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MULTIPLE SCLEROSIS AND ITS SYMPTOM MANAGEMENT THROUGH SUPPLEMENTATION AND DIETARY PLANNING

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DEDICATION

It is with the utmost pride and pleasure that I dedicate my Honor's Thesis to my heroes: my mom, dad, and not-so-little brother. Without your constant encouragement, support, and tough love when I need it most, I would not be half of the person I am today. I would not have been able to accomplish the feats that I have to this point, nor would I be able to conquer the endeavors that I will. You have each been a perfect example of tenacity and determination in my life. I will be lucky to become half of the people that you each of you are. I am forever grateful for your love and support.

All of my love…always,

Lindsey Jane

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With love,

Lindsey Davis

Abstract

Multiple Sclerosis (MS) is an autoimmune, neuroinflammatory disorder that is characterized by the breakdown of myelinated axons in the Central and Peripheral Nervous Systems. It is a potentially debilitating autoimmune disease that affects almost 1 million people in the United States, and nearly 2.5 million people worldwide. The precise etiology of MS is still being researched, but much progress has been made towards understanding the molecular mechanisms and impactful ways to treat this disease. While there is still no cure, new treatment plans are constantly being orchestrated in effort to alleviate the burden that MS carries. Combination treatment plans have statistically proven to be the most beneficial in caring for the mind and body of MS patients. With new medications such as Larazotide, which aims to control leaky junctions, and dietary systems like the Paleolithic diet, that seeks to reduce saturated fat intake and inflammatory progenitors, the outlook for symptom management of MS is positive.

KEY WORDS: Multiple Sclerosis, HLA-DRB1, demyelination, Paleo, Larazotide, combination treatment, EAE

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Introduction

Multiple sclerosis is an autoimmune inflammatory disorder characterized by the demyelination of axons in the Central and Peripheral Nervous Systems. Diagnosis of this disorder can be difficult to pinpoint, and even harder to treat effectively. Multiple Sclerosis is predicted to impact roughly 915,000 individuals in the United States alone, in 2017.[1] Women are 2 to 3 times more likely to be affected by this disease and there are several environmental, genetic, and immunologic factors that have been identified in the onset of Multiple Sclerosis. Some of these factors include genetic predispositions, exposure to certain viruses, and cigarette smoking.[2] MS affects the individuals through their activities of daily living (ADL), it tears away at their mental stability, their physical stability, and can even put stress on their social relationships. While there is no cure for MS, there is a great need for one. There are many viable symptom management options, but because the exact immunological mechanism that induces Multiple Sclerosis is not known, there is no cure. How then do we move past only symptom management and get to the eradication of progressive axon demyelination altogether?

Multiple Sclerosis is known to be the active demyelination of axons in the Central Nervous System (CNS).[1] The degeneration of the myelin sheath can occur along the spinal cord, cerebral cortex, deep gray matter, and most notably for this study: the white matter of the brain.[1] There are several classifications denoting the severity and pattern of multiple sclerosis manifestation. The first type is Relapsing-Remitting Multiple Sclerosis.[1] This is the most common form of MS, occurring in roughly 85% of presented cases.[3] Here, patients are characterized as having stable neurological time spans with intermittent relapses of acute exacerbations.[1] Over time, the debilitation increases and the body is actively broken down by repeated exacerbations. An exacerbation is simply a flare up, or the worsening of one's medical

condition.[4] The second identified classification of Multiple Sclerosis is Primary Progressive.[1] In this form, the neurological condition of whomever is affected continually worsens; there is no relapse or remittance.[1] There are not typically very many exacerbations, only a few around the time of diagnosis, however steady debilitation is guaranteed. The third category of Multiple Sclerosis is known as Secondary Progressive.[1] In this case, there is an initial pattern of relapse and remittance, but this trend eventually declines into a steady neurologic deficiency.[1] Finally, the newest and last classification of Multiple Sclerosis is known as Clinically Isolated Syndrome.[1] CIS is initiated by some type of preliminary neuroinflammation or brain trauma, which induces MS-like symptoms, and while not initially diagnosed as MS, can manifest into the presentation of chronic Multiple Sclerosis at a later time.[5]

Review of Literature

After extensive research has been conducted, it has been made apparent that a combination-style approach has proven to be the most beneficial in treating individuals diagnosed with Multiple Sclerosis. Treatment through mind and body exercise, dietary planning, and pharmaceutical supplementation have all been looked to for the improvement of daily living in the midst of such a potentially debilitating diagnosis. As investigations are continued and research pushes forward, it is important to expand upon previously established treatment methods and compile these with new discoveries in an effort to eradicate the onset and experience of Multiple Sclerosis.

CNS VS PNS

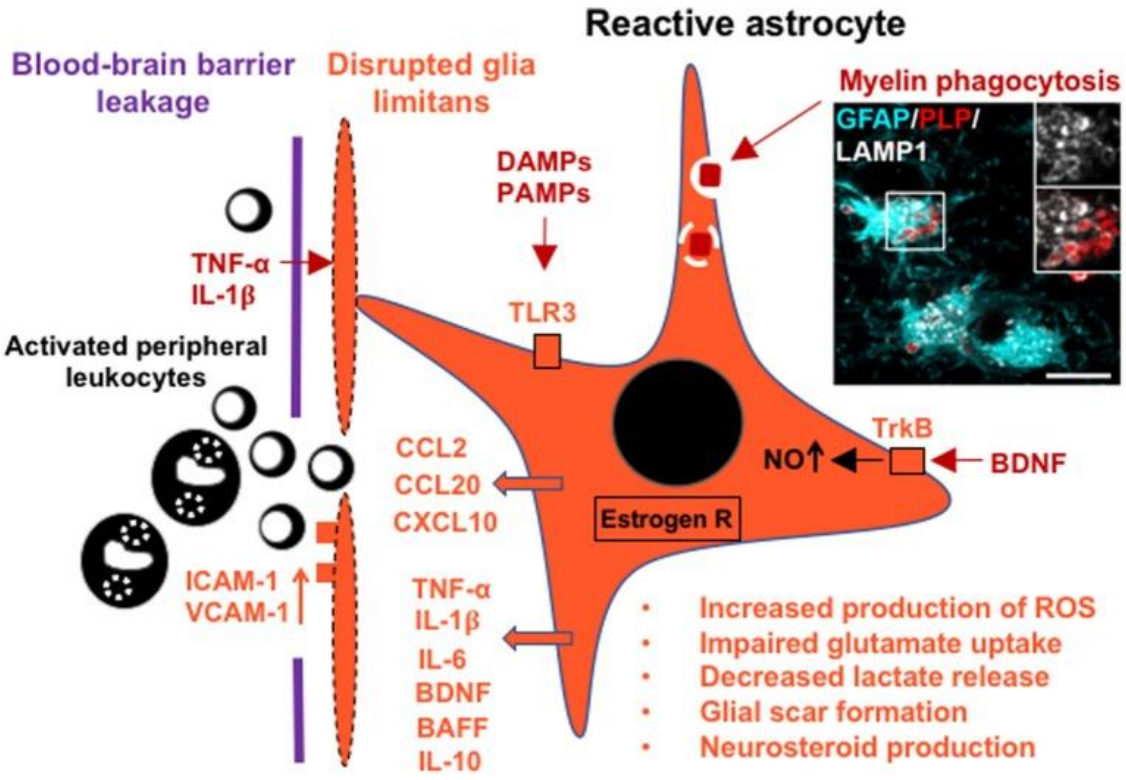
The nervous system is a compilation of two main components: The Central Nervous System (CNS) and the Peripheral Nervous System (PNS). These two systems collectively are responsible for every aspect of our wholistic wellbeing. They control our thoughts, emotions, responses, memories, and every other physiological process that occurs in living beings.[6] The CNS consists of two integral parts. These parts are respectively known as the brain and the spinal cord.[6] These organs are then comprised of two main types of cells: neurons and glia.[7] Glia cells are more numerable than neurons, but neurons are the main components of brain activity. However, neurons would not be able to function in their designated manner if it were not for the supporting glial cells.[7] There are four types of glial cells. They are known as microglia, astrocytes, ependymal cells, and oligodendrocytes.[8]

Microglia are typically known as the garbage men of the Central Nervous System. They are essential to the health and function of the CNS, as they are the acting immune response.[9] As immune cells, microglia release oxygen reactive products and inflammatory molecules in

response to immunological threats.[9] Due to the nature of these cells there is conclusive evidence inclining that microglia are directly involved in the destruction of the myelin sheath that occurs in neurodegenerative diseases, like Multiple Sclerosis.[10]

Astrocytes are a type of supporting glial cell. They make up approximately 30% of the functioning glial cells in the CNS.[11] Astrocytes are the defenders of the brain. They are responsible for eradicating any threat that is posed to the integrity of the CNS health.[12] Their main functions include that of regulating synapses, neurons, processing networks, and cognitive functions.[13] This means that astrocytes are widely responsible for much of the function of the brain. In addition to the aforementioned responsibilities, astrocytes are involved in regulating ionic homeostasis, and inflection of synaptic plasticity.[13] They also act as a secondary barrier behind the Blood Brain Barrier (BBB), which aims to limit the infiltration of peripheral immune modulators that do not belong in the CNS.[11] Astrocytes often are functionally and morphologically connected to oligodendrocytes by gap junctions.[11] In connection with neuronal synapses, the network of astrocytes and oligodendrocytes form a tripartite synapse that helps regulate the conduction of neuronal synapse conduction.[11] Figure 1 demonstrates the morphology and functionality of astrocytes. The action of astrocytes are often triggered by oxidative stressors and inflammatory progenitors.[11] Thus, astrocytes play an active role in Multiple Sclerosis by forming lesions, accumulating lymphocytes, and directly causing structural damage to neurons when their desired function is compromised.[11]

Figure 1 Reactive Astrocyte in Multiple Sclerosis Diagram[11]



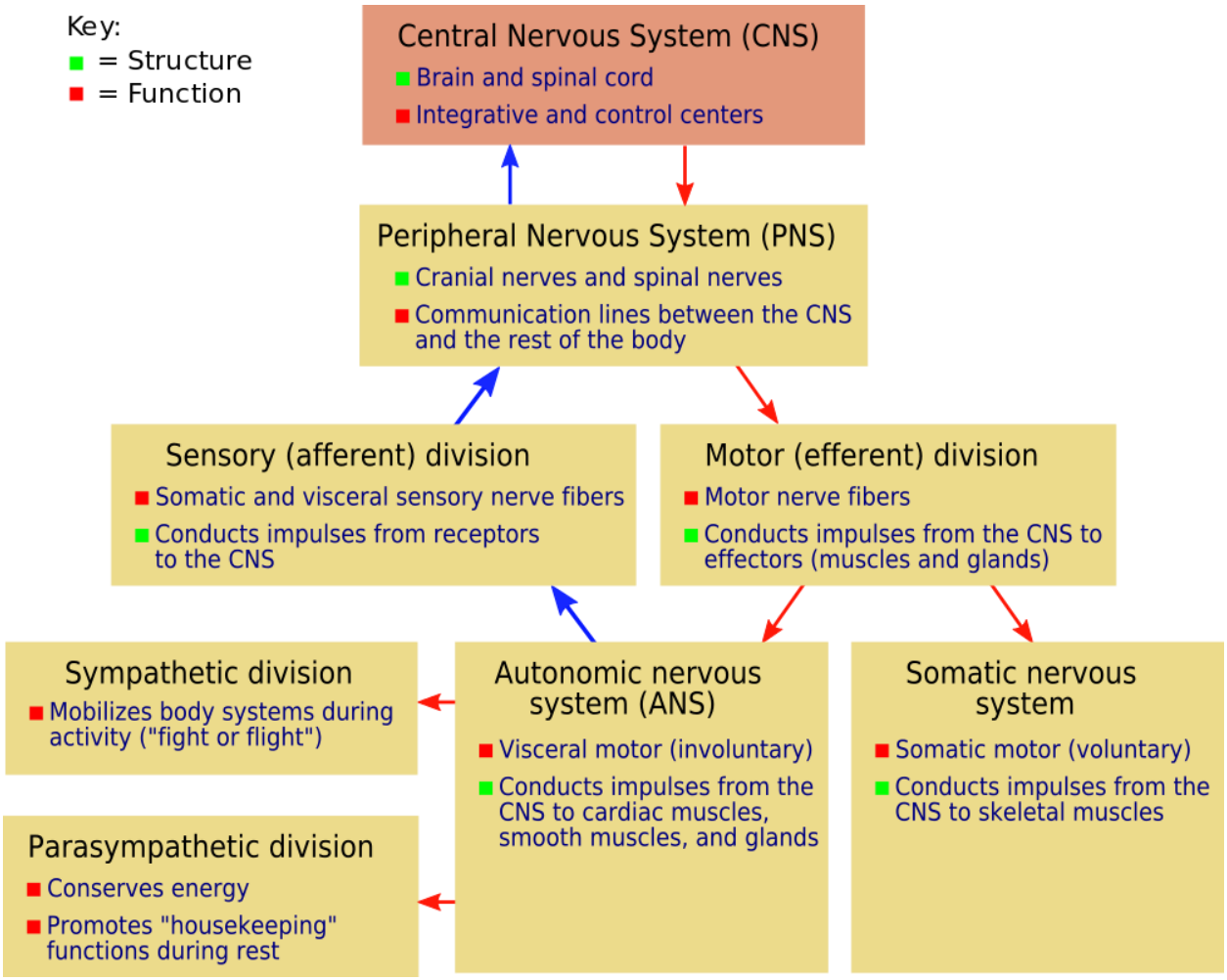
Oligodendrocytes, in comparison to astrocytes are the type of glial cells that are responsible for actually myelinating the axons of neural cells in the CNS.[14] Only five to eight percent of the cells in the CNS have the potential to turn into oligodendrocytes, and of this small percentage, the grey and white matter if the cerebral cortex receive relatively equal distrobutions.[15] Grey matter typically receives a slightly smaller amount of oligodendrocyte progenitor cells.[15] These specialized glial cells myelinate the axons of neurons through continual extraction and retraction of cell body processes to sense neighboring cells and ensure uniform spacing between nodes of myelin.[15] Due to the expensive metabolic activity that occurs while formulating the myelin sheath, oligodendrocytes in general are more susceptible to encountering cytotoxic and exocytotoxic factors like those that occur in and trigger Multiple Sclerosis.[15]

Schwann cells, in conjunction with oligodendrocytes are responsible for myelination as well. However, they myelinate the nervous cells in the Peripheral Nervous System. These cells are radially and longitudinally polarized, which allows for a bilamellar configuration.[16] The double membrane organization allows for the myelin sheath to formulate itself between the two outer-lying lamella.[16] Schwann cells orchestrate this type of morphology through circumferential wrapping around neural axons in the same fashion that oligodendrocytes do.[16]

The PNS in comparison to the CNS consists of the rest of the nervous system; the peripheral nerves that receive signals from sensory receptors and other stimuli and relate them back to the brain (or spinal cord). Figure 2 shows a simplistic breakdown of the different categories of the nervous system. The PNS encompasses all parts of the neural system, with regard to peripheral and autonomic responses.[17] The PNS is primarily composed of 43 different nerve segments.[18] There are 12 pairs of cranial nerves and 31 pairs of spinal nerves that are responsible for the conduction of nerve impulses to the body. The PNS is then divided into two different conduction divisions: The Sensory system, in which nerve impulses are conducted in afferent pathways, otherwise known to receive nerve impulses through sensory receptors and conducted towards the CNS, whether it be to the brain or spinal cord. The other division of the PNS is known as the Motor system, which conducts nerve impulses in efferent ways, or away from the nervous system and into the Somatic or Autonomic nervous systems. The Somatic nervous system is then comprised of all of the parts in the body that are responsible for voluntary control of body movements by the skeletal system.[18] These processes include systems and physiological functions that can be consciously influenced, such as moving our limbs.[19] The Autonomic system on the other hand is responsible for all of the physiological processes that are not usually effected by conscious processes.[19] This system is always active. It is responsible for

regulating the mechanisms in the body that are in charge of things such as breathing, blood pressure, libido, other and metabolic processes.[19] The Autonomic nervous system is in charge of the operation of the sympathetic, parasympathetic, and enteric nervous systems.[20] The sympathetic and parasympathetic systems are both comprised of afferent and efferent pathways, thus they are capable of relaying sensory input and motor output to the Central Nervous System.[20]

Figure 2 Breakdown of CNS and PNS[21]



Action Potential and Saltatory Conduction

Without nerve signals firing and conduction of action potentials through the nervous system, our body would not function. When a neuron is at rest, it is said to be at resting membrane potential.[22] At this state, all ligand and voltage gated channels are closed, thus creating a negative charge within the cell. Resting membrane potential occurs when there are more Potassium (K+) ions inside the cell compared to the outside where there are more Sodium (NA+) ions.[22] While this is the case, the concentration gradient of ions across the cell membrane is always changing, and does so very quickly. Neurons are partially permeable to Na+ ions. Due to this, Na+ is constantly moving in and out of the cell by way of leak channels.[22] In comparison to Na+, neurons are *very* permeable to K+ ions. As a result, much larger amounts of K+ ions flow in and out of the cell through leaky channels.[22] The ideal state for the cell is at resting potential which is roughly -40 to -90 millivolts (mV), so in effort to maintain this negative state, a Sodium-Potassium pump is continually sending 3 Na+ ions out of the cell and moving 2 K+ ions into the cell.[23]

Action potentials occur when there is a sudden change in charge across the cell membrane[24] There are three main components that trigger an action potential. Before these three things occur, an event must take place that triggers the depolarization of the cell.[24] The first event itself is depolarization of the cell. Depolarization is a positive shift in the charge from resting membrane potential.[25] When this happens, there is a greater influx of Na+ ions into the cell body.[22] Due to the negative charge that is extended in the axon of a neuron, these positive sodium ions are shoved towards the surrounding axon, thus depolarizing it.[22] During this time, the charge of the cell propels higher than the equilibrium charge.

The next event that takes place in generating an action potential is repolarization. Repolarization is the event that brings the cell back down to equilibrium by stopping the influx of NA+ ions, and opening the K+ channels, which allow more K+ to flow out of the cell, bringing the charged down even further.[22] After the cell is brought back down to equilibrium, the cell undergoes the third vital event: hyperpolarization. Hyperpolarization occurs when K+ channels stay open for an extended amount of time. When this happens, the membrane potential plummets lower than the equilibrium charge.[22] After these three events occur, an action potential is generated.

While all of this is happening very quickly, speed and efficiency are vital for the nervous system. In unmyelinated nerve cells, Sodium-Potassium pumps and channels extend all along the length of the neural axon.[26] On the other hand, myelinated neurons only have these pumps and channels in the Nodes of Ranvier.[26] The contrast in necessary activation of these voltage-gated channels is very important here. Due to the necessity of multiple voltage gates needing to be opened through depolarization, the propagation of action potentials in unmyelinated nerves takes much longer than that of myelinated nerves.[26] The conduction of action potentials in myelinated neural fibers is called saltatory conduction.[27]

Implications of MS

As a chronic neurodegenerative disease, Multiple Sclerosis has the capacity to do a significant amount of damage to the physiology of the body. Some physical symptoms of MS include sensory disturbances, gait impairments, vision problems, urinary, intestinal, and sexual dysfunction, cognitive impairment, and vertigo issues.[28] Potential specific vision impairments include loss of sight in one or both eyes, pain with eye movement, or blurred and double vision.[29] Slurred speech is also a potential manifestation of MS.[29]

In addition to the physical detriment that MS has on individuals, the implications of Multiple Sclerosis extend to mental and social health as well. Multiple Sclerosis is comorbid with several different mental health implications. The most commonly connected disorders to MS are Depression and Bipolar Disorder.[30] Major Depression manifests in similar ways that Multiple Sclerosis does. They both produce severe fatigue, and the lack of desire to go out and do things. When they are paired together, the repercussions of depression amplify the lack of drive, lack of energy, declined sexual function, and overall perception of health.[30] An individual's work experience can be effected as well. One of the preliminary steps in obtaining a new job is a survey towards the end of your application asking whether or not you are affected by a disability.[31] Multiple Sclerosis is on this list of disabilities. Granted, you are not mandated to reveal this information, and can opt out of responding, but that induces anxiety about whether or not your employer will notice that you opted out instead of responding with not having a disability. In the self-reporting National Health and Wellness Survey, it was noted that of the 196 respondents that were effected by RRMS, there was a pattern of absenteeism, an increased amount of health care resource utilization, and overall lower work productivity compared to those individuals who were not impaired by MS.[32] It was also made known that as symptoms of MS worsen, so does the lack of productivity and health care resource usage.[32] Multiple Sclerosis is the most common neuroinflammatory disease among working-age individuals (20-60 years).[32] The added stress of dealing with burdens that are out of your own control, with the stress of an average workload on top of these things can only lead to mental, emotional, and financial strain.

Comorbidities of MS

As aforementioned, Bipolar Disorder and Major Depressive Disorder are common comorbidities of Multiple Sclerosis. Major Depressive Disorder, more commonly known as Depression, is characterized by the DSM-5 as either having a loss of interest or pleasure, or a depressed mood with 5 additional criteria for a minimum of two weeks.[33] Some of these symptoms include changes in appetite, trouble sleeping, decrease in energy, feeling worthless or guilty, thoughts or suicide, and difficulty concentrating.[33] Depression is a very serious illness. It impacts 1 in every 15 adults in any given year, and at least 1 in 6 people will experience depression at some point in their lives.[33] It has been found that women are far more likely to experience depression and that one-third of women will endure a major depressive episode at some point in their lives.[33] While this is true, the most typical age of onset for Depression is in the late teens to mid-twenties.[33] In relation to Multiple Sclerosis, it has been identified that there is likely an inflammatory component to experiencing depressive symptoms.[34] With MS being a neuroinflammatory condition, it is not surprising that there be a correlation between the two of these ailments. The location of MS caused lesions have been studied in correlation to depression as well, and it has been identified that individuals with lesions in the brain are more likely to experience depressive symptoms than those that only have lesions in the spinal cord region.[35] While there is a strong connection between symptoms of Multiple Sclerosis and those of Major Depressive Disorder, they are two completely different entities. It is important to monitor the prevalence of depressive symptoms, as they can deteriorate the longevity of an individual already suffering from MS. It is important to keep patients active, engaged, and stimulated.

Bipolar Disorder (BD) is another common comorbidity of Multiple Sclerosis. Bipolar Disorder, which was formerly known as manic depression, is characterized as a mental health

disorder that leads to extreme mood swings, both highs and lows.[36] The highs of Bipolar Disorder are known as manic states in which an individual is likely to appear abnormally upbeat or wired, having increased energy or agitation, heightened distractibility, less of a need for sleep, and an exaggerated sense of wellbeing/euphoria.[36] In extreme cases of mania, an individual may experience psychotic episodes, and in this instance would need to be hospitalized.[36] On the other end of the disorder, the depressive symptoms are exactly as to be expected; the same as that of major depression. As it is important to regulate depressive symptoms with MS, it is also vital to regulate the cause and relationship to manic episodes, as both MS and BD trigger such things.[37] It has been found in some cases that the likely connection between Bipolar Disorder and Multiple Sclerosis is due to the increased amount of demyelination an individual experiences.[37] Due to this, and a variety of other factors, individuals with Multiple Sclerosis are two times more likely to develop Bipolar Disorder than the general population.[37]

Molecular Components of MS

One of the most direct linkages to the onset of Multiple Sclerosis include a mutation of the HLA-DRB1 gene.[38] There is a specific locus on this gene, identified as HLA-DRB1*15:01, that is one of the biggest risk factors in developing MS.[38] When this gene is hypomyelinated, or "under"-myelinated, there is a prominence of this gene expression in monocyte carriers.[38] What this means is that when the gene encoding for myelination is already under-myelinated, there is likely to be a continued defect in myelination that is replicated into cells that are continually produced from this genetic mutation.

It is known that HLA class II loci code for molecules that are involved with the formation of peptide antigens to T cells.[38] When this gene is mutated, there is a multiplication of 3 times the likelihood of an individual to develop MS.[38] However accurate this genetic component may

be, only about half of the loci responsible for the deterioration that occurs in MS has been identified.[38] This is due to the genetic variation and possible molecular mechanisms that can take place within each individual. The combination of environmental triggers, and genetic components make the full etiology incredibly difficult to pin down.

Two of the most likely molecular mechanisms involved in the development of Multiple Sclerosis include molecular mimicry of self-antigens and infectious agents, and the bystander activation of autoreactive immune T cells.[39] Self-antigens are any molecule or chemical group that act as antigens and are responsible for inducing antibiotic activity.[40] Both of these pathways are involved with the mutation or manipulation of the HLA-DRB1 gene. The HLA (human leukocyte antigen) gene is specifically responsible for making a protein that plays a vital role in our immune system.[8] It is categorized as a class II beta chain paralog, which is important for illustrating peptides from extracellular proteins, and then presenting antigens to the histocompatibility complex when necessary.[41] The histocompatibility complex is the part of the immune system (present in both humans and mice) that is responsible for binding to peptides of threatening molecules, grabbing them, and presenting them to the immune T-cells to be terminated.[42]

The first mechanism, involving molecular mimicry, occurs when molecules or chemical groups act as antigens and are responsible for inducing antibiotic activity.[40] This can be problematic in the production of healthy brain cells. The HLA (human leukocyte antigen) gene is specifically responsible for making a protein that plays a vital role in our immune system.[8] If the brain's histocompatibility complex is activated beyond reason, attacks begin to occur in healthy regions of the brain, as there are no "unhealthy" regions or molecules to be destroyed. The second mechanism that may induce inappropriate demyelination likely occurs through bystander

activation of T Cells.[39] T Cells are a type of lymphocyte (white blood cell) that is key in immune system responses, adaptive immunity, and protection.[43] When there is an overactivation of T Cells, healthy cells appear dangerous to lymphocytes and the healthy axon of neurons is attacked rather than left alone to function as intended.

Myelination and Demyelination

The myelin sheath was first described in 1717 by Anton van Leeuwenhoek as a 'nervule' with 'fatty parts' around it.[15] Myelinated nerve fibers are vital for saltatory conduction between neurons.[16] Saltatory conduction is the propagation of action potentials on myelinated nerve fibers.[27] In unmyelinated nerves, action potentials are generated through the opening of Sodium-Potassium pumps that span along the entire cell membrane. As mentioned before, in myelinated nerves though, action potentials are generated and conducted through the axon, but Sodium-Potassium channels only exist in Nodes of Ranvier. Nodes of Ranvier are gaps in myelinated regions of the axon.[44] The myelin sheath itself is comprised of several layers of protein and lipid lamella that rapidly conduct nerve signals.[45] The constricted nature of the lamellae help insulate the axons and diminish the leakage of action potentials, rather than losing ions the ions that are essential for generating membrane potentials.[26]

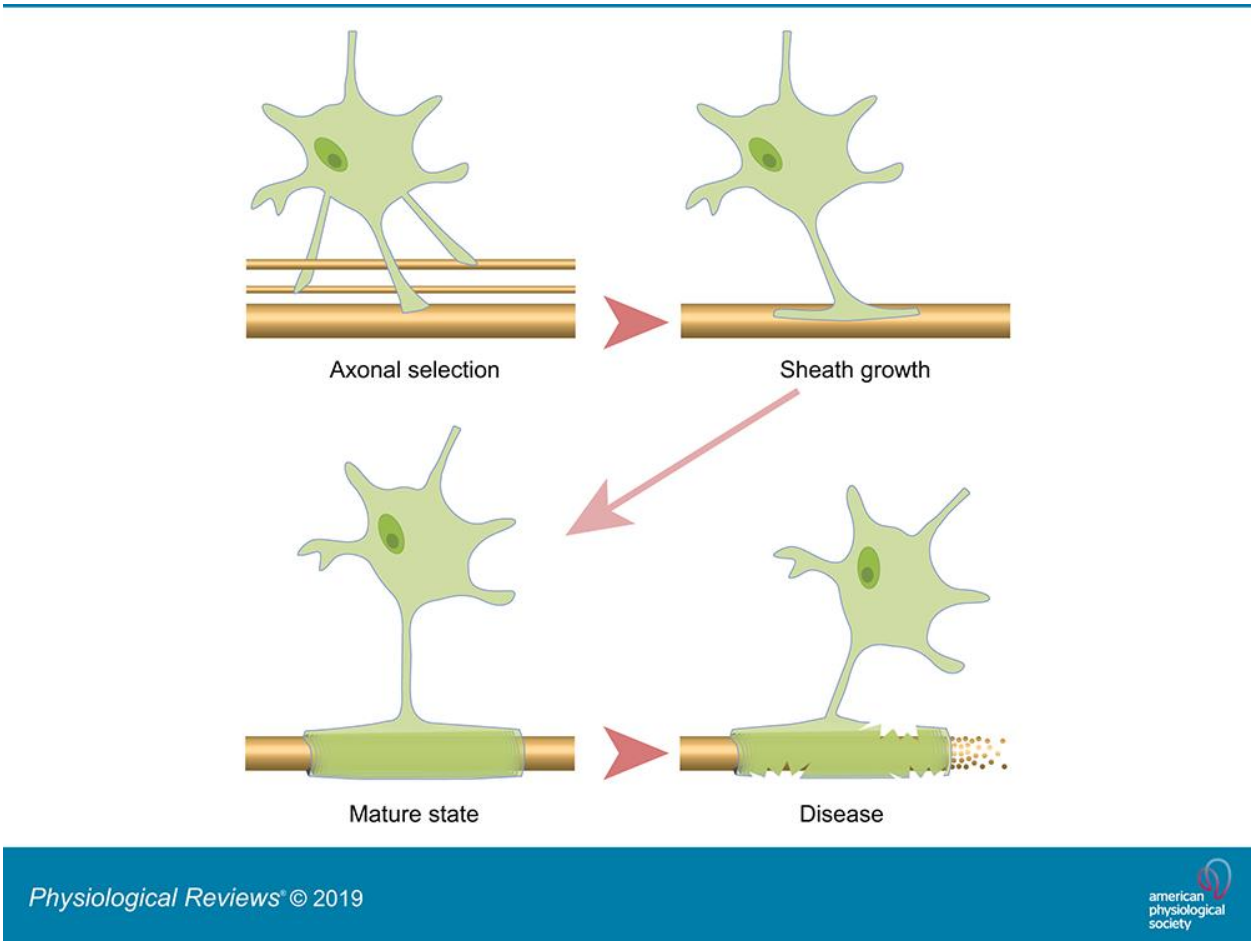
In Multiple Sclerosis, the myelin sheath that insulates and propagates axons for saltatory conduction is deteriorated. This process is known as demyelination. Demyelination is the unraveling of the myelin sheath that insulates axons in the Central and Peripheral Nervous Systems.[46] When this happens, the conduction of electrical signals in the brain are slowed and have the potential to halt altogether, thus causing serious neurological conditions.[46] The myelin sheath is an extended and modified plasma membrane that is wrapped around neuronal axons in a spiral manner.[47] Schwann Cells and Oligodendrocytes are responsible for the formulation of

the myelin sheath. Schwann Cells are in charge of building the myelin sheath in the Peripheral Nervous System, and Oligodendrocytes are responsible for the same in the Central Nervous System.[47] Schwann cells and oligodendrocytes are only capable of myelinating segments of axons, thus between each section of myelin, there is a space known as a Node of Ranvier.[47] Figure 3 characterizes the way Schwann cells and Oligodendrocytes myelinate axons in segmented regions, thus leaving room for Nodes of Ranvier. These nodes are vital for the conduction of electrical impulses. The way that axons, myelin, and their electrical impulses collaborate is through conduction and insulation. In unmyelinated regions, fibers have local circuits that drive ionic currents into active regions of the membrane.[47] These local circuits then, with access to extracellular components, depolarize each section of the "free" axon.[47] In order to continue to the next node, the electrical impulse must travel through the myelinated region. The myelinated region has a higher capacitance, indicating that depolarization/excitement of the axon can occur at a much faster rate than if the axon were not insulated.[47] Essentially, electrical signals flow in local currents to depolarize the axon and create neurological functions. Each section that is to be depolarized is connected by segments of myelin that increase the speed of depolarization and insulate the spaces between each Node of Ranvier.

In Multiple Sclerosis, the myelin that insulates axons and transduces electrical signals, is broken down. This deterioration occurs as a response to T cells infiltrating parts of the Central Nervous System that they do not belong. When this happens, an exaggerated inflammatory response is evoked and initiates the breakdown of the myeline sheath.[48] The demyelination that occurs in MS alters saltatory conduction and slows action potential propagation which poses a threat to blocking neural conduction altogether.[49] In addition to disrupted impulses and neural conduction, demyelination also has a direct effect on axon health and morphology.[48]

When an axon is demyelinated, it has been observed that the conduction of an electrical impulses is blocked from continuing down the axon, and disperses distally instead.[48] This leaves two options for the axon and its potential. One implication is that instead of saltatory conduction occurring, the conduction is continuous and slow down the axon.[48] The other potential result is that there is a continued block of impulse, causing extended demyelination and deleterious chemicals like nitric acid begin to build up.[48] This leads to direct breakdown of the axon.[48]

Figure 3 Myelination[45]



Diagnosing and Treating MS

The onset of symptoms of Multiple Sclerosis can look relatively similar to a variety of other illnesses such as vasculitis, HIV, Lyme disease, thyroid dysfunction, and several other nutritional deficiencies.[1] Specific symptoms include fatigue, decline in vision (diplopia or unilateral optic neuritis, painful eye movements), asymmetric limb weakness, balance issues, awkward gait, sensory disturbances, constipation, and erectile dysfunction.[1] These all can be differentiated from other ailments and targeted to MS through various evaluations.[1] All of these symptoms come to characterization through the part of the CNS that is affected. Multiple Sclerosis, as aforementioned is characterizable by the lesions and demyelination that occurs in multiple areas of the Central Nervous System.[1] Several types of imaging produce the best ways to identify these lesions and give opportunity for a timely diagnosis. MS is a progressive disorder that can be extremely detrimental if left to run its course. Imaging processes such as MRI, Diffusion Tensor Imaging, Functional MRI, Positron Emission Tomography, Spinal Cord Imaging, and Iron Imaging.[50,51] Magnetic Resonance Imaging (MRI) produces a lateral image of the brain which is capable of showing the sizes of lesions in cortical regions and gray matter.[51] MRI is vital for the evaluation of long-term prognosis.[50] The progression of demyelination can be observed through MRI analysis.[50] Figure 4 shows MRIs where lesion formation and growth is evident. Diffusion Tensor Imaging is another form of MRI that allows for diffusion-weighted tracking of molecules through biological tissues.[50] This process allows for quick evaluation of structural connectivity or lack thereof in the brain.[50] Functional MRI allows for evaluation of Cerebrospinal Fluid (CSF) distribution and blood oxygenation. The areas of the brain that are increasingly activated by the CSF and oxygenated blood have consistently been prominent in individuals ailed with Multiple Sclerosis.[50] Positron Emission Tomography allows for the

visualization of the uptake of cerebral glucose consumption, when there is high level of uptake, it can be concluded that that specific region of the brain that has increased utilization of energy by activated inflammatory cells.[50] Iron imaging, is very useful in tracking the demyelination of axons. Iron is the most abundant metal in the brain and is found concentrated in myelin and oligodendrocytes.[51] This imaging process allows for the visualization of oxidative damage.[51]

While the implications of Multiple Sclerosis are vast, so are the treatment options. It is typical for physicians to prescribe a combination of pharmaceutical and nonpharmacologic treatment plans. Some of the most common pharmaceutical treatments include oral administration or injection of corticosteroids and DMTs (dimethyltryptamines).[1] The most prevalent form of orally administered drug is known as Tecfidera (dimethyl fumarate).[52] This drug is designed to help manage symptoms of Multiple Sclerosis and has good clinical evidence supporting its efficacy.[52] It's goal is to produce antioxidative effects and immunomodulatory effects.[52] Both of these are helpful in reducing the sizes of lesions and a decrease in the amount of relapses that occur (in correlation to the amount of time that an individual has habitually taken a DMF supplement).[52]

Another up and coming pharmaceutical option looked at for treating MS is a supplement known as Larazotide. Larazotide is an octapeptide inhibitor that is currently used in the treatment of Celiac disease (CD).[53] CD is a common chronic immune disorder that is triggered by gluten.[54] In other words, CD is a gluten intolerance. This disease acts through an unwarranted immune response to gluten-derived peptides.[55] It has been identified that HLA-type genes are genetic factors in triggering the activation of T-lymphocytes that in turn produce a reaction in the small intestine.[55] The goal of Larazotide in treating CD is to regulate the tight junctions that allow for the undesired gliadin protein to infiltrate the endothelial cells of the gut biome.[54] Gliadin is the

water insoluble protein of the gluten complex that triggers the inflammatory lymphocyte response.[54] In comparison to CD, MS is also affected by HLA derived gene performance and like the gut biome, the BBB is permeated by unwanted antigens that trigger T-cell production. The idea is that Larazotide will work in the same manner for MS as it does for CD: tightening the leaky junctions in barriers that should not otherwise be permeated.

On the other hand, a nonpharmaceutical option that can potentially help in symptom management of MS is a patient-specific dietary plan. Monitoring a patient's diet helps regulate several mechanisms that could potentially be triggers of MS and other health implications such as body weight, cholesterol levels, and other vascular conditions that affect the course of MS.[56] Nutrition management also helps aid in improving the overall health and energy levels of a patient. Actively being involved in the preparation and acknowledgement of the nutrients that are being put in your body is known to make a significant impact on the overall wellbeing of an individual. Several nutrition plans have been implemented as symptom management for patients with MS. The goal of many of these diets is to lower the intake of carbohydrates, increase Omega-3 levels, and increase Vitamin D levels as well. Each of these components have been linked in some way or another to the onset and progression of MS.[57] Vitamin D and Omega-3 are especially important for the sustainability of an individual's overall health, so increasing the wellbeing of a compromised individual is likely to be beneficial.

One specific dietary plan that has been shown to be helpful in regulating inflammatory progenitors is the Paleo diet. This diet incorporates free-range meats, organic fruits, vegetables, and nuts.[58] It aims to avoid processed foods, grains, dairy, and legumes. An important aspect of the Paleo diet is the avoidance of saturated fats which are known to cause inflammation.[56] It is known that red meat is linked to a decreased amount of adiponectin, increased plasma CRP

(which is a biomarker for systemic inflammation), thus it has been observed that a reduction of red meat consumption has led to overall healthier outcomes for individuals.[59,60] Dairy is avoided here as well, as there has been an observed correlation between MS patients and a heightened immune response to dairy antigens.[56] By avoiding such things, it is hopeful that there will be an optimization of mitochondrial, myelin, and neurotransmitter function, and overall longevity of MS patient's lives.[58]

Other forms of treatment (nonpharmacological) include practices that target areas where there are deficits produced by MS.[1] These focus areas include cognitive function, motor strength, mental resilience, physical ability, and lack of drive/energy.[1] Exercise and mindfulness training are great practices for targeting these things. Activities that target physical function are beneficial for the maintenance and prolonged existence of the physical capacity that is known to degenerate as MS progresses.[1] Mindfulness training and cognitive activities on the other hand focus on the mentally beneficial practices that encourage being aware of one's own thoughts, and focusing on being aware of your own presence in the moment.[1]

While there is still no cure for MS, it is important to acknowledge the necessity of generating a healthy state for the mind, body, and spirit. Eating healthy and staying active both mentally and physically are all likely to help combat fatigue and minimize the depression and anxiety that are commonly induced by Multiple Sclerosis. This is why combination methods consisting of both pharmaceutical and non-pharmacological treatments are often prescribed to individuals diagnosed with MS.

Figure 4 T1W1 MRI[61]

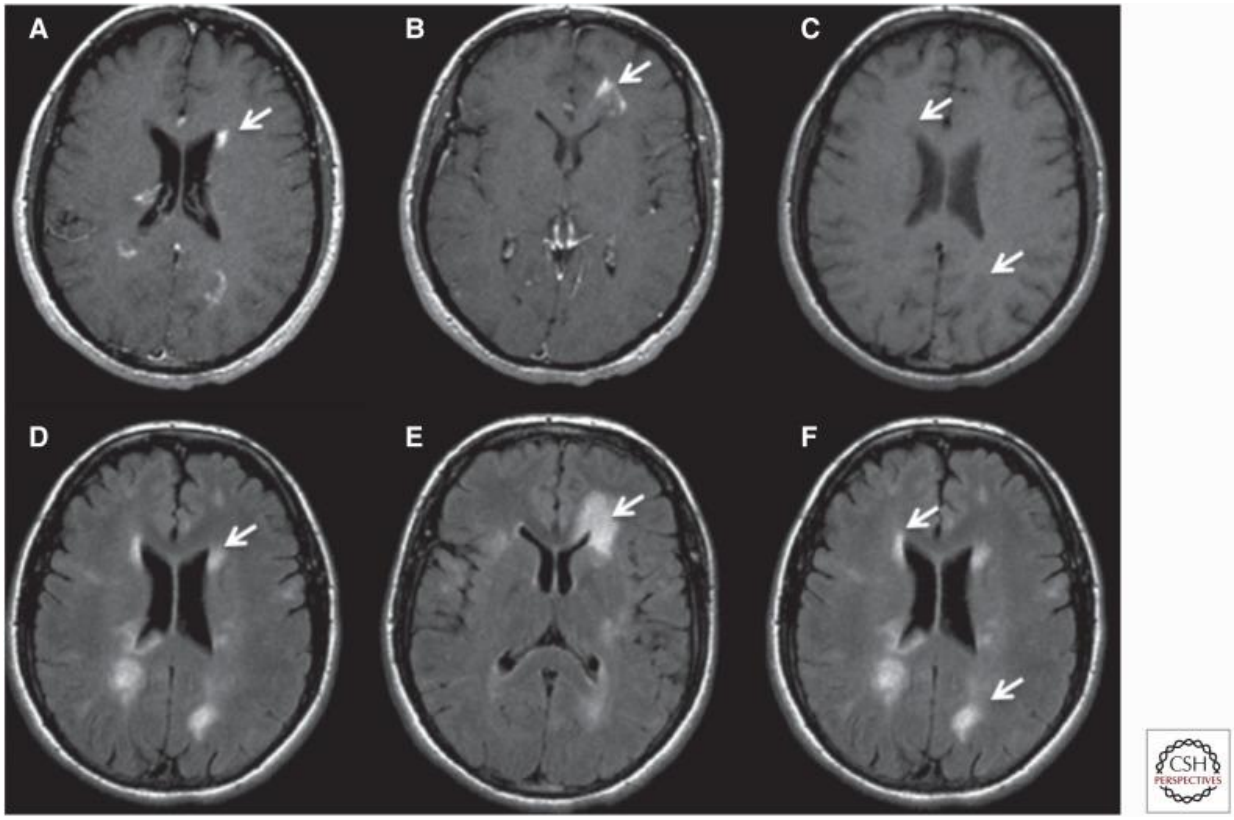


Figure 2 T1-weighted imaging shows lesions in white matter of brain (indicated by arrows)

Call to Action

While treatment and diagnosis processes are not infallible, there is progress being made in the realm of Multiple Sclerosis. Even with this progress though, MS produces a compromised lifestyle for those that are impacted, and it is never guaranteed how much time you have or how your body will individually be affected. In 2016, there was a reported 2,221,118 individuals diagnosed with a prevalent case of Multiple Sclerosis.[62] It is second only to congestive heart failure in terms of medical expenses every year, with a median estimate cost of $31,386.[30] This is a 10.5% increase since 1990.[62] Clearly, even with the advancement that has taken place over the last 30 years, it is essential that more progress be made for patients and their families. While

Multiple Sclerosis is not what one would call a “common” disease, it is prevalent enough to give rise to a call to action. The typical age of diagnosis is between 20 and 40 years of age, the prime of an individual’s lifetime.[29]

The implications of MS do not end with just the patient. The average maternal age in the United States as of 2018, was approximately 27 years old.[63] This age is right in the midst of the average time of diagnosis and onset of MS. What this means for individuals diagnosed in this timeframe is that while trying to start a family or new marriage, they have the potential to experience great changes in their sexual function and ability, accrue great medical debt, and experience even more mental strain. This is only one of the several detriments that MS can impose on an individual and their relations. Progress towards a cure for MS is underway, but more knowledge is essential, and it is vital that treatments become more effective.

Proposal

In an effort to eradicate lesion formation and symptom experiences in those diagnosed with Multiple Sclerosis, it is my goal to implement a combination treatment (non-pharmacological and pharmacological) plan based on what is known as the Paleolithic Diet, and through Larazotide supplementation. The Paleo nutrition plan is known to reduce triglyceride consumption, saturated fat intake, and eliminate most consumable inflammatory triggers. Larazotide is currently only used for experimental purposes in treatment of Celiac Disease, as it has not yet been approved by the FDA for treatment of other disorders. Regardless, the compound itself is an octapeptide inhibitor, whose goal is to regulate paracellular permeability, especially in leaky gap junctions. This is the same desired goal while implementing it in this treatment plan for MS. The idea is that like the gut biome, the Blood Brain Barrier can be manipulated into preventing or allowing ions to pass through or be blocked. Larazotide has been effective thus far in regulating channels in the gut biome, so in this instance, it is the goal that Larazotide block antigens that are unwelcome in the CNS.

Specific Aims

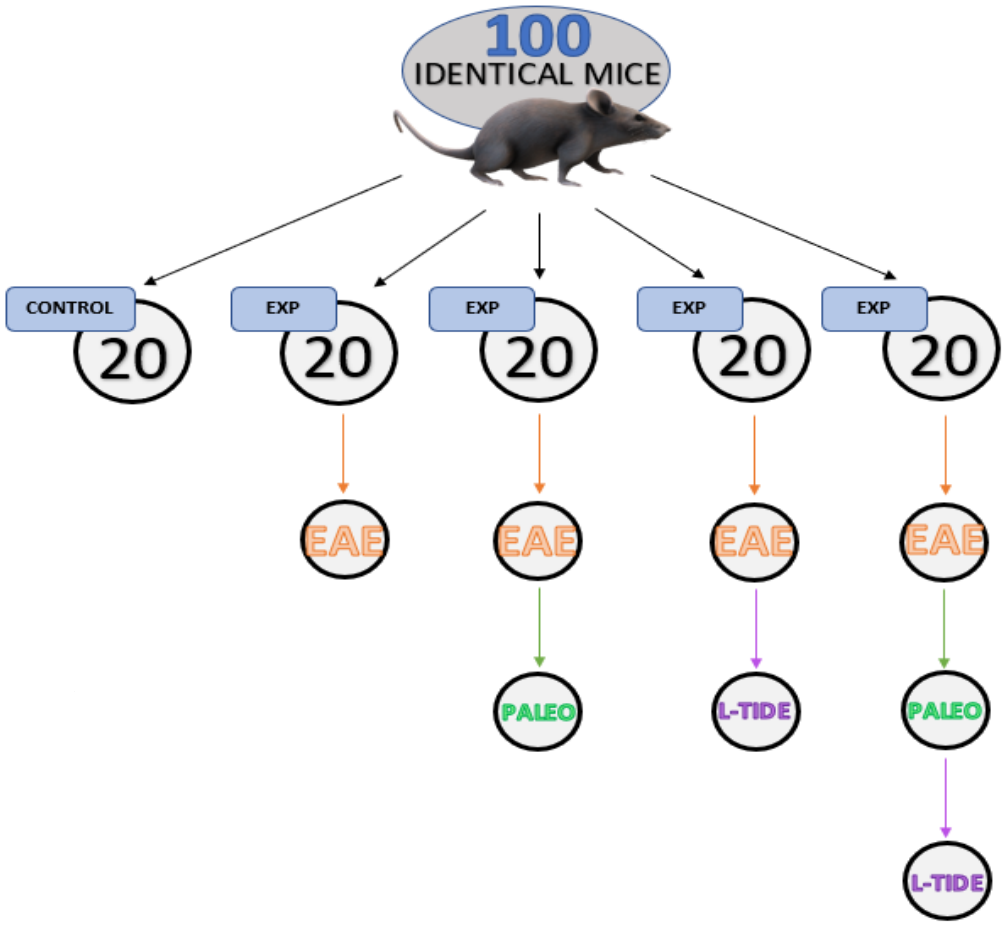
The first specific aim of my proposal is to develop a treatment plan that is successful in diminishing the formation of lesions and symptoms experienced in Multiple Sclerosis. This is to be accomplished with the combination of Larazotide supplementation and Paleo Diet nutrition planning. My second specific aim is to discover whether there is a correlation specifically between implementing the Paleo Diet nutrition plan and lesion and/or symptom development caused by MS. The third and final specific aim that I would like to focus on in this study is whether Larazotide influences the symptom management and lesion formation implemented by MS independently. Further explanation regarding these specific proposals will be demonstrated in the following content.

Methodology

In accordance with human ethical standards, the preliminary trials of this research proposal are to be conducted on rodent models. Mice models were decided upon because they are known to be cost effective, genetically malleable, the genome is fully mapped, and there is a vast amount of morphological similarities in brain structure between humans and mice.[64] In this study, 100 genetically identical mice specimen will be observed in a longitudinal fashion over a span of one month. To ensure seamless comparability, the mice will be split into five separate, equal groups. Figure 5 illustrates the breakdown of each group. The first group of 20 mice will be the control group. This group will be left to conduct a normal life without any manipulation in diet, supplementation, or infection. They will be contained for observation, but nonetheless left to do as they please. The second group of 20 mice, however, will be the preliminary experimental group. This group will be subjected to experimental autoimmune encephalomyelitis (EAE) vaccination.[65] EAE is the most common form of mouse experimental virus intended to mimic autoimmune, neuroinflammatory disease, Multiple Sclerosis. EAE was decided upon as it is one of the most reliable mimicry agents of MS, therefore making it incredibly useful for studying molecular mechanisms of MS and testing developing pharmacological solutions.[65] Vaccination is the preferred method of administration as it allows for guaranteed entrance of the disease. However, the vaccination is not EAE alone. It will be introduced in conjunction with a variety of bacterium and incomplete Freund's adjuvant. Incomplete Freund's adjuvant is prepared from non-metabolized oils and is known to be well-manageable, but evokes a heightened antibody response.[48] This antibody response enables the perforation of the Blood Brain Barrier, thus inducing inflammation and demyelination in the CNS of the mice. The typical onset of EAE symptoms occur in roughly the first five to seven days after introduction.[66]

The next group of 20 mice will also be vaccinated with EAE, but in addition they will be treated with the Paleolithic Diet. The goal of this treatment is to see a reduction of inflammation in neural regions, a diminished amount of lesion formation, and fewer expressed symptoms. Following this group of 20, the next 20 mice will be vaccinated with EAE and treated with Larazotide supplements. The goal of this treatment then is to observe a diminished amount of lesion formation, less inflammation, and fewer symptom expressions here as well. Larazotide is intended to tighten leaky junctions in the BBB, so this should be attainable. The final group of 20 mice will again be vaccinated with EAE, but they will be treated with a combination of both the Paleo Diet and Larazotide supplementation. The goal of this treatment is to see both a reduction of inflammatory factors, a diminished quantity of lesions, and still a decreased expression of symptoms. While the goal of all the treatment groups is the same, the same results are not guaranteed. This is why each treatment method is isolated.

Figure 5 Mouse Group Chart



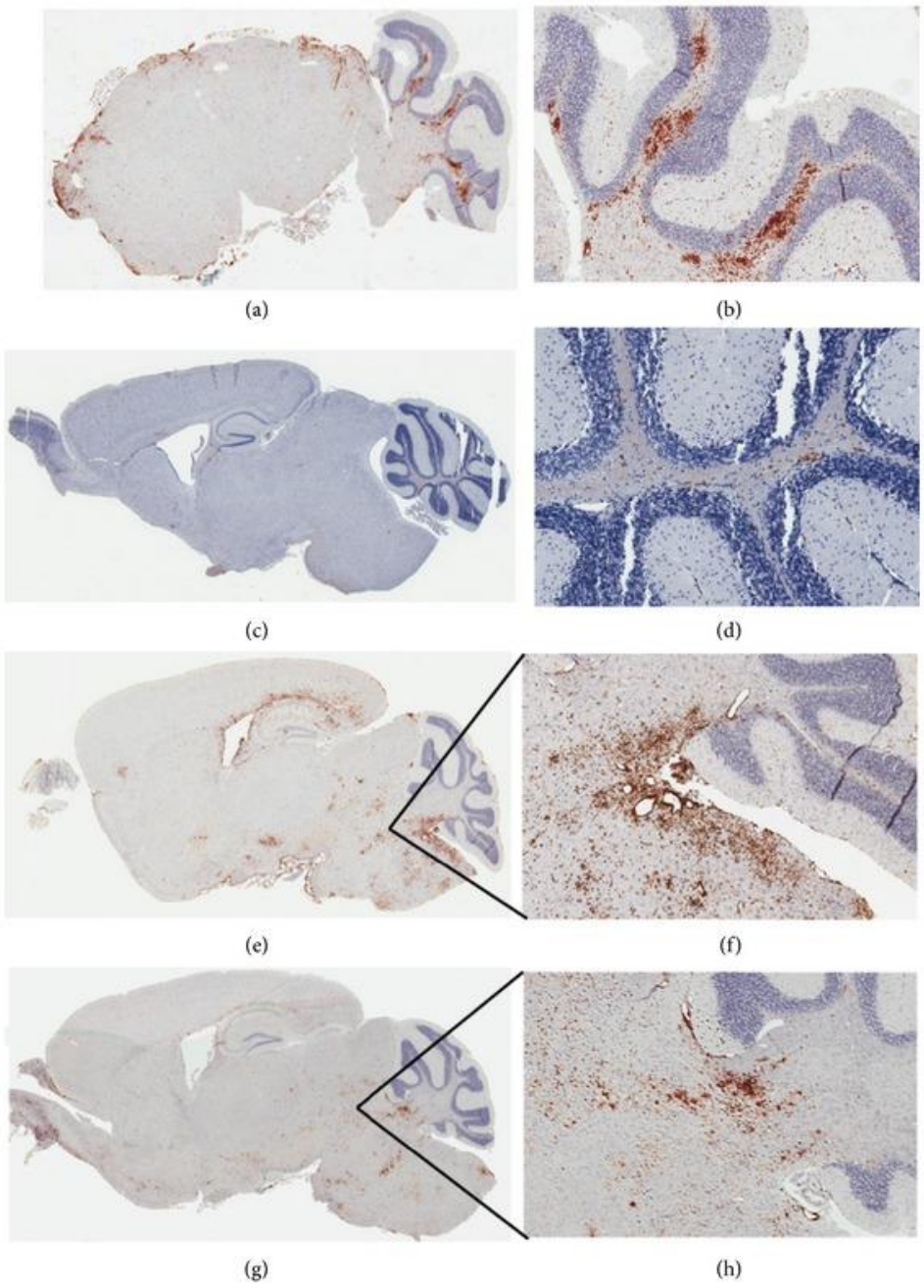
Before any manipulation occurs however, micro-slices of 20 mice will be harvested to observe the unaltered brain morphology. Once the compound has infiltrated the brain, myelin-specific T cells will be activated, thus commencing the deterioration and proliferation of the myelin sheath that insulates the mouse's neuronal axons.

During the course of this month, close observation of the mice will take place. Both qualitative and quantitative measurements will be recorded. Qualitative observation will include direct reporting of the behavior and habits (altered or unchanged) of all 5 mouse groups. Quantitative observation will require the periodic euthanasia of mice from each group. After the

mice have been euthanized, harvested brain slices will allow for the observation of brain morphology and lesion formation or lack thereof. Each region of deterioration and every lesion that is formed or otherwise shrank, will be measured by an electronic caliper to ensure precision and accuracy. The goal is to eliminate as much human error as possible. As symptom management is an extrinsically observable characteristic to document, the mice will continually be monitored, and observable symptoms will be documented.

The goal of the Paleo Diet and Larazotide supplementation is to ultimately reverse or diminish the size of the lesions that have manifested in response to the EAE and reduce the frequency of symptom exacerbations. The use of a control and experimental group will allow for constant comparison between the treated and non-treated groups to find out if there is in fact an effect on the brain morphology and symptom prevalence in these mice. The ultimate desired effect between all observed groups is to identify a factor that is responsible for inflammation reduction, tightening of leaky channels in the BBB that allow for overactivation of lymphocytes, and improve the overall health of the mice affected by EAE.

Figure 3 EAE Induced Mouse Brain[67]



Conclusion

The goal of this proposal is to observe a lessened amount of symptom experiences, a slowed progression or lack thereof of lesion development, and an overall improvement of the wellbeing of individuals affected by Multiple Sclerosis. Incorporating both Larazotide and the Paleo nutrition plan attacks MS from two different angles, which will hopefully increase the likelihood of efficacy.

The goal of Larazotide usage is to mend the challenges that come with HLA-DRB mutation and eliminate inappropriate perforation of the BBB. Larazotide is a synthetic peptide that is currently only used in experimental processes.[68] The role of this protein is to regulate paracellular permeability functions and correct the leaky junctions that allow for transmittance of antigens to places they should not be. Larazotide is most typically used in experimental treatments of Celiac Disease, but in this case with Multiple Sclerosis, leaky junctions need to be corrected so that the blood brain barrier is not susceptible to the attacks it has potential to spring upon on itself.[69] While we have only seen Larazotide used for confronting Celiac Disease, we believe that the same mechanisms can be applied to the treatment of MS. Because we have labeled two mechanisms that are involved specifically with perforation of the BBB, it is our effort to eliminate that extra perforation of unwanted bacterium and antigens that are known to take place. As a peptide inhibitor, we hypothesize that the incorporation of Larazotide will prevent unwanted antigens from crossing the BBB threshold thus eliminating the hyperactivity of the histocompatibility T-cell immune response.

Along with the Larazotide supplementation, implementing the Paleo nutrition plan is designed to help reduce inflammatory triggers. The Paleolithic Diet (Paleo Diet) is plan based on the hunting and gathering style of humans during the Paleolithic period which ranges from

roughly the beginning of time to about 10,000 years ago.[70] During this vast timespan, the hunter-gatherer style of cultivation was prominent. This is what the paleo diet consists of: ingesting items that do not require technological cultivation. Food such as fruits, vegetables, fish, lean meats, wild game, and nuts constitute for the basis of this meal plan. The goal of implementing a strict diet like this is to refamiliarize the body with the compounds that the human digestive system was designed to process. In doing this, many factors that influence Multiple Sclerosis are lessened or eradicated all together. The goal of this incorporation is to include a lessened amount of triglyceride consumption, a lowered amount of saturated fats, and one of the most influential factors being a reduction of inflammation. The reason we believe this method to work is due to the reduction of inflammatory progenitors. If we reduced the amount of red meat and triglyceride consumption, which are both known to be involved in inflammatory processes, then we believe there will be a lessened amount of risk factors that could potentially trigger Multiple Sclerosis exacerbations.

Next Steps

As mice and humans are very different study groups, there will be some necessary modifications to the treatment plan for further studies. In accordance with human ethical standards, if the desired outcome of depletion of lesions and symptoms is accomplished in our mice models, then the goal would be to implement this study in a voluntary human clinical trial. It would be ideal to observe patients with a variety of MS types, but with a concentration on Relapsing and Remitting MS. With this, there will be 4 treatment groups per type of MS. For example, for RRMS there would be one study group that does not receive any type of treatment. This will be the control. The second group will participate in the Paleo diet. The third group will

be supplemented with Larazotide. The fourth and final group will receive the combination treatment, with both the Paleo diet and Larazotide supplementation combined.

Instead of harvesting brain tissue periodically, as done with the mice, it will be necessary to take frequent MRI's and observe lesion size in patients. Symptom experiences will still be observed and recorded as they were with the mice, however more detail will be able to be given by the individual patients themselves. For example, specific feelings and emotional detail will be able to be described. If the desired results are not obtained by the mice however, it would be necessary to reevaluate treatment plans.

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