NEURODEGENERATIVE DISEASES AND THE MICROBIOME: LESSONS LEARNED FROM MOUSE MODELS

by Alexander C. Maue, Ph.D.

Humans play host to a community of trillions of microorganisms collectively termed the microbiome. Investigations into the microbiome and how it impacts human health represent one of the most rapidly growing fields of study. A simple Pubmed search for "'microbiome" demonstrates a greater than 700% increase in the number of publications from 2010 to 2017. During this time period, key links between the microbiome and various conditions and disease states have been demonstrated including, but not limited to, cancer progression and treatment, metabolic disorders and inflammatory bowel disease.

The gastrointestinal tract represents the largest reservoir of microbes within the body, with the colon possessing up to 70% of all the organisms comprising an individual's microbiome[1]. Thus, a high concentration of microbiota exists in close proximity to the enteric nervous system (ENS). The ENS comprises greater than 100 million nerves residing within the lining of the gut[2]. Research has shown that the intestinal microbiome regulates the functions of the ENS and provides feedback to the central nervous system (CNS)[3].

It is well established that a bidirectional pathway of communication exists between the gastrointestinal tract and the CNS, which is critical for maintaining homeostasis and is coined the gut-brain axis. This close relationship has also led some to term the gut as the 'second brain'[4]. Disruptions to this bidirectional pathway have been demonstrated to have impacts on satiety, behavior, stress responses and brain biochemistry[4]. As more evidence continues to accumulate, it appears that shifts from a healthy, normal microbiome composition to an altered form, defined as dysbiosis, can disrupt the gut-brain axis, is associated with disease states and may be an early event in pathogenesis.

The laboratory mouse has served an important role in demonstrating the impact of the microbiome on the gut-brain axis, in part owing to the availability of germ-free animals, homogenous genetics of inbred strains, knockout and transgenic mice, and the relatively low cost of performing studies. To date, there remains limited high-quality data regarding changes in the composition of microbial communities or production of microbial-derived metabolic products in human patients with brain or gut-brain disorders[5]. Furthermore, results from clinical studies with human patients examining the microbiome with regard to neurologic health have largely been correlative rather than focused on either the cause or effects of specific microbial changes. Thus, mouse models continue to be valuable in advancing the field of gut-brain axis research. This article reviews key findings on the role of the microbiome in mouse models of neurogenerative disease. Given the relatively large amount of research being conducted on specific diseases, this review focuses on data generated in models of multiple sclerosis (MS), Alzheimer's disease (AD) and Parkinson's disease (PD).

Multiple Sclerosis

MS is a demyelinating disease whereby the insulating cover of neurons in the CNS is damaged. The impact of this damage is that the relay of messages within the CNS is altered or stopped completely, resulting in the development of neurological symptoms that vary in type and severity. MS is generally characterized by relapsing and progressive forms. To date, the etiology of MS remains unknown, but it is commonly suspected that a combination of genetic and environmental factors contributes to its development[6]. Hallmarks of MS include both demyelination and inflammation.

To study the experimental induction of MS, researchers have utilized mouse models of experimental autoimmune encephalomyelitis (EAE)[7,8]. Generally, in mouse models of EAE, C57BL/6 or SJL mice are immunized with a CNS-specific antigen in combination with a powerful adjuvant to break immunologic tolerance and drive an autoimmune response, resulting in the demyelination of nerve cells. Affected animals display both cognitive and motor deficits that mirror aspects of MS. Recently, the use of these models has begun to incorporate examinations of the microbiome, and have delineated potential roles for it in the initiation and progression of disease.

The commensal flora has an important role in maintaining immune homeostasis. Ochoa-Reparez et al demonstrated that gut microbiota are a critical factor in the development of regulatory T cells, a population of immune cells that modulate inflammatory responses and suppress autoimmune responses[9]. In this study, prior to the induction of EAE, a study group of animals was administered antibiotics in their drinking water for seven days to reduce the gut flora. Compared to control animals, mice that had their gut microbes ablated by treatment exhibited a significantly lower disease score. This finding was consistent between SJL and C57BL/6 disease models. To understand the mechanism(s) underlying a reduction in disease, the researchers examined the cytokine responses and populations of cells in immune tissues. Treatment with antibiotics led to a reduction in the production of proinflammatory cytokines. Furthermore, the research team observed an increase in the presence of regulatory T cells and production of the cytokine interleukin-10 (Il-10), which are key factors in dampening inflammation.

Additional research has shown that either specific gut microbiota or microbial compounds can affect the course of EAE. Germ-free mice have significantly attenuated EAE disease compared to animals with a more conventional microbiome[10]. In standard specific pathogen free (SPF) mice, treatment with Lactococcus spp. ameliorated EAE severity by inducing regulatory T cells that produced Il-10 and reduced the expression of pro-inflammatory cytokines[11]. Similar effects were reported with a strain of *Pediococcus acidilacti*[12]. *Bacteroides fragilis* is a bacterium with known immunomodulatory effects. Specifically, the production of a polysaccharide, polysaccharide A (PSA), by *B. fragilis* induces the production of suppressive regulatory T cells that can provide protection against disease[13]. In contrast, Lee et al showed that the addition of specific bacteria can exacerbate disease development[10]. The association of germ-free mice with segmented filamentous bacteria (SFB) led to the development of a population of T cells expressing Il-17, a cytokine which drives inflammatory responses and disease following EAE induction.

Alzheimer's Disease

Alzheimer's disease (AD) is a degenerative brain disease and the most common form of dementia. It is estimated that upwards of 20 million people worldwide are living with AD[14]. Like other neurodegenerative diseases, the etiology of AD is unknown but thought to be caused by a combination of genetic and environmental factors. A disease that typically manifests later in life, initially with short-term memory loss, progresses with cognitive and functional impairments that eventually lead to loss of life. With life expectancy on the rise globally, concomitant with an increased incidence of AD, this disease remains a major burden on human health.

AD is characterized by the extracellular deposition of amyloid-β (Aβ) peptides in senile plaques and the intracellular accumulation of neurofibrillary tangles[15]. In addition, neuro-inflammation is a consistent pathological feature of AD. Understanding that the intestinal microbiota impacts the gut-brain axis, researchers have searched for links between the microbiome and AD. Several findings suggest that alterations in the intestinal microbiome affect both brain function and disease progression in AD models. Studies utilizing germ-free mice have shown that in comparison to control animals with an intact microbiome, animals devoid of a microbiome possess deficits in non-spatial and working memory tasks as well as reduced expression of brain-derived neurotrophic factor (BDNF), which is critical for synaptic plasticity and cognitive function[16]. Similarly, mice treated with antibiotics to alter the gut microbiota exhibit decreased cognitive function and BDNF expression[16].

Several transgenic mouse lines exist that are useful for modeling AD in that they recapitulate the development of amyloid plaques and/or neurofibrillary tangles[17]. Minter et al utilized the APPSWE/PSlΔ9 mouse to examine the effects of antibiotic-mediated disruption of the gut microbiota on amyloid deposition[15]. Starting at two weeks of age, APPSWE/PSlΔ9 male and female mice were either treated with a high-dose cocktail of antibiotics or a vehicle control by oral gavage for one week before being placed on a lower maintenance dose of antibiotics provided in their drinking water until the study's end. Six months after starting the mice on the treatment regimen, brains were harvested

from a cohort of animals, sectioned and subjected to histology to detect Aβ plaque deposition. Amyloidosis was shown to be significantly reduced at this timepoint in treated male animals compared to non-antibiotic treated control animals and was associated with higher levels of soluble Aβ in the brain. Additionally, they observed reduced levels of inflammatory mediators and reduced inflammation surrounding the plaques observed in antibiotic-treated animals. Interestingly, these same findings were not observed in female APPSWE/PSlΔ9 mice, suggesting that gender differences may exist.

Using the 3xTg-AD mouse model, Bonfili et al. tested the effects of probiotic administration on the development of disease in this model[18]. The 3xTg-AD model is a triple-transgenic mouse that incorporates both amyloidosis and neurofibrillary tangles. In this model, pathology can be detected as early as three to four months of age with regard to the deposition of Aβ peptides. Two groups of mice were administered either water alone or a novel formulation of nine live bacterial strains including *Streptococcus thermophilus*, bifidobacteria and lactobacilli for four months. Mice treated with the probiotic formulation performed better in cognitive tests and exhibited decreased levels of brain damage compared to control animals. Probiotic administration correlated with increased levels of short chain fatty acids (SCFA) in the feces of treated mice and significantly decreased plasma concentrations of pro-inflammatory cytokines. These results are important in showing that modification of the gut microbiome can induce positive effects on neuronal pathways and may represent a future therapy for AD.

Parkinson's Disease

Parkinson's disease (PD) is a progressive neurodegenerative disease that primarily affects motor functions, over time progressing to some behavioral and cognitive problems including dementia. In 2015, PD affected over 6 million people worldwide[19]. Like MS and AD, the cause of PD is unknown and there is no cure. Gastrointestinal dysfunction is an important non-motor symptom in PD patients and may precede the onset of motor symptoms by years[20], suggesting a potential role for the intestinal microbiota in affecting the nervous system. Indeed, a survey of the fecal microbiomes between PD patients and health controls revealed that the composition of the gut microbiota in PD patients is altered[21].

In a landmark manuscript, the Mazmanian laboratory demonstrated that microbiota drives disease progression in a mouse model of PD[22]. The researchers utilized an alpha-synuclein overexpressing (ASO) mouse which is characterized by progressive deficits in motor functions and defects in gut motility. In humans, unregulated alpha-synuclein expression is associated with a higher risk of PD[23]. ASO mice that possessed a complex microbiota displayed significant deficits in motor function compared to wild-type littermate controls harboring the same microbiota profile. Interestingly, when ASO mice were re-derived to be axenic, or germ-free, they exhibited ameliorated deficits in motor function compared to ASO mice with conventional microbiota. These observations were not seen in germ-free wild-type and conventional wild-type mice, suggesting a genetic-microbial interaction.

In PD, motor deficits coincide with the aggregation of alpha-synuclein in the brain. The presence of microbiota in the ASO mouse was associated with increased formation of alpha-synuclein aggregates and fibrils in the frontal cortex of the brain compared to brains examined from germ-free ASO animals. These aggregates were also shown to be immune stimulating, shown by activation of microglial cells in the brain.

Sampson et al. also wanted to determine if active gut-to-brain signaling during adulthood was responsible for the observed microbiota effects on the nervous system or if the influence of the microbiota was established during gestation[22]. To demonstrate that post-natal microbial signals modulate the PD phenotype, germ-free ASO mice were associated with SPF microbiota and wild-type ASO mice were treated with antibiotics to ablate the intestinal flora. The authors observed that antibiotic-treated mice displayed little motor dysfunction and resembled axenic mice. Conversely, germ-free ASO mice associated with SPF microbiota displayed significant alpha-synuclein-dependent motor dysfunction. The authors state that while not ruling out developmental signals, these findings do demonstrate active signaling by microbiota through the gut-brain axis.

SCFAs are microbial metabolites that provide signals that modulate various cell types, including microglia[24]. Sampson et al. showed that SCFAs alone are sufficient to promote alpha-synuclein neuroinflammation[22]. Germ-free ASO and germ-free wild-type mice were fed a mixture of SCFAs and displayed increased levels of microglia activation compared to untreated mice. Significantly impaired motor performance was seen in SCFA-treated ASO mice compared to untreated control mice. Interestingly, when germ-free ASO mice were administered heat-killed bacteria the same deficits in motor performance were not seen, suggesting that microbiota produce metabolites (e.g. SCFAs) that drive the disease phenotype in this PD model.

In perhaps their most remarkable finding, the authors associated germ-free ASO mice with microbiota from either PD patients or healthy controls. Using the same panel of motor performance tests, PD microbiota was shown to drive increased impairments in motor function compared to healthy donor microbiota. These findings demonstrate that in a genetically susceptible host (e.g. ASO), dysbiotic microbiota can contribute to disease development particularly in this mouse model of PD.

References

1. Sekirov, I., Russell, S. L., Antunes, L. C. M. & Finlay, B. B. Gut Microbiota in Health and Disease. Physiol. Rev. (2010). doi:10.1152/physrev.00045.2009
2. Rao, M. & Gershon, M. D. The bowel and beyond: The enteric nervous system in neurological disorders. Nature Reviews Gastroenterology and Hepatology (2016). doi:10.1038/nrgastro.2016.107
3. Mayer, E. a, Tillisch, K. & Gupta, A. Gut / brain axis and the microbiota. J. Clin. Invest. (2015). doi:10.1172/JCI76304.Several
4. Ochoa-Repáraz, J. & Kasper, L. H. The Second Brain: Is the Gut Microbiota a Link Between Obesity and Central Nervous System Disorders? Curr. Obes. Rep. (2016). doi:10.1007/s13679-016-0191-1
5. Simre, M. et al. Intestinal microbiota in functional bowel disorders: A Rome foundation report. Gut (2013). doi:10.1136/gutjnl-2012-302167
6. Glenn, J. D. & Mowry, E. M. Emerging Concepts on the Gut Microbiome and Multiple Sclerosis. J. Interferon Cytokine Res. (2016). doi:10.1089/jir.2015.0177
7. Chu, F. et al. Gut Microbiota in Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis: Current Applications and Future Perspectives. Mediators Inflamm. (2018). doi:10.1155/2018/8168717
8. Constantinescu, C. S., Farooqi, N., O'Brien, K. & Gran, B. Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). British Journal of Pharmacology (2011). doi:10.1111/j.1476-5381.2011.01302.x
9. Ochoa-Repáraz, J. et al. Role of gut commensal microflora in the development of experimental autoimmune encephalomyelitis. J. Immunol. (2009). doi:10.4049/jimmunol.0900747
10. Lee, Y. K., Menezes, J. S., Umesaki, Y. & Mazmanian, S. K. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. Proc. Natl. Acad. Sci. (2011). doi:10.1073/pnas.1000082107
11. Rezende, R. M. et al. Hsp65-producing Lactococcus lactis prevents experimental autoimmune encephalomyelitis in mice by inducing CD4+LAP+ regulatory T cells. J. Autoimmun. (2013). doi:10.1016/j.jaut.2012.07.012
12. Takata, K. et al. The lactic acid bacterium pediococcus acidilactici suppresses autoimmune encephalomyelitis by inducing IL-10-producing regulatory T cells. PLoS One (2011). doi:10.1371/journal.pone.0027644
13. Ochoa-Repáraz, J. et al. A polysaccharide from the human commensal Bacteroides fragilis protects against CNS demyelinating disease. Mucosal Immunol. (2010). doi:10.1038/mi.2010.29
14. Mayeux, R. et al. Epidemiology of Alzheimer Disease. Cold Spring Harb. Perspect. Med. (2012). doi:10.1101/cshperspect.a006239
15. Minter, M. R. et al. Antibiotic-induced perturbations in gut microbial diversity influences neuro-inflammation and amyloidosis in a murine model of Alzheimer's disease. Sci. Rep. (2016). doi:10.1038/srep30028
16. Jiang, C., Li, G., Huang, P., Liu, Z. & Zhao, B. The Gut Microbiota and Alzheimer's Disease. Journal of Alzheimer's Disease (2017). doi:10.3233/JAD-161141
17. Elder, G. a, Gama Sosa, M. a & De Gasperi, R. Transgenic Mouse Models of Alzheimer's Disease. Mt. Sinai J. Med. A J. Transl. Pers. Med. (2010). doi:10.1002/msj.20159
18. Bonfili, L. et al. Microbiota modulation counteracts Alzheimer's disease progression influencing neuronal proteolysis and gut hormones plasma levels. Sci. Rep. (2017). doi:10.1038/s41598-017-02587-2
19. GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet (London, England) (2016). doi:10.1016/S0140-6736(16)31012-1
20. Verbaan, D. et al. Patient-reported autonomic symptoms in Parkinson disease. Neurology (2007). doi:10.1212/01.wnl.0000266593.50534.e8
21. Scheperjans, F. et al. Gut microbiota are related to Parkinson's disease and clinical phenotype. Mov. Disord. (2015). doi:10.1002/mds.26069
22. Sampson, T. R. et al. Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. Cell (2016). doi:10.1016/j.cell.2016.11.018
23. Soldner, F. et al. Parkinson-associated risk variant in distal enhancer of α-synuclein modulates target gene expression. Nature (2016). doi:10.1038/nature17939
24. Kim, C. et al. Neuron-released oligomeric α-synuclein is an endogenous agonist of TLR2 for paracrine activation of microglia. Nat. Commun. (2013). doi:10.1038/ncomms2534

Dr. Alexander Maue is Director of Microbiome Research Services at Taconic Biosciences. Previously he was Head of the Campylobacter Immunology Laboratory at the Naval Medical Research Center. Trained as an immunologist, his research focus was on enteric diseases and the development of vaccines and therapies to prevent illness.

Summary

Neurodegenerative diseases, including MS, AD and PD, are a global health concern accounting for a significant amount of morbidity and mortality. Despite efforts, the causes of these diseases are yet to be discovered. Continued research into the influence of intestinal microbiota on the gut-brain axis shows promise for improving human health. Probiotics and manipulations of the microbiome could potentially be developed as prophylactic measures or future curative therapies. However, researchers should aim to understand the limitations of these models and express care in extrapolating results to the human condition. Owing to their many advantages, mouse models have been instrumental in determining potential mechanisms of disease and future therapies for these and other neurodegenerative diseases. ■