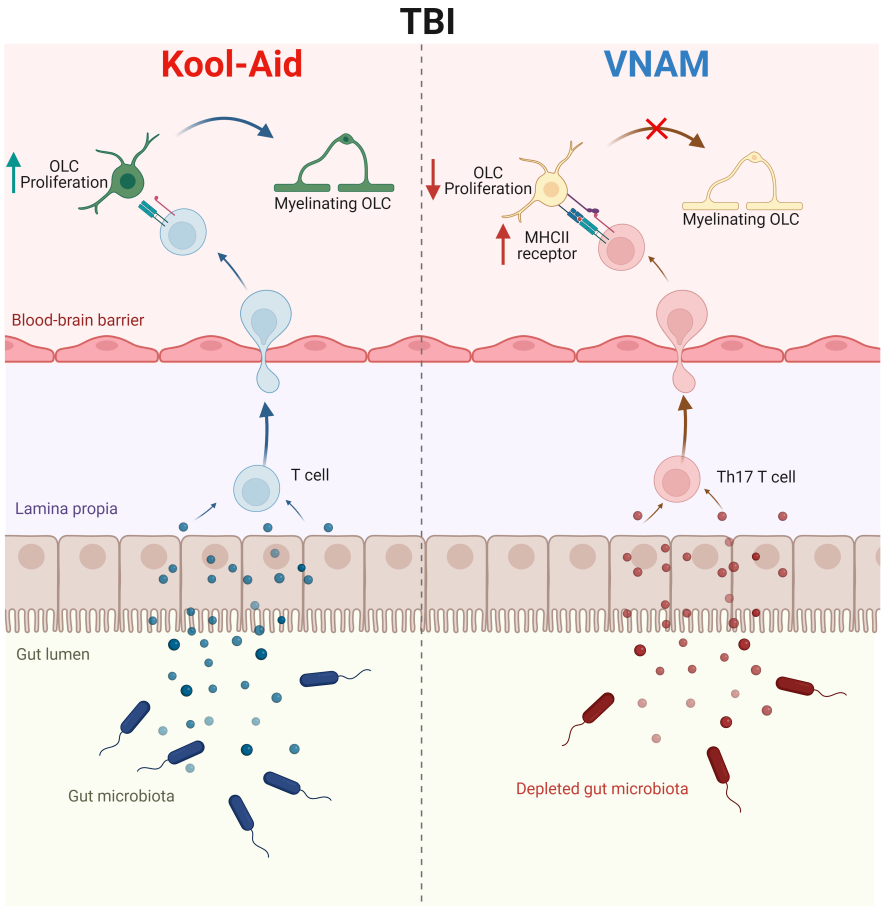

Figure 1 | **Representative illustration of the proposed mechanism for the impact on myelination following TBI with gut microbiota depletion.**
Post-traumatic remyelination is hindered by a decrease in the proliferation of OLCs and the accumulation of myelin debris in animals with depleted microbiota. This could occur through T cell interaction with OLCs via the MHCII receptor, leading to a reduction in their ability to proliferate and a potential increase in their immunomodulatory functions. Created with BioRender.com. MHCII: Major histocompatibility complex II; OLC: oligodendrocyte lineage cells; TBI: traumatic brain injury; VNAM: vancomycin, neomycin, ampicillin, metronidazole.

of bidirectional communication between T cells and OLCs, we explored the presence of MHC-II in OLC after injury and gut microbial depletion. We found that when *in vitro* OLCs were exposed to T cells derived from injured gut microbiota-depleted mice, they exhibited increased MHC-II expression. Furthermore, Olig1 and MHC-II co-localization significantly increased in peri-contusional CC of injured antibiotic-depleted mice compared with injured controls. These results provide further evidence for the potential activation of immunomodulatory functions in OLCs. Our findings indicate a bidirectional mechanism by which T cells and OLCs might directly interact through MHC-II.

In summary, our investigation into TBI and early gut microbial depletion reveals a profound impact on WMI recovery through T cell modulation of OLCs. We observed impaired white matter remyelination, characterized by decreased OLC proliferation and myelin debris accumulation, in animals with depleted microbiota early after TBI. Furthermore, FMT in germ-free mice showed a direct impact of the gut microbiota on WMI. This evidence indicates that the gut microbiota has the potential to modulate post-TBI white matter recovery via regulation of OLCs. Since the gut microbiota are known to regulate the immune cell response after TBI, we further demonstrated that the absence of T cells and gut-microbiota depletion had a significantly reduced impact on myelin repair and OLC proliferation following injury. These data suggest that T cells play a key role in gut microbial and brain communication in the setting of TBI. Future studies should focus on the precise molecular mechanism of the gut microbiota modulation of the immune activation of T cells.

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Date of submission: September 9, 2024
Date of decision: October 23, 2024
Date of acceptance: November 6, 2024
Date of web publication: December 7, 2024

https://doi.org/10.4103/NRR.NRR-D-24-01061
How to cite this article: *Shumilov K, Friess S (2026) Gut microbiota modulates oligodendrocyte lineage cell response after traumatic brain injury. Neural Regen Res 21(1):310-311.*

Open peer reviewer: *Andrew Octavian Sasmita, Max Planck Institute for Multidisciplinary Sciences -City-Campus, Germany.*
Additional file: *Open peer review report 1.*

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P-Reviewer: Sasmita AO; C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y