Review Article

Multiple sclerosis: Unraveling the neuropathology and nechanisms of neurodegeneration

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Abstract

Multiple Sclerosis (MS) is a debilitating autoimmune disease affecting the central nervous system and has been the focus of intense research for the past 20 years. A better understanding of immune-related pathogenic mechanisms is necessary for the development of: (1) novel methods to monitor disease progression, (2) earlier diagnoses through unveiling new biomarkers, and (3) the invention of more effective and personalized MS treatments therapies. Several disease modifying treatments (e.g., natalizumab, fingolimod and beta interferons) have been approved for MS; however, with no cure, the current treatment paradigm has shifted to the notion of 'no evident disease activity'. While many of these Food and Drug Administration approved MS treatments have been shown to reduce the number of relapses and lesions, the paradox concerning MS treatments, namely the overuse of T cell activation as a target, necessitates the need for identifying entirely new contributors to disease pathology. MS pathogenesis has been associated with many theories, ranging from the release of proinflammatory cytokines from macrophages and microglial cells to B cell-derived demyelinating antibodies. As such, these hypotheses should be considered in addition to the role of T cells when developing novel MS treatment plans and identifying aligning biomarkers. Recently, clinical trials (ClinicalTrials.gov Identifier: NCT00040482, NCT00342134, and NCT00342134) investigating autologous hematopoietic stem cell transplantation have demonstrated success in MS. Future research should focus on identifying non-invasive biomarkers, such as blood concentrations of miRNAs, as an indication of underlying pathology and to aid in early diagnosis, tracking disease progression and identifying more effective and personalized MS treatments.

Keywords: Multiple sclerosis; therapies; pathogenesis

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Introduction

The immune system is a complex network of interacting components including cells, tissues and organs that fight invading microorganisms by maintaining the body's lines of defense.[1] The body's ability to do so is likely attributed to its ability to differentiate between "self" and "non-self", resulting in complex immunological communication.[1] When immune surveillance reveals that foreign cells carry "non-self" markers, a response is activated.[1] Millions of cells, each subset with their own orchestrated functions, signal and send information to each other to further activate inflammatory signaling cascades by producing chemicals, such as cytokines.[1] The body recognizes and responds to non-self markers (antigens) by secreting specific chemicals and activating specialized cells to destroy them[1]. If the body is left undefended, foreign invaders such as bacteria, parasites, fungi and viruses can cause infections, leading to a variety of adverse health effects.[1]

The lymphatic system is organized throughout the body to maintain optimal protection.[1] Lymphocytes are specialized immune cells, and reside in lymphoid organs such as the thymus, bone marrow, spleen and lymph nodes, where they are able to impart immunity.[1] Primary lymphoid organs including the bone marrow and thymus are responsible for the development and maturation of lymphocytes while secondary lymphoid organs, such as lymph nodes, tonsils and the spleen, are involved in generating immune responses and fighting infection by interacting with non-self pathogens.[1,2] The bone marrow gives rise to all lymphocytes and following multiplication, they enter the bloodstream for further specialization into mature lymphocytes.[1,2]

The central nervous system's (CNS) ability to regulate immune and inflammatory responses is dependent on the interactions between brain structures and their microenvironment.[3] Specifically, the blood brain barrier (BBB) is a structure within CNS capillaries composed of tight endothelial junctions that limits access of circulating immune cells, antigen presenting cells (APCs) and inflammatory cytokines to maintain the integrity of the CNS.[3] In a systemic inflammatory response, if these cells and cytokines within the bloodstream were to gain entry into the brain, this could have deleterious consequences. In normal conditions, only a small percentage of immune cells, cytokines and chemokines, such as interleukin (IL)-1, tumor necrosis factor alpha (TNF-α) and IL-6 enter the CNS.[3,4] Immune privilege refers to the features present in the CNS to impede potentially damaging immune reactions, such as the BBB and incompetent APCs.[5] Typically, APCs express costimulatory molecules and exposure to foreign antigens in their activation; however, APC incompetence refers to the lack of MHC and costimulatory molecule expression within CNS macrophage populations, which helps regulate T cell responses.[6]

In many neurodegenerative diseases such as Multiple Sclerosis and Alzheimer's Disease, CNS pathology is accompanied by BBB dysfunction which permits penetration through the BBB and elicits a response by resident brain cells, such as microglia and astrocytes.[3] Neurons in pathological states release inflammatory signals, resulting in activation of oligodendrocytes. Oligodendrocytes, the cells that support myelin production are highly vulnerable to ROS-induced

injury (or other specific molecules) produced by immune reactions due to their structure.[3] Specifically, oligodendrocytes have high concentration of polyunsaturated fatty acids, low anti-oxidative enzyme glutathione concentrations and free radical scavengers which cause lipid peroxidation.[7,8] Immature oligodendrocyte progenitors are even more susceptible to oxidative stress due to higher levels of pro-apoptotic proteins.[8]

This invasion of the BBB resembles typical leukocyte infiltration which involves chemoattraction, adhesion and diapedesis across capillary walls. The entry into brain parenchyma occurs in two steps: (1) migration across the vascular wall and (2) glial limitans.[9] Following migration across endothelial cells in a mature CNS capillary, these invading substances interact with glial limitans, also known as astrocyte foot processes, which participate in tight junction function, contributing to normal vessel structure integrity.[10] Endogenous or exogenous leukocyte sources elicit CNS immune responses that may serve pathological functions in multiple sclerosis (MS), namely the damaged areas called lesions, created by infiltrating macrophages and T cells inflicting damage upon oligodendrocytes, myelin, and their underlying nerves.[9] The role of immune cells in the pathogenesis of MS as well as the standard and potential treatments in the pipeline will be explored in this review.

Multiple sclerosis

Multiple Sclerosis (MS) is a neurodegenerative and chronic inflammatory disease of the grey and white matter in the CNS, characterized by demyelination with relative sparing of axons.[11,12] Specifically, the activation of local macrophages by autoreactive T helper cells (Th1) destroys the insulating layer around nerves called myelin.[11] Myelin allows for the transmission of electrical messages along nerve cells and the disruption of these myelin sheaths slows or stops these impulses, resulting in neurological problems. Grey matter contains neuronal cell bodies, dendrites and terminals which participate in synapses, the site of communication between neurons.[13] As the cell body is vital for neuronal life, grey matter damage primarily includes neuronal death as the cell is unable to support its axon or dendrites.[13] White matter, denoted by its colour, is composed of the axons connecting neurons of grey matter.[14] White matter injuries sever communication between areas of grey matter, which result in the inability to relay information, such as motor or sensory, to muscles or skin.[15] MS is associated with a wide spectrum of disabilities such as motor impairments, sensory deprivation, decreased cognitive function and cerebellar and brainstem dysfunction.[11] The autoimmune pathogenesis of MS involves many immune cell groups, and its pathological mechanisms have been oversimplified in the past with the sole implication Th1 cells in plaque formation and demyelination.[12] However, current research is indicating differently with a focus on B cells and the role of demyelinating antibodies in MS pathology.[12] Specifically, autoreactive B cells infiltrate the CNS, and once expanded, these B cells may produce antibodies that attack myelin.[12] These antibodies can be identified in the CSF of patients and increase with disease progression and the number of active lesions, which may serve as a diagnostic tool.[16] The use of next generation sequencing, a tool

that determines nucleic acid sequences, has allowed for the analyzation of B cell receptors to characterize B cell populations found in MS, which suggests that there is therapeutic potential by targeting B cell subtypes.[16] Evidence for this stems from research evaluating the role of anti-CD20 B cell depleting therapies such as rituximab, ocrelizumab and ofatumumab.[17,18] MS is marked by fluctuating periods of exacerbation and remission, and is characterized by four clinical presentations: relapsing remitting MS (RRMS), primary progressive MS (PPMS), secondary progressive MS (SPMS), and progressive relapsing MS (PRMS).[8]

Pathogenesis

The pathogenesis of MS emphasizes the expansion of autoreactive T cells that cross the BBB, their role in activating microglia, and the demyelinating effect of microglial products.[11] The infiltration of monocytes is made possible by the breakdown of the BBB tight junctions due to reactive oxygen species (ROS).[19] Autoreactive Th1 cells interact with the endothelial cells through cell adhesion molecules (CAMs), which are involved in binding other cells and allow for their trafficking across the BBB).[19] This migration across the BBB increases subsequent permeability for other leukocytes as a result of their initial release of pro-inflammatory cytokines).[19] These Th1cells activate macrophages and microglial cells via IFN-γ, TNF-α or IL-2, which stimulates reactive microglia to express MHC II molecules, highlighting their role in further T cell activation.[11,12] These cytokines also activate transcription factors responsible for Th1 differentiation, such as T-bet and Stat-4, causing cells to differentiate into their Th1 phenotypes.[20] As the primary effector immune cell in the CNS, microglia have phagocytic, antigen presenting and cytokine generation abilities.[21] Activated microglia release proteases, TNF-α, reactive oxygen species (ROS) and metalloproteinases, which damage oligodendrocytes and myelin.[11] ROS-generating enzymes of myeloid cells include myeloperoxidase, xanthine oxidase and NADPH oxidase, which produce ROS, accumulating in sources such as endothelial mitochondria, microglia and astrocytes.[22] These cells are stimulated by inflammation, O^{2+}, H_2O_2, and hydroperoxides which damage endothelial cells by activating kinase pathways and transcription factors, including NF-kB, poly-ADP ribose polymerase.[22] These factors activate inflammatory genes such as ECAMs, MMPs, and iNOS[23]. iNOS recruits NO^{+}- releasing cytokines compromising BBB function by reorganizing the architecture of endothelial TJs including the disruption of zona occuludens-1 and occludin interactions (proteins that help create a scaffold for TJs) and breakdown of extracellular matrix which affects the structural support of TJs.[22,23] As the disease progresses, BBB pathology allows for increasing damage and becomes more vulnerable to ROS effects and immune cell infiltration due to its already weakened state.[22] This highlights the treatment of more advanced cases where the worsened pathology dictates an approach that may be able to deal with the more extensive damage.[24] The combination of these factors contributes to the chronic demyelinated lesions evident in MS.

There are different immune cells involved in demyelination; Figure 1 encompasses the distinct demyelinating roles of T cells, B cells and macrophages. As aforementioned, the

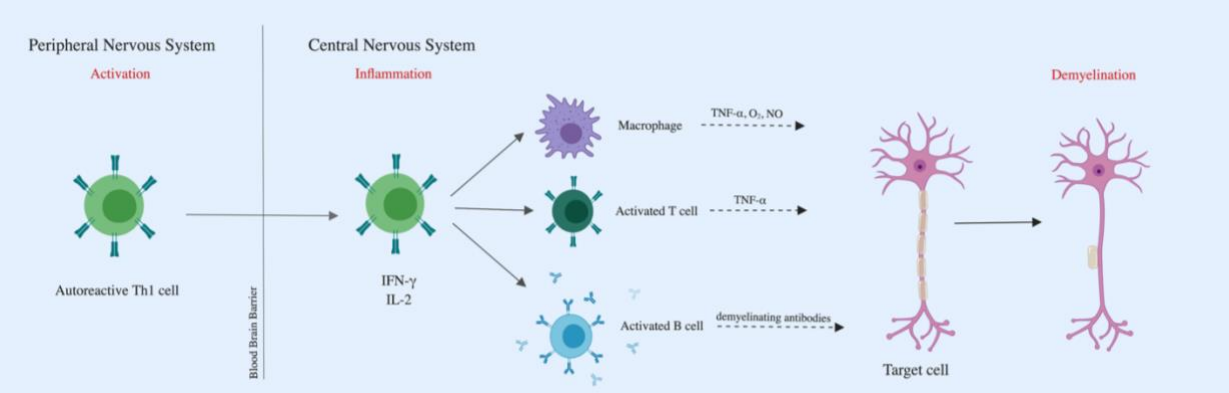

Figure 1: T cell, macrophage and B cell models of MS pathogenesis. Autoreactive T cells penetrate the BBB and secrete Th1 proinflammatory cytokines that stimulate activation of T cells, macrophages and B cells, which induce demyelination through proinflammatory elements, ROS and demyelinating antibodies.

inflammatory response is exacerbated by the toxic mediators of macrophages or ROS in response to T-cell activation. However, the demyelination process is not solely attributed to Th1 responses, as Th17 cells have been recognized in inducing EAE (experimental autoimmune encephalomyelitis), the animal model of an inflammatory demyelinating disease.[25] While autoreactive Th1 cells initiate spinal cord inflammation, myelin-specific Th17 cells promote cell infiltration into brain structures, thus highlighting the unique roles these cells have in allowing inflammatory cells to enter the CNS.[25] The cytokine profile of Th17 cells suggests that these cells promote an inflammatory response by secreting IL-17, a pro-inflammatory cytokine, and

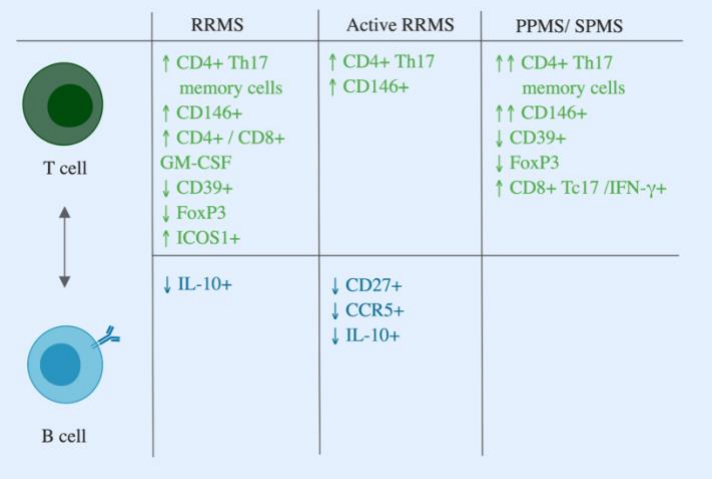

Figure 2: T and B cell marker expression reflects MS disease subtype and disease progression. As disease burden increases, T cells demonstrate increased levels of CD4+ Th17 cells as well as CD146+ expression. However, when RRMS is in the relapsing stage, B cells express decreased levels of CD27 and CCR5. Edited from Jones et al., 2017.

this may be a factor in lesion topology.[25] B cells contribute to the demyelinating process in both antibody-dependent and independent mechanisms. B cells can produce anti-myelin oligodendrocyte glycoprotein antibodies or act in an antibody-independent manner by processing and presenting antigens to T cells, using an EAE model.[25] These antibodies bind myelin antigens which impairs oligodendrocyte metabolism resulting in extensive damage such as disrupted axonal calcium homeostasis, cytoskeleton degradation, axonal transport blockage and the activation of other proteases to further disrupt axonal processes (Table 1).[12,20] As MS disease burden progresses, T and B cells reflect this by their cell surface marker expression, outlined in Figure 2.[26]

Table 1: Summary of MS neurodegenerative disease model and mechanisms, including prevalence, risk factors, disease pathology and standard of care treatments.

Multiple Sclerosis		References
Prevalence	● 4000 new cases of MS diagnosed every year in Canada	[49]
Risk Factors	● Mutations identified in HLA locus and other chromosomes (eg. 10p15, 5p13, and 1p36) ● Pathogens and viral infections may provide an environment to increase susceptibility to MS	[20,29]
Pathology	● MS is associated with demyelination and irreversible acute axonal injury ● Active lesions result in tract degeneration and neuronal atrophy	[12]
Standard of Care Treatment	● FDA approved MS treatments address symptoms such as inflammation ● GA or IFN-β products are delivered subcutaneously or intramuscularly to regulate pro-inflammatory cytokine secretion and T cell activation ● Escalation and induction DMTs mitigate relapse and the formation of new lesions	[29,33]

Demyelination processes between individuals vary and this suggests a heterogenous nature in the progression of MS.[12] Irreparable acute axonal injury is caused by demyelination present in active lesions.[12] Tract degeneration and neuronal atrophy are the cause for MS-associated symptoms, with the severity of axonal loss correlated to the number of toxic mediators (NO, proteases) released by infiltrating macrophages and Th1 cells in active lesions.[12] Affected areas include the optic nerve, cerebrum, brain stem and cerebellum and spinal cord which translate to symptoms such as vision loss, cognitive impairments, sensory and motor deficits and ataxia, illustrated by Figure 3.[27]

Stark comparisons can be drawn between myelin reactive T cells in those with and without MS. In patients with MS, antigen-specific cells are activated, whereas the same cells in

patients without MS are naïve.[20] The chemokine receptors expressed on reactive T cells and the cytokines released are aligned towards a Th1 response and more inflammatory in those with MS.[20]

Complement proteins opsonize oligodendrocyte and myelin membranes via 29,39-cyclic nucleotide 39-phosphodiesterase (CNP) for phagocytosis by microglial membrane receptors, further highlighting their role in MS pathogenesis.[11] Astrocytes typically suppress the phagocytic, antigen presenting, and cytokine producing functions of microglia to support axonal regrowth and stimulate remyelination within the CNS.[11] Upon exposure of IL-4, astrocytes,

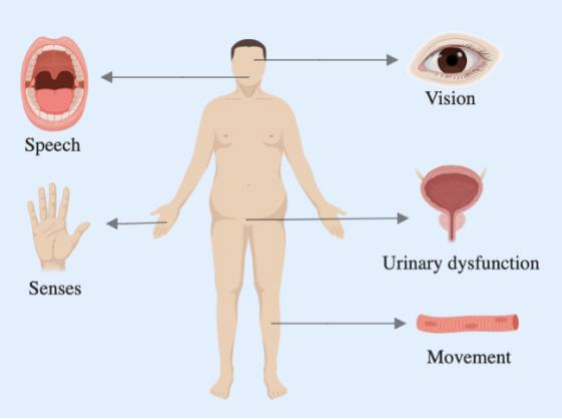

Figure 3: The infiltration into the BBB and its related brain structures translates to symptoms such as vision loss, cognitive impairments, urinary dysfunction and sensory and motor deficits.

which express IL-4 receptors, release neurotrophic factors such as nerve growth factor, glial-cell-line-derived neurotrophic factor and basic fibroblast growth factor (bFGF).[11] Astrocytes also respond to infiltrating immune cells by inducing apoptosis.[11] Other cells present in active lesions that may contribute to the inflammatory response include granulocytes, eosinophils and Th2 cells.[12]

As demonstrated by Figure 4, the infiltration of immune cells across the BBB in degenerative diseases highlights the interaction between microglia and astrocytes, by inhibiting microglial phagocytic properties to propel the neurodegenerative processes.[28] The disease is characterized by increasing neuronal death translating to motor and/or cognitive impairment.[21]

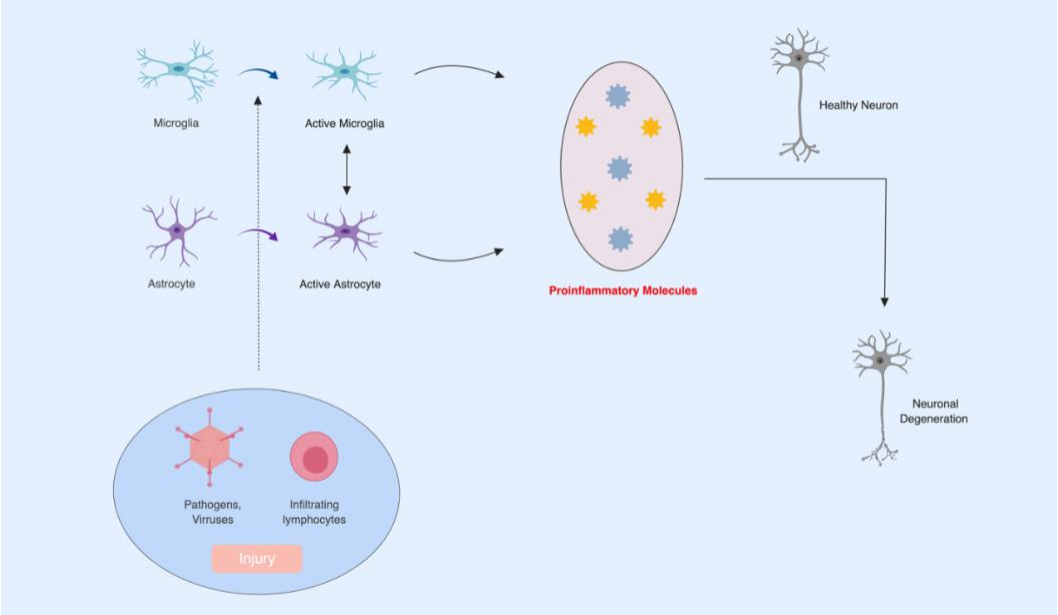

Figure 4: Microglia and astrocytes undergo morphological changes to their activated states following interaction with immune cells that have infiltrated the BBB. These autoreactive cells inflict injury upon the CNS and as such, microglia and astrocytes release proinflammatory signals, such as cytokines, that result in the perpetual mutual activation of these cells. Generally, these cells return to their resting state, however in patients with MS, there is a persistent activation, which triggers downstream cascades, ultimately causing neuronal degeneration.

Etiology

Though there is no distinct trigger leading to the development of MS, genetic and environmental risk factors are postulated to contribute to a person's development of the disease.[20] Identified loci associated with MS include the DR antigens in the HLA locus on chromosome 6p21, specifically the HLA-DRB1*1501-DQB1*0602 haplotype (DR2).[29] Other chromosomes with MS-susceptibility include: 10p15, 5p13, and 1p36.[29] In terms of environmental factors, exposure to vitamin D, a protective factor, as well as infectious agents become increasingly important before the age of 15 in the development of MS.[29] Pathogens and viral infections such as the human herpesvirus type 6, Epstein Barr virus, mycoplasma pneumoniae, upper respiratory tract infections and bacterial urinary tract infections provide an appropriate environment for the development of MS by stimulating toll-like receptors (Table 1).[20, 29] Toll-like receptors (TLRs) expressed on immune cells, such as microglia, recognize pathogen-associated molecular patterns on pathogens, causing them to produce proinflammatory cytokines.[30] This results in the reactivation of myelin-acting autoreactive T cells in the CNS.[29] The TLR signalling pathway plays a role in oxidative stress, including the production of ROS.[29] The multifactorial MS model suggests that all of these factors contribute to oligodendrocyte and neuronal cell death.

Diagnosis

Due to the spectrum of symptoms experienced with cases of MS, there is great diversity in the presentation of MS across individuals. Some individuals may seek out ophthalmologists and orthopedic surgeons, who will then refer them to a neurologist if MS is suspected.[31] CIS is the first presentation of an individual's neurological episode, caused by localized inflammation and demyelination or damage incurred across brain structures.[31,32] CIS presentation may include acute unilateral optic neuritis (inflammation of the optic nerve), partial myelitis (inflammation of the spinal cord) or brainstem syndrome, including symptoms such as difficulty breathing, speaking and swallowing.[31] However, the onset of MS may be slowly progressive, with neurological symptoms that evolve over months.[31] The diagnosis of MS is made by assessing neurological symptoms characteristic of MS, such as sensory or motor impairments, and CNS lesions (this combination is illustrated in Figure 5).[31] As per the 2016 European Magnetic Resonance Imaging in Multiple Sclerosis (MAGNIMS) network, MRIs can help determine the extent of histopathological MS features, such as inflammation, the state of myelination, gliosis, axonal loss and presentation of CSF oligoclonal bands.[32] Spinal cord MRIs may be recommended if the individual has myelopathy (spinal cord disease) or inconclusive MRI brain findings.[31]

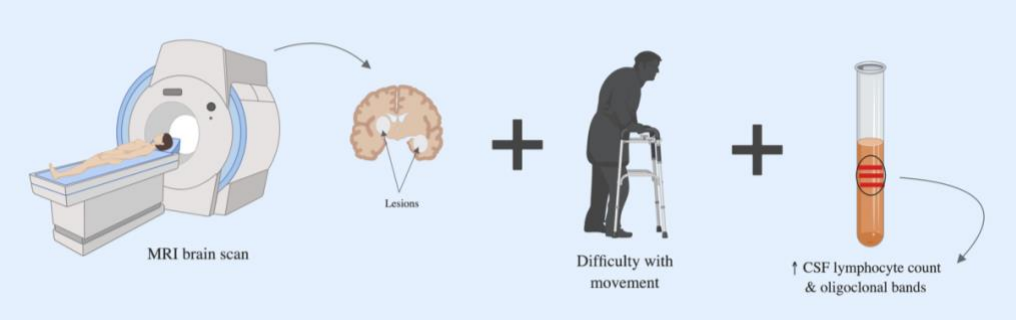

Figure 5: Diagnosis of MS is made by evaluating three factors: neurological symptoms (ex. motor impairment), the presence of CNS lesions on MRI brain scans and laboratory testing (CSF oligoclonal bands).

Current guidelines for an MS diagnosis are the 2017 McDonald criteria, which emphasizes the distinction between typical clinically isolated syndrome (CIS) and MS, where it is only applied to individuals who already have a greater likelihood of having MS, as CIS usually predates the other forms of MS.[32] The combination of patient history, physical examination and results of imaging and laboratory testing are necessary to make a reliable MS diagnosis.[32] As with other diagnostic tools, the trade-off between sensitivity and specificity exists with new considerations implemented in the 2017 revision in order to improve these scores.[32] Additional criteria as per the 2017 revisions include: the requirement of one periventricular lesion, the inclusion of both symptomatic and asymptomatic lesions in order to better understand the dissemination of lesions across space and time and the use of cortical lesions in the determination of MS (this was previously neglected as standard MRIs have limited ability to

illustrate cortical lesions).[32] The difference between the 2017 revision and prior versions illustrates the importance of CSF analysis and considering possible differential diagnosis.[32] MRIs can be used to confirm the diagnosis, however, in some cases, further testing, such as a CSF examination and neurophysical testing may be warranted.[31] CSF findings may reveal an increased white cell count, mainly lymphocytes and the presence of oligoclonal bands.[32] Other conditions that can be mistaken for MS include acute disseminated encephalomyelitis (ADEM) and neuromyelitis optica spectrum disorder (NMOSD).[31] The association between aquaporin-4 IgG levels and NMOSD, has confirmed the difference between MS and NMOSD.[31] As clinical, imaging and laboratory testing of ADEM and MS overlap, the additional requirement of encephalopathy (altered level of consciousness and cognitive deficits) is necessary in differentiating ADEM and MS.[31]

The MRI criterion provides different recommendations for the diagnosis of RRMS and PPMS. RRMS diagnoses require evidence of dissemination in space with >1 T2 lesions in at least two of 4 brain areas including periventricular, juxtacortical, infratentorial and spinal cord regions.[31] PPMS requires dissemination in space with a ≥1 T2 brain lesions in at least one of three sites including periventricular, juxtacortical and infratentorial regions and ≥2 T2 spinal cord lesions.[31] As T2 lesion loads are lower in PPMS, CSF may also be required, with positive results requiring ≥2 OCBs and/or elevated IgG levels.[31]

Standard of Care Treatment

Current treatments address MS symptoms and inflammation but are unable to reverse the immune-mediated damage.[29] The FDA approved IFN-beta products are delivered subcutaneously or intramuscularly and are recombinantly produced in *E. coli* bacteria or ovarian hamster cells.[29] This treatment has been used for over 20 years and is used to control the secretion of pro-inflammatory cytokines and T cell activation (Table 1).[29]

Currently there are 14 escalation and induction disease-modifying therapies (DMT) which serve to reduce relapse and formation of new lesions, characterized by nerve damage, but are unable to "cure" the disease.[33] Traditional first line therapies, such as glatiramer acetate (GA) and IFN-β, have been used for two decades, and now prompt research into pre-treatment predictors to optimize individual prognosis and treatment.[33] DMTs differ in administration, mechanistic profiles and side effect, but also vary in responsiveness between individuals.[33,34] The heterogeneity in severity and unpredictability in treatment response require the identification of individualized biological markers to guide treatment decisions with the greatest promise in responsiveness (Table 1).[33]

Clinicians use a trial and error approach but prescribing non optimal treatments impedes the critical treatment window to prevent the transition to secondary progressive MS.[33] Genetic biomarkers will inform clinicians of a patient's response to a given treatment using sequence variation analysis.[33] By identifying polymorphisms expressed in patients with MS, pharmacogenomics evaluates how *a priori* markers predict one's most safe and effective

treatment.[33] As suggested by the literature, future directions for MS treatment requires the adoption of an entirely new approach. This approach to MS treatment is the defined target of no evident disease activity (NEDA), which indicates no relapse, disability progression and MRI activity (MS lesions).[35] An early, more aggressive approach in advanced MS treatments, refocuses expectations to preserve reserve neuronal capacity.[35] Using the length-dependent axonopathy hypothesis, MS progression initiates at pathways with the longest axons and harnessing neuronal systems with reserve function may promote recovery.[35] Combination therapies in advanced MS employs different classes of MS treatments, such as anti-inflammatory therapies, those that target specific innate or adaptive immune mechanisms (activated glia, B cells or T cells), therapeutic monoclonal antibodies and neuroprotective or remyelination therapy.[35]

With no cure, MS patients continue to experience worsening neurological function, uncontrolled clinical symptoms, and staggered periods of remission and as such, the prognosis of MS focuses on quality of life and disability, highlighted in Figure 6.

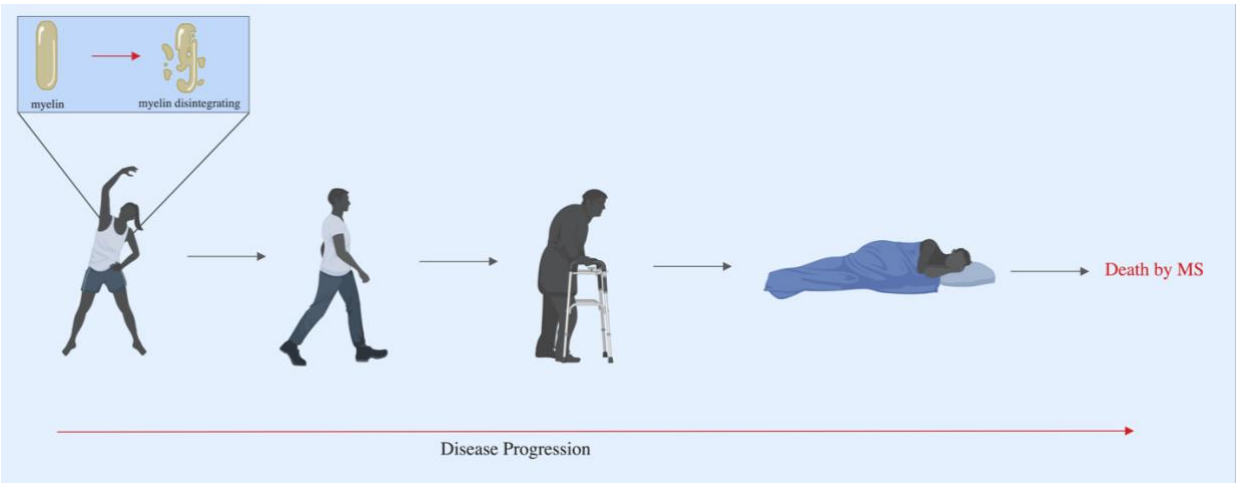

Figure 6: The expanded disability status scale quantifies the disability experienced by patients with MS. As the disease progresses, individuals may transition from no disability to minimal signs of MS, moderate disability in one system or mild disability up to 4 systems with no impairments in walking. As demyelination and the immune cell load becomes too great, this translates to increased motor deficits, with the inability to walk, and may result in MS-related death.

Discussion

Leading research ideologies concerning MS at this time are rooted in investigating better methods to diagnose and treat the disease, including discovering biomarkers, placing an emphasis on training physicians and developing novel treatments.

As the McDonald criteria was based on the data accumulated from academic MS speciality centers, further validation of this criteria is needed in diverse populations without typical presentation of CIS, such as investigating pediatric and late-onset MS patients with

comorbidities in primary practice settings.[32] The 2016 MAGNIMS Criteria will also need to be further validated, and improved by implementing a MRI feature to determine the chronicity of lesions during a patient's first assessment.[32] Increasing field strength imaging may serve to be more sensitive in detecting lesions and distinguishing MS lesions from other conditions, however, more research is needed to determine its efficacy and limitations.[32] With a greater emphasis on precision medicine and advanced technology, MS diagnostic tools will need to be further validated as there is currently no test that can solely diagnose MS.[32]

Biomarkers can aid in early diagnosis, tracking disease progression and identifying effective PD treatments. While there is a lack of clinically validated diagnostic biomarkers, ongoing research is investigating potential biomarkers to support MS treatment.[32] Due to the many targets that have been widely implicated in MS pathogenesis, there is great heterogeneity in the pathogenic mechanisms for the disease which remains widely unknown and to address this, a focus on affected proteins and genes may be beneficial. Screening miRNAs up or downregulated may reveal the underlying pathology of MS, the transition from CIS to MS and medication effects.[36] The stable nature of miRNAs makes the detection of circulating miRs in biofluids simple with methods such as qt-PCR, miRNA array analysis, or next generation sequencing.[36] Following (disease modifying treatment), miR-150 levels were altered in a disease-specific manner.[36] miR 128-3p and miR 191-5p were upregulated in patients with primary progressive MS with differential miR 128-3p levels in comparison to those with secondary progressive MS.[37] miR-128 was upregulated in naive CD4+ T cells in three forms of MS: relapsing-remitting, primary and secondary progressive.[37] MiR-128 suppresses Th2 cell differentiation, promotes the production of Th1 proinflammatory mediators and is involved in the p53, ErbB and TCR signalling pathways, all of which are implicated in MS pathology.[37] MiR 191-5p, involved in cellular differentiation and development, was upregulated in both forms of progressive MS but was downregulated in RRMS patients treated with natalizumab.[37]

Inflammasome signalling proteins also represent potential in reflecting MS pathology.[38] An inflammasome is a multiprotein complex involved in the activation of inflammatory responses and secretion of cytokines such as IL-1β and IL-18. In EAE, IL-1β contributes to the pathogenesis of the disease, however in humans, there is conflicting data with regards to their contribution.[38] Specifically, Caspase-1, apoptosis-associated speck-like protein (ASC) and IL-18 were elevated in the serum of MS patients, with ASC (AUC: 0.9448, CI: 0.9032-0.9864) and caspase-1 (AUC: 0.848, CI: 0.703-0.9929) as the most promising serum biomarkers.[38] The cut-off used for ASC was 352.6 pg/ml with 84% sensitivity and 90% specificity, while the Caspase-1 cut-off was 1.302 pg/ml with 89% sensitivity and 56% specificity.[38] Increasing the sensitivity and specificity compromised the value of the other measure. ASC acted in a dose-responsive manner, where protein levels increased with greater severity, with an AUC of 0.7596 (CI: 0.5437-0.9756).[38] As such, ASC may be a promising biomarker in MS, however, the prognostic potential of these different biomarkers needs to be further studied with regards to this line of research (Figure 7). More studies with larger sample sizes will aid in determining cut-offs to

optimize sensitivity and specificity when using biomarkers levels as a diagnostic tool in conjunction with physical examinations and imaging.

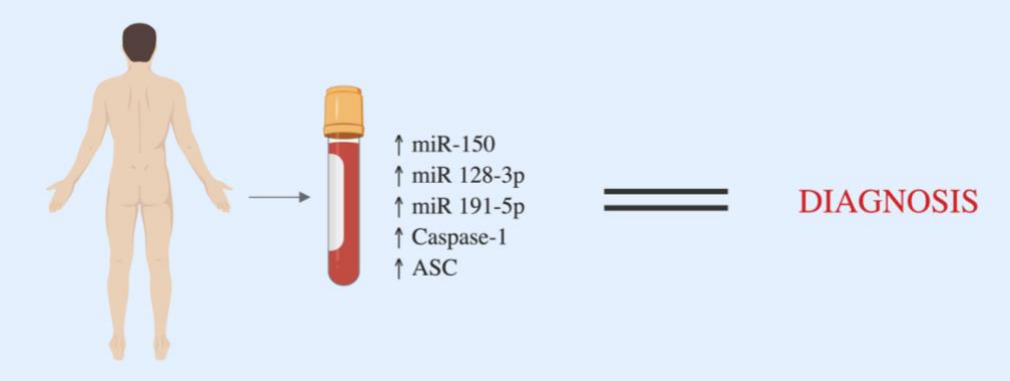

Figure 7: Non-invasive blood biomarkers may serve as a diagnostic tool in conjunction with other tests. Micro-RNA and inflammasome signalling proteins have been observed to be elevated in the serum of patients with MS, however, further tests with larger sample sizes are needed to determine their diagnostic and prognostic potential.

In the past 15 years, great strides have been made to improving the care of MS patients by highlighting the role of earlier individualized treatment plans to prevent long-term irreversible damage.[39] However, while FDA-approved treatments try to slow the progression of the disease or control symptoms experienced by MS patients, these medications are not completely effective.[40] Recently, a greater emphasis has been placed on the merits of medical marijuana in reducing patient-reported symptoms.[40] The only alternative medicine intervention with strong evidence is cannabinoids, where nabiximols, oral cannabis extract and synthetic tetrahydrocannabinol were effective in reducing spasticity and centralized pain.[40] However, the association between cannabis use and risk of schizophrenia and other cardiovascular conditions needs to be considered. As marijuana is now legal in many jurisdictions, adequate education to patients is becoming increasingly important.[40] Spasticity inflicts over 85% of patients with MS, with 17% of patients experiencing severe spasticity. Over 2/3 of patients experience MS-related pain that greatly interferes with their lives.[40] This pain manifests itself in the form of headaches, neuropathic arm or leg pain, back pain and painful spasms by CB1 receptors in the brain and peripheral nerves which process pain.[40]

DMTs are the standard approach in treating MS, however future research is needed to improve physician decision-making with regards to administering DMTs.[41] Areas for future research include: effects of DMT-related outcomes beyond trial outcomes to be better informed of the generalizability of these treatments; subpopulation DMT efficacy to identify optimal treatments for specific groups; long-term effects of using high-potency DMTs early in disease course, changing DMTs or discontinuation; and differences in DMTs for MS and CIS.[41] While DMTs have become integrated into the treatment of MS patients, there are still many questions remaining regarding treatment variation that can be addressed with pragmatic clinical trials.[41] For instance, siponimod has recently been recognized as one of the first DMTs that may be able

to slow the progression of SPMS.[42] Siponimod is a DMT that inhibits the entry of lymphocytes from lymph nodes into the BBB by acting on a selective sphingosine-1-phosphate receptor.[42] The EXPAND phase 3 clinical trial identified a 21% risk reduction of disability in a 3-month time period in patients with SPMS.[42] It has a similar safety profile to other sphingosine-1-phosphate receptor modulators and patients were exposed to the drug for 18 months.[42] Younger patients with greater disease burden, less disability and shorter disease duration were more likely to benefit from siponimod treatment.[42] Perhaps, the use of this drug will be restricted to specific patient populations, however, more studies are needed to evaluate the optimal subpopulation.

Novel treatments are constantly being developed and these represent new opportunities to treat MS. With a greater understanding of the mechanisms involved in MS, many researchers are trying to target specific pathways. For instance, hematopoietic stem cell transplantation (HSCT) has been used primarily as a cancer therapy but has garnered attention in its applicability to MS. Autologous HSCT (aHSCT) has been used in phase I and II trials in patients with advanced MS that have exhausted their DMT options.[43] The risk inherently associated with this therapy only makes it suitable for aggressive forms of MS.[43-47] AHSCT depletes autoreactive cells, such as Th17 cells and mucosal-associated invariant T cells, through the conditioning regimen and anti-thymocyte globulin (ATG).[43] Immune reconstitution allows for the expansion of diverse and specific CD8+ and CD4+ T cells, with B cells undergoing a similar process to reinstate immunological tolerance.[43] These clinical trials and case series have demonstrated efficacy and safety in IV administration of autologous bone-marrow derived HSCs in MS.[43-47] There are many more clinical trials being conducted that focus on different signalling pathways and this may also contribute to the personalized MS treatment paradigm.

Ibudilast, a phosphodiesterase inhibitor, suppresses the inflammatory pathway by acting on cell signalling molecules such as IL-1β, TNF-α, and IL-6.[42] The drug was tested in phase 2 SPRINT-MS randomised trials with 255 progressive MS patients where it reduced the rate of brain atrophy by 48% compared to a placebo.[42] This drug may serve as a promising therapy for another reason, namely its use for drug repurposing, which is the application of a drug that has already been developed for another condition.[42] Ibudilast is used in Japan and Korea to treat asthma and cerebrovascular disorders.[42] This is an interesting realm to explore in future studies as it may provide more opportunities for MS treatment.

Another approach in MS treatment is integrating formal risk management and decision-making training in medical schools and scientific institutions.[48] It has been suggested that clinicians have limited training in decision-making and risk management, reflected by therapeutic inertia (TI).[48] TI is the lack of initiating and intensifying treatment when therapeutic goals are unmet, leading to suboptimal treatment decisions, poor clinical outcomes and greater patient disability.[48] Studies suggest that 50-70% of clinicians do not intensify therapy when it is indicated by guidelines.[48] This may be partially due to *default bias* where clinicians continue to use options selected by others despite the availability of others.[48] This may contribute to the resistance in escalating therapy.[48] However, this may have also be caused by uncertainty of

relapse, insurance barriers, etc.[48] As such, being aware of one's biases and developing educational interventions may mitigate errors and optimize therapy.

Conclusions

The immune system plays a significant role in the neurodegenerative disease progression of MS and propelling neuroinflammation via pro-inflammatory cytokines. A pathological hallmark of MS is a dysfunctional BBB, allowing neuronal and peripherally derived cells, such as B cells, autoreactive T cells and activated microglia, to initiate inflammatory processes and contribute to disease pathogenesis. Oligodendrocytes are fundamental in the protection of the central nervous system, however, during disease processes, namely the demyelination of neurons and neuronal injury propelled by cytokines and signalling molecules such as IFN-γ, TNF-α, ROS, and proteases. As such, the environment exacerbates disease burden through the secretion of proinflammatory cytokines, further propelling neural degeneration. These powerful signalling mechanisms cause extensive damage, illustrated by symptoms reflecting nerve damage, across different sense modalities such as vision and sensory impairments. The functional impact that this has on individuals is profound and as such, controlling MS remains paramount. Future directions involve identifying minimally invasive disease-specific biomarkers, new diagnostic tools, and developing new treatments. Blood biomarker concentrations may inform potential MS biomarkers and aid in the unique treatment model of MS in which targeting these biomarkers may alleviate disease severity. As there were recent revisions in the McDonald criteria, this data must be further tested in populations to evaluate its sensitivity and specificity, but the development of more sensitive imaging would better distinguish MS lesions from other hyperintensities. Clinical trials are being conducted concerning the development of novel treatments, and in 2018 alone, there has been ground-breaking advancements in this field. These treatments have been informed by expanding our understanding immune cell involvement in MS and exploring untraditional methods of managing MS. These results suggest that the future holds great promise with research being conducted concerning the availability of biomarkers to aid in MS diagnosis and tailored treatments to manage disease progression.

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