Spinal cord injury and the human microbiome: beyond the brain–gut axis

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In addition to standard management for the treatment of the acute phase of spinal cord injury (SCI), implementation of novel neuroprotective interventions offers the potential for significant reductions in morbidity and long-term health costs. A better understanding of the systemic changes after SCI could provide insight into mechanisms that lead to secondary injury. An emerging area of research involves the complex interplay of the gut microbiome and the CNS, i.e., a brain–gut axis, or perhaps more appropriately, a CNS–gut axis. This review summarizes the relevant literature relating to the gut microbiome and SCI. Experimental models in stroke and traumatic brain injury demonstrate the bidirectional communication of the CNS to the gut with postinjury dysbiosis, gastrointestinal-associated lymphoid tissue–mediated neuroinflammatory responses, and bacterial-metabolite neurotransmission. Similar findings are being elucidated in SCI as well. Experimental interventions in these areas have shown promise in improving functional outcomes in animal models. This commensal relationship between the human body and its microbiome, particularly the gut microbiome, represents an exciting frontier in experimental medicine.

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At the cellular level, spinal cord injury (SCI) is a polyphasic insult.⁴³ First, direct mechanical injury results in damaged cell membranes, ischemia, and accumulated neurotransmitters. Secondary processes increase the size of the injury, causing cell death propagated by cascades of free radical production, energy failure, immune cell invasion, and glutamatergic excitotoxicity. In the chronic stages of SCI, astroglial scar formation, continued demyelination, syrinx formation, and other regenerative processes can continue for years at the cellular level.

It should be no surprise that the multiple injury phases involve systemic modifiers. At least a part of this process involves the brain–gut axis, the major bidirectional communication pathway between the brain and the gastrointestinal (GI) system that incorporates both afferent and efferent signals involving neuronal, hormonal, and immunological pathways.⁴² To date, most of the existing research following CNS injury pertaining to the micro-

biome involves studying changes to the gut microbiome following stroke or traumatic brain injury (TBI).^{4,26,38,39,42,48,49,52} Thus, there is a paucity of research involving SCI and the bidirectional influence on the gut microbiome. The purpose of this paper is to review the literature published on the microbiome and highlight the changes that occur following SCI. By developing an understanding of the role that the microbiome has in potentiating inflammation or protecting against secondary injury, it may be possible to enhance patient care, identify therapeutic targets for neuroprotection, and potentially improve outcomes.

Brief History of the Human Microbiome

Shortly after the Human Genome Project was completed, the Human Microbiome Project became the next frontier for understanding the human “supra-organism.”⁴⁴ The microorganisms living within and on the human body

ABBREVIATIONS GALT = GI-associated lymphoid tissue; GI = gastrointestinal; IL = interleukin; MIP-2 = macrophage inflammatory protein 2; SCI = spinal cord injury; TBI = traumatic brain injury; TNF- α = tumor necrosis factor- α .

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outnumber human cells, and symbiotic relationships are being explored in healthy and disease states.³⁵ The microbiome is recognized as a source of genetic diversity, component of host immunity, and effector on nutrient and drug metabolism.¹⁵ Knowledge of the taxonomic and functional composition of the gut microbiome has grown exponentially with the use of 16s ribosomal RNA encoding and metagenomic technologies, which have replaced the previous generation of culture-based approaches.^{1,15} Early investigations into the microbiome and disease states were related to obesity and inflammatory bowel disease.^{24,45} Subsequently, non-GI correlations with pathological conditions were noted in cardiovascular disease, asthma, and even autism.^{12,19} As the field has progressed, the gut microbiome has been linked to essentially all organ systems.

Microbiome and Injury

The link between surgical critical illness, systemic inflammation, and pathological changes in gut bacteria is decades old.^{7,27} Trauma, burns, sepsis, and surgical injuries can result in a constellation of dysfunctions in intestinal transit, expression of nutrient transporters, and mucosal barrier changes.³⁰ These alterations allow bacterial dysbiosis in the gut, which leads to the development of a systemic inflammatory response. Unsurprisingly, the maintenance of the integrity of the intestinal mucosal epithelium is multifactorial; gut bacteria may be the most important factor, along with intraluminal contents, pancreatic enzymes, hepatobiliary factors, and the enteric and central nervous system.^{13,30,36}

An unstable microbiome during injury more closely resembles that of an infectious state. The intestinal microbiota becomes fundamentally disrupted within hours of injury, with quantitative reductions of 1000-fold compared to healthy controls.¹⁸ Furthermore, among patients with sepsis, the composition of the gut microbiome dramatically changes such that communities of ultra-low-diversity pathogens emerge and trigger further virulence in the host.⁵³ Krezalek and colleagues noted that these disturbances were predictive of sepsis-associated mortality in their patient population.²⁵ Intestinal wall damage leads to inflammation of the mucosal barrier, which results in an altered GI tract with higher levels of nitrate and abnormal gradient of mucosal oxygen.⁹ As a result, the changes to the environment lead to a proliferation of Proteobacteria such as *Pseudomonas aeruginosa* and *Escherichia coli*, as well as certain Firmicutes including *Staphylococcus aureus* and *Enterococcus* species.

Burn injury is an obvious model for gut biome research given its prevalence and clinical observations that 75% of all deaths after severe burns are related to sepsis and infectious complications.⁸ Demonstrated in both human and murine models, burn injury alters the structure of the intestinal biome to promote gram-negative aerobic bacteria and leads to increased intestinal permeability to allow bacterial translocation to normally sterile sites.¹⁰ Overall, healthy bacteria such as those in the Lachnospiraceae family are reduced, while Enterobacteriaceae family flourish, species often found in septic patients.^{8,10} The integrity of the gut epithelium itself may have a fundamental role

in the development of sepsis following burn injury. These findings support the concept that trauma in many forms causes disruption in the gut, which in turn acts in a pathological manner.

The Feed-Forward Effect on the CNS–Gut Axis in Injury

The use of stroke models to investigate the brain–gut axis has given interesting insight into a brain–gut feed-forward loop.³⁷ Murine large-volume stroke models demonstrated increased gut permeability and bacterial translocation associated with clinical poststroke infections.^{4,40} In human patients, Yamashiro et al. found that ischemic stroke is associated with gut dysbiosis and systemic inflammation.⁵¹

In contrast to stroke, TBI appears to offer independent disruptions to intestinal integrity. Clinically, TBI can result in chronic dysfunction of the GI system, and disability.²² Murine-TBI models have shown ultrastructural gut changes within 3 hours of injury; epithelial cell tight junction integrity is impaired, and tight junction proteins that normally maintain close cellular contacts (claudin and occludin) have decreased expression.¹⁷ Experimental blockades of these protein changes ameliorate post-TBI increases in gut permeability.^{3,5} This diminished integrity and loss of tight junction protein expression may be caused by increased tumor necrosis factor- α (TNF- α) and myosin light chain.^{32,34,46} However, Ma et al. found that moderate-level TBI in mice elicited intestinal histopathology independent of inflammation.²⁶ They observed histopathological increases in mucosal depth and smooth muscle thickening, increased expression of glial fibrillary acidic protein and *SOX10* expression (associated with enteric glial cell activation), and functional increases in paracellular flux at 28 days postinjury.

One potential explanation for the ability of the brain to affect gut integrity independent of inflammation involves the enteric nervous system and neuroendocrine signals.^{13,21,30} One part of the enteric nervous system, vagal motor activity, has been shown to be impaired after TBI, and intervention via closed-loop stimulation has shown improvement in functional recovery in two different murine studies.^{2,31} Such results show promise in elucidating the CNS–gut axis and its role in secondary processes after brain injury.

New investigations are now underway that examine the relationship of microbiome and SCI, particularly the concept that the brain–gut axis may be a component of a larger CNS–microbiota–gut axis. Kabatas et al. demonstrated temporal GI changes after SCI in a rat model.²⁰ They found decreased gastric emptying and slowed GI transit 4 weeks after SCI. Notably, they were unable to show such changes in the acute phase (within 48 hours). This delay was linked to neurotransmission, as demonstrated by slowing of the inhibitory junction potentials regulated by decreases in GI vasoactive intestinal polypeptide and neuronal nitric oxide synthase. Lastly, they demonstrated histopathological thinning of the GI mucosa and muscle layers at 4 weeks postinjury.

O'Connor et al. used a rat SCI model to investigate

microbiome and proinflammatory cytokine changes.²⁹ At 8 weeks postinjury, their SCI group showed significantly higher levels of Bifidobacteriaceae and Turibacteraceae families, with depletion of *Clostridium saccharogumia* species compared to controls. The analysis of concomitant proinflammatory cytokines found that interleukin (IL)-1 β , IL-12, and macrophage inflammatory protein 2 (MIP-2) were significantly correlated with microbiome changes in animals with SCI. MIP-2 and TNF- α were significantly higher at the 4-week analysis than at the 8-week analysis after SCI.

Kigerl et al. made similar observations.²³ These investigators demonstrated gut dysbiosis occurs with SCI, and that premorbid dysbiosis leads to poorer neurological outcomes and neuropathological findings postinjury. Analysis of proinflammatory cytokines also found increased expression of TNF, IL-1 β , and IL-10 in the mesenteric lymph nodes by postinjury day 7. GI-associated lymphoid tissue (GALT) inflammatory response was marked by increases in B lymphocytes, CD8-positive T lymphocytes, dendritic cells, and macrophages with reductions in CD4-positive T lymphocytes. Clinical investigation into human gut dysbiosis after SCI seems warranted based on these laboratory findings in animal models.

The Feedback Effect on the Brain–Gut Axis in CNS Injury

The more surprising feedback effect of the gut on the CNS has been demonstrated in several experiments. Ma et al. found mice subjected to a bacterial-diarrhea infection model 4 weeks after TBI showed increased astrocyte and microglia inflammation with corresponding increases in brain lesion sizes.²⁶ Our group has recently shown that significant changes in the gut microbiome are evident within 2 hours following a moderate TBI induced in rats. A decrease in the relative abundance of traditionally beneficial bacteria was observed, specifically among bacteria within the Firmicutes phylum, with concomitant increases in families that contained pathogenic bacteria including Bacteroidaceae in the phylum Bacteroidetes and Enterobacteriaceae and Pseudomonadaceae in the phylum Proteobacteria. Moreover, the changes observed in the bacterial diversity correlated with post-TBI, MRI-determined lesion volume and loss of behavioral function in the animal model.²⁸ Two laboratories have independently demonstrated the brain–gut pathway after stroke, which affects gut dysbiosis, neuroinflammatory injury response, and functional outcomes.^{4,39} Singh et al. showed germ-free mice recolonized with poststroke microbiota had larger stroke lesions. Conversely, Benakis et al. used antibiotics to alter the composition of the microbiota to reduce infarct volumes in mice subjected to transient middle cerebral artery occlusion. Both groups found benefit of therapeutic fecal transplantation on stroke lesions and functional outcomes. Certain probiotic treatments were neuroprotective in murine stroke models.⁴¹ These experiments demonstrated immune cell-dependent mechanisms as evident by intestinal IL-10, IL-17, regulatory T cells, T-helper, and $\gamma\delta$ T-cell changes in intestinal and CNS tissues correlating with stroke lesions and functional outcomes (Fig. 1).

The first study examining this link in SCI was by Kigerl et al.²³ Antibiotic-induced dysbiotic mice showed more severe SCI lesions; they showed that resuscitation with probiotics improved postinjury recovery. One potential mechanism opined by the authors involved reductions in the amount of available neuroactive bacterial metabolites, including serotonin precursors and short-chain fatty acids. This comes from work that has shown these metabolites can bypass the blood-brain barrier to influence CNS structure and function and induce T-cell differentiation in the gut, which plays a role in the CNS injury response.^{11,14,33,47}

Taken together, these findings indicate that SCI elicits a dysbiosis response in the gut, and suggests the gut, in turn, can modulate the neuroinflammatory response in the CNS. Given the influence of the composition of the gut microbiome on the homeostasis of the CNS, the interplay of these bacterial populations in digestion, immunity, disease, and health is of great interest. Developing further understanding of this interplay in healthy and pathological conditions following CNS injury may prove invaluable.

Limitations

In microbiome research, it is paramount to closely examine causation and mechanisms over correlation and bystander effects.¹⁶ Additionally, germ-free or antibiotic-treated mice have often been used as controls, raising important questions as to the validity of these models for comparison. As is the case in most animal research, consideration must be paid to rodent and human differences including anatomy, diet, baseline/healthy microbiota, immunology, comorbidities, and social dynamics.⁴⁹ Although some laboratory successes have been published, translational trials have tempered enthusiasm. Studies into the benefit of probiotics in TBI remain inconclusive.⁶ Fecal microbiota transplantation has been attempted for Parkinson's disease, multiple sclerosis, autism, and chronic fatigue syndrome, with little explanation of therapeutic mechanism.⁵⁰

Future studies investigating the temporal changes in the microbiome after SCI could offer insight into the many comorbid conditions faced by patients years after SCI. Changes in neuroenteric innervation, gut mobility, neurogenic bladder, and even locomotion may lead to microbiome changes that predispose patients with chronic SCI to infections including septicemia, urinary tract infections, *C. difficile* colitis, and decubitus ulcers.

Conclusions

Changes in the gut microbiome have been demonstrated in a wide variety of injury models and appear to be affected by an array of local and systemic phenomena. Bacterial translocation and sepsis following polytrauma and burn injuries illustrates the impact of the microbiome following systemic injuries. A similar “pathobiome” can be seen following CNS injury, with the potential for targeted interventions to mitigate secondary damage via the brain–gut axis and altering the neuroinflammatory feedback loop. Acute phase changes including bacterial metabolites, intestinal permeability, and GALT-associated immunity warrant further exploration as intervention points in SCI. Crossover applicability in treatments for SCI, TBI, and

FIG. 1. CD4-positive (CD4⁺) regulatory T cells (T_{reg}) serve as a fulcrum in the balance of proinflammatory and antiinflammatory immune modulation. In the gut, microbiota are sampled or may translocate, resulting in an immune response. The CD103-positive dendritic cells (CD103⁺DC), a type of antigen-presenting cell of the intestinal mucosa, have been found to drive the proliferation of T_{reg} cells while the microbiota is in a steady state. Subsequent activity by T_{reg} cells results in suppression of an inflammatory response by multiple mechanisms. A central focus of the current stroke research is the T_{reg} cell-mediated influence on γδ T cells. γδ T cells have the potential to cause either inflammatory or antiinflammatory effects, but the influence of T_{reg} cells while gut microbiota remain in a steady state urges a reduced inflammatory state. T_{reg} cells additionally produce IL-10, further suppressing T helper (T_h)1 and T_h17 responses.

stroke via the gut microbiome is possible. Enthusiasm for new applications of this science must be tempered while mechanisms are elucidated to exclude correlation and bystander effects.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Wallace, Sayre, Patterson, Nicholson, Grandhi. Analysis and interpretation of data: Grandhi. Drafting the article: all authors. Critically revising the article: all authors. Reviewed submitted version of manuscript: Wallace, Hilton, Grandhi. Approved the final version of the manuscript on behalf of all authors: Wallace. Study supervision: Sayre, Hilton, Grandhi.

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