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Advances in Multiple Sclerosis

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Abstract

Multiple Sclerosis (MS) is one of the most prevalent chronic inflammatory, demyelinating and neurodegenerative diseases of the central nervous system in young adults. Worldwide, there are more than 2 million patients suffering from MS. This disorder is a heterogeneous, multifactorial, immune-mediated disease that is influenced by both genetic and environmental factors. The pathological hallmark of MS is the accumulation of demyelinating lesions in the white and grey matters of the brain and the spinal cord which may lead to neuro-axonal damage. Focal lesions are caused by the infiltration of immune cells, including T cells, B cells and myeloid cells, into the parenchyma of central nervous system with associated injury. In the initial phase of this disease, relapses occur. These are reversible episodes of neurological deficits which usually last for days or weeks, and termed as clinically isolated syndrome and relapsing–remitting MS. In due course, permanent neurological deficits and clinical disability become prominent which are termed as secondary progressive MS. However, a few patients develop a progressive disease course from beginning, termed as primary progressive MS. The present review focuses on enhanced perceptive on roles of inflammatory cells, oxidative stress and mitochondrial dysfunction in the disease course along with the expansion of many novel and efficient treatments that can substantially combat, decline or delay the disease progression and disease related activities. Current developments in treatment of multiple sclerosis such as antioxidant therapy, selective modulation of immune system have also been reviewed.

Keywords: Multiple sclerosis; Inflammation; Epidemiology; Etiology; Oxidative stress; Adaptive immune cells

Introduction

Multiple Sclerosis (MS) is a multi-factorial autoimmune disease of the central nervous system of our body. It is characterized by chronic inflammation, demyelination, loss of axons and neurons. Patients may develop neurological symptoms such as motor, sensory, and cognitive impairment depending on the site of demyelinating lesions. In general, symptoms of MS patients are ataxia, numbness, muscle spasms, walking difficulties, fatigue, pain, bladder or visual problems, depression and dementia [1].

Frequently, MS patients suffer from Chronic Neuropathic Pain (CNP), a non-motor associated symptom. It is a long-lasting chronic pain affecting nearly 60% of MS patients and dramatically decreases their quality of life [2]. Since MS is a multi-factorial disease, its etiology is complex. In major, inflammation is the driver of the pathology. Additionally, oxidative stress also contributes to tissue

injury and promotes existing inflammatory response. MS disease is inflammatory in nature; therefore, most widely used therapeutic approach for combating MS is to target the immune response.

Corticosteroids in limited dose are used to treat acute attacks of MS. However, steroids have severe side effects; therefore, they should not be used for chronic treatment. Recently, many immune-modulatory agents have been approved as disease-modifying therapies for MS. There are adjuvant drugs including antidepressants which have been employed to treat CNP [3]. Despite of development of all these therapeutics, all MS symptoms are not efficiently treated. Still, treatment options for sensory impairments are limited and non-efficient [2,4]. Therefore, there is a need to develop novel therapeutics that possesses the capacity to target both motor and sensory MS disease. The present review ought to summarize the contribution of inflammation along with oxidative stress in the pathology of MS. Current therapeutic developments that may improvise treatments of MS have also been discussed.

Epidemiology and Etiology of Multiple Sclerosis

There are around 2.5 million people suffering from MS all over the world and the counting is increasing continuously [4]. People may develop this disease at any time in the life; however, it is observed that majority of persons get diagnosed with MS at an age between 20 to 40 years. Women are generally two to three fold more affected with this disease compared to men [1]. Similar sex differences are found for MS associated CNP as well as CNP in general [2]. The factors that impact the incidence of MS include variable epidemiology such as ethnicity, geographical location and other environmental factors all around the world.

The genetic factors also play an important role in the development of MS. It has been observed that normal individual has nearly 0.2% life time risk to develop MS while siblings of MS patient have a 10- to 20-fold higher risk of developing the disease [5].

The susceptibility for MS has been impacted by mutations in specific Human Leukocyte Antigen (HLA) variants within the major Histocompatibility Complex (MHC) gene complex. It depicts the relevance of the immune system in the development of MS. It is a complex genetic autoimmune disorder and involves a polygenic etiology as well as a huge number of MS-associated genes outside the MHC locus [6].

Clinical Demonstration of the MS

The Relapsing-Remitting Course (RRMS) is the most prevalent form of MS. The RRMS is dominated by central and peripheral inflammation and it leads to loss and injury (demyelination) in axons and neurons. Due to accumulation of demyelinated portions, various neurological signs and symptoms develop in the course of RRMS. It may evolve years when it is converted into Secondary Progressive MS (SPMS). It is reported that around 15% of MS patients do not get relapses after clinical onset. They directly develop a Primary Progressive (PPMS) disease [1]. The average age of clinical onset is nearly 40 years. The average age is almost similar in SPMS and PPMS patients [7]. Around 60% of MS related CNP patients also suffer with significant disability and depression [2]. MS associated pain syndromes are broadly divided into two classes of pain; primary pain that is caused by inflammation, demyelination and neuro-degeneration, and the secondary pain which is caused as a result of indirect consequences of the CNS lesions [8]. MS associated CNP patients can experience a wide range of CNP symptoms including continued dysesthesia pain in the lower extremities, paroxysmal pain, which is divided into Lhermitte's phenomenon and trigeminal neuralgia along with thermal and mechanical sensory abnormalities [2,3,8].

Pathology of MS

Demyelinating plaques within the white and grey matters of CNS are the main hallmark of MS pathology [1]. The quantity as well as quality of these lesions along with their locations is variable with time. It is a crucial determinant of the clinical outcome. The driving force for the de-myelinating lesions is the inflammatory reaction caused due to autoimmune response. MS is classically referred as a disorder related to T cell-mediated autoimmunity [9]. Previously, it was reported that initiation of MS occurs by an

adaptive immune response directed against CNS antigens. Certainly, inflammation and demyelination occur when activated auto-reactive T cells infiltrate in CNS. In response to this infiltration, there is an up regulation of pro-inflammatory mediators, thereby activating microglia/macrophages resulting into demyelination.

It has also been evident from the reports that B lymphocytes and the innate immune response also contribute towards MS pathology [10]. It is reported that oxidative injury and subsequent mitochondrial damage resulted in neuro-degeneration [11]. Some reports suggested that MS is a primary inflammatory disease, in which immune-mediated mechanisms drive the demyelination and tissue injury all through different stages and in all different courses [12]. Some other reports suggested that MS is a primary neurodegenerative disease, modified and amplified by the inflammatory process [13]. Non-immune-mediated mechanisms also contribute towards MS pathology as it is reported that oligodendrocyte apoptosis in MS lesions and tissue damage can even take place without interference of lymphocytes or peripheral macrophages [14].

Likewise, MS-associated CNP develops through central inflammation, demyelination, and neurodegeneration [2]. The rodent Experimental Autoimmune Encephalomyelitis (EAE) model revealed that in course of disease progression, peripheral nerves suffer major pathologic changes after neuro-degeneration in the CNS [15]. Infiltration of lymphocytes into peripheral nerves and macrophage activity in the dorsal root ganglion symbolize peripheral CNP pathology [2]. It is revealed that peripheral inflammation demyelination and nerve lesions may contribute to MS-related CNP [16].

Inflammation in MS

Role of Adaptive Immune Cells

The presence of CD4+ and CD8+ T cells in the inflammatory lesions within the CNS has been reported. The meninges in progressive MS revealed the presence of ectopic germinal centers that comprised B cells and other immune populations [17]. It signified the role of the adaptive immune system in pathogenesis. It is reported that MS patients showed two types of inflammation. In acute and relapsing MS, the Blood-Brain Barrier (BBB) becomes leaky and focal bulk invasion of T and B cells into the white matter leads to the classical active demyelinated plaques [9].

There is correlation between lymphocyte invasion and cytokine activity in the CNS. Increase in the disease activity has been related to elevated expression of inflammatory cytokines. In primary stages of MS, a slow and gradual increase in T cells and B cells accumulation occurs and major blood brain barrier damage is observed in the connective tissue spaces of the brain. In the second type of inflammation, subpial demyelinated lesions occur in the cortex and diffused neuro-degeneration in the grey or white matter get promoted [9]. CD4+ T cells are particularly reported to contribute in the initiation of immune response in MS patients. However, it does not play a major role in the effector stage of CNS that includes inflammation and immune-mediated demyelination and neuro-degeneration [17]. The pathology of MS gets increased by elevated secretion of Interferon Gamma (IFN γ) and Interleukin-17 (IL-17) by CD4+ T cells. These cells act as the pathogenic initiators of MS [17,18]. At the remitting stage of MS, patients have elevated levels of IL-22 in the CNS [19]. Infiltration of additional lymphocytes in the CNS is promoted by secretion of IL-22, amplifying the inflammatory cascade [20]. CD8+ T cells are the major lymphocytes present in active MS lesions. These cells are potential contributors to MS pathology. In order to perform their cytotoxic function, CD8+ T cells require MHC class I expression and presentation. MHC class I is constitutively expressed by all cells, but in active MS lesions. Expression is gradually up regulated on astrocytes, oligodendrocytes, neurons, and axons. This expression makes these cells potential targets for CD8+ T cells in disease course [21].

Granzymes induced cytotoxicity has also found its part in CD8+ T cell-mediated neuronal injury [22]. Axonal injury is also found correlated with the infiltration of CD8+ T cells into lesions [23].

T helper cell-mediated pathology gets induced by the cytokines IFN γ and IL-17 secreted by CD8+ T cells. Regulatory T Cells (TREGS) also have an impact on MS pathology. In immune system, TREGS act as the master regulators that can suppress autoimmunity and contribute in tissue regeneration. The function of TREGS is to express T Cells Receptors (TCRS) that are able to recognize self-antigens and get activated by self-antigens. TREGS have functional deficits in MS patients. However, there is no change in the frequency of TREGS in the peripheral blood of MS patients. Only, the immune-modulatory function of TREGS is impaired in MS patients [24].

Role of B cells in MS

In MS patients, B-cells also contribute in adaptive immune inflammation in the CNS [25]. The Cerebrospinal Fluid (CSF), the meninges and the brain parenchyma of MS patients are reported to have clonally expanded B cells [26].

The CD20+ B cells are dominant components of the lesions in early disease course while plasma cells are major components in later course of disease that include lesion maturation and progressive stage of the disease [25]. The role of B cells in MS disease course include development of ectopic lymphoid follicles within the CNS, antigens presentation to T cells, secretion of cytokine and chemokine leading to production of auto-antibody in the CNS [22]. It is revealed from the CNS of MS patients that B cells led pathogenic effect as they produced factors that trigger demyelination and neuro-degeneration *in vitro* [27,28].

Role of Macrophages/Microglia

Microglia and monocyte derived macrophages exhibit significant roles in MS [29]. Particularly encephalitogenic T cells interact and activate macrophages, and it is vital for inflammatory demyelination in MS. When macrophages are fully activated, they release cytokines, chemokines and other inflammatory mediators, thereby intensifying the neuroinflammation and neuropathology [30]. Unlike bone marrow-derived macrophages, microglial cells originate from the embryonic yolk sac. They represent a self-perpetuating CNS-specific glial cell population [31].

Microglial cells play a significant role in clearance of apoptotic cells, synaptic pruning, and the formation of mature neuronal circuits during development under physiological conditions. They are also involved in diverse brain processes such as synaptic plasticity, learning, cognition and memory in adults [32]. They stand as a first line of defense for CNS. They are crucial part of CNS immune system and exhibit brain protection and maintain homeostasis. They hastily sense damage or pathogen associated signals. Subsequently, they get activated to release pro-inflammatory mediators such as IL-1 β, isoform nitric oxide (iNOS), chemokines and TNF. Further they activate and recruit peripheral immune system cells to infiltrate the CNS [33].

Diverse phenotypes of microglia with multiple functions have been detected in the chronic neurodegenerative disease [34]. Microglia cells are known to perpetuate neuro-inflammation and disease pathogenesis. Microglial cells drive chronic neuro-inflammation in the absence of Blood Brain Barrier (BBB) breakdown and significant infiltrating immune cells [35]. Normally, the microglia maintain homeostasis, however, in MS, neuronal synaptic plasticity is lost resulting in synaptic loss and thus cognitive decline [36].

Non-microglial cells involved in MSs

There are several types of non-microglia myeloid cells present in healthy CNS including Barrier-Associated Macrophages (BAMS) and CNS Dendritic Cells (DC) [37]. These cells (BAMS and DC) are primarily present in boundary regions like the meninges, perivascular spaces and the choroid plexus [37]. BAMS are long-lived while CNS DC are bone marrow-derived and short-lived. The CNS DC performs a function of representation of CNS auto-antigens to activated T cells which is a critical function for the initiation of CNS-directed T cell autoimmune disease [38].

Next to CNS injury, CNS-resident macrophages and microglia get activated. If CNS gets recruited with some additional blood-born monocytes, the BBB get disrupted and neurological symptoms become clearly evident.

Particularly during the effector stage, monocytes infiltrate in the CNS and get differentiated into monocyte-derived macrophages and release the pro-inflammatory mediators. It directly promotes towards demyelination. An inflammatory cytokine namely IL-1 β is majorly expressed in activated macrophages, microglia and monocytes; it significantly contributes towards the development of MS. IL-1 β contributes towards the differentiation of T cells into Th17 cells via the STAT3 pathway aggravating inflammation in the CNS [39]. Likewise, demyelinated lesions of MS patients have harmful monocyte-derived macrophages also [40].

Myelin antigens are first sensed by microglial cells [41]. Adaptive immunity gets activated and intensified when microglia gets transformed into Antigen-Presenting Cells (APCS). In MS disease course of demyelination and remyelination, microglial APCS activate T cells [36]. Therefore, it can be inferred that microglia play a significant role for the targeting of adaptive immune cells to the CNS [42]. On activation, microglia express class I and II MHCs. Microglia present antigen and activate adaptive immune cells. Moreover, microglia expresses co-stimulatory molecules including B7-1 and B7-2. These interact with CD28 present on T cells to stimulate proliferation, differentiation, and cytokine secretion. They also stimulate CTLA4 to promote T cell induced apoptosis [43]. Activated microglia, subsequently to their interface with adaptive immune cells, can secrete cytotoxic cytokines and oxidative products such as NO and ROS radicals in MS lesions thereby advancing towards oxidative stress and finally leading to destruction of myelin [36]. Depending on their activation state, macrophages and microglia show a high plasticity. These have been roughly classified into two phenotypes namely M1 has proinflammatory and M2 has pro-repair and anti-inflammatory nature.

Proinflammatory mediators, such as TNF, IL-1 β, and IFNγ characterize M1 polarized cells. M1 cells are potent APCS and can activate adaptive immunity. M2 polarized cells are characterized by anti-inflammatory mediators, such as IL-4, IL-10 and transforming growth factor - β (TGF-β). These cells contribute in immune-regulation [44]. Also, M2 microglia promotes oligodendrocyte differentiation. It is also reported that depletion of microglia impairs remyelination [45].

Myelin debris has been reported to be a potent inhibitor of differentiation process of Oligodendrocyte Precursor Cell (OPC) into myelin-forming oligodendrocytes [46].

M2 polarized microglia also promote remyelination by secreting anti-inflammatory mediators; for example, IL-4 enhances oligo-dendrogenesis [47] and also suppresses Th1 macrophage reaction including release of macrophage Inflammatory Protein (MIP) and activin A, thereby promoting differentiation of oligodendrocytes [45]. It can be inferred that if we get the better knowledge regarding the cellular and molecular mechanisms of the threshold control of the microglial polarization between pro-inflammatory to pro-repair phenotypes, it can be significant in drug designing. This can promote the beneficial functions of the cells and can positively reverse the inflammatory process of demyelination in MS. This can also provide neuro-protection and neuro-repair in other neurodegenerative diseases also.

Oxidative Stress and Mitochondrial Dysfunction in Multiple Sclerosis

Redox Homeostasis and Oxidative Damage

Energy is produced as the end-product of the mitochondrial electron transport chain by oxidative metabolism. Mitochondria also incorporate components of the respiratory transport chain and recruit free radicals producing enzymes.

Free radicals are chemical entities having an unpaired electron in their outer orbit capable of inducing reactivity. On receiving an electron, oxygen becomes superoxide anion radical (O2-) and then subsequent addition of other molecules form secondary Reactive Oxygen Species (ROS) like Hydrogen Peroxide (H_2O_2) and hydroxyl radical (OH). Often, in response to endogenous and exogenous stimuli such as cytokines, xenobiotics, pathogens and radiations, cellular ROS are formed [48]. Nitric Oxide (NO) is another free radical having an unpaired electron which belongs to the Reactive Nitrogen Species (RNS) family. ROS such as nitric oxide, superoxide anion act as regulatory mediators in signaling processes. Free radicals along with their derivatives regulate vascular tone, sense oxygen tension and enhance the signal transduction from various membrane receptors such as the antigen receptor of lymphocytes. They also modulate oxidative stress responses, thereby maintaining homeostasis [49]. Free radicals combat oxidative damage and also maintain redox homeostasis when cells are challenged by metabolic and temporary environmental stressors via endogenous feedback mechanisms directed towards continuously balancing nucleophiles and electrophiles [50]. For example, upon NO stimulation, redox signaling acts by the self-inhibition of neuronal NO synthases which in turn gets converted into a catalytically

inactive ferrous-nitrosyl complex [51]. Sometimes, feedback loop gets disturbed by any of these challenges such as a permanent harmful challenge, an inappropriate defense response, an inefficient nucleophilic feedback, and oxidative damage and breaching of the physiological redox steady state.

Antioxidants (both enzymatic and nonenzymatic) efficiently delay or inhibit oxidation of substrate at low concentrations and maintain redox homeostasis. Endogenous enzymatic antioxidants are Superoxide Dismutase (SOD), Glutathione Peroxidase (GSHPX) and Catalase (CAT) and the non-enzymatic antioxidants are vitamins C and E, carotenoids and plant polyphenols. Some cofactors such as copper, zinc, iron, manganese support enzymatic antioxidants and convert dangerous oxidative products to hydrogen peroxide (H_2O_2) and finally to water [52].

Nuclear factor erythroid 2-related factor 2 (Nrf2) is an important regulator of the antioxidant defense system. It is a transcription factor that binds to a DNA sequence called Antioxidant Response Element (ARE). Upon activation by drug-metabolizing enzymes like cytochrome P450, Nrf2 regulates the response against high electrophiles and oxidants, and removes and detoxifies dangerous metabolites [53]. Nrf2 exhibits another significant function that is the inhibition of the NF-κ b pathway and inhibition of inflammation through thereby decreasing cytokine production and oxidative responses [54].

Oxidative Damage of Mitochondria leading to Cell Death

ROS activates the apoptosis and promotes tissue damage *via* intrinsic mitochondrial pathway. Further, it also causes triggering of permeabilization of outer membrane and translocation of cytochrome c, Apoptosis-Inducing Factor (AIF) and Second Mitochondria-Derived Activator of Caspases (SMAC) from mitochondria to the cytosol. These factors induce cytosolic apoptotic signaling events or promote nuclear chromatin condensation and DNA fragmentation by translocation of Apoptosis Inducing Factor (AIF) from the cytosol to the nucleus [55].

The Permeability Transition Pore (PTP) is essential to promote the mitochondrial permeabilization and the release of apoptotic signals. PTP spans the inner and outer mitochondrial membrane. It is huge size pore protein mainly composed mainly of three component proteins: The Voltage-Dependent Anion Channel (VDAC) and Cyclophilin D (CYPD) and Adenine Nucleotide Translocase (ANT) [56,57].

The Mitochondrial Permeability Transition Pore (MTPTP) acts as a calcium-dependent and voltage-gradient channel which permits the entry of solutes size up to 1.5 KDa across the usually impermeable IMM. An alteration in the membrane permeability results in depolarization of the trans-membrane potential, mitochondrial swelling, escape of small solutes and proteins and further loss of oxidative phosphorylation [58].

ROS exhibits both direct and indirect effects on formation of MTPTP. The oxidation of the thiol groups of IMM causes changes in the membrane conformation that induces formation of disulfide bond and aggregation of protein [59]. VDAC also regulates the MTPTP by exposing amino acids to the inter-membrane space or cytosol. Therefore, they become readily accessible for oxidation. Thus, VDAC plays a role of a mediator in ROS-induced apoptosis [60]. Similarly, ROS exhibits direct effect on ANT also. The difference arises in binding of ANT and CYPD [61].

The mitochondrial Ca^{2+} overload also acts as another important inducer of PTP opening. Ca^{2+} interacts with CYPD when high amount of Ca^{2+} gets accumulated in the mitochondrial matrix [62]. This interaction leads to induction of opening of the MTPTP and thereafter leading to formation of ROS and free fatty acids, thus exacerbating the opening of MTTPTP.

Due to loss of membrane permeabilization, MMP gets dissipated and if the overload of Ca^{2+} persists, MTPTP will continue to remain open leading to solutes accumulation in the mitochondrial matrix. Eventually, there will be rupture of outer mitochondrial membrane releasing the contents of inter-membrane space followed by leaking of pro-apoptotic signals into the cytoplasm resulting in

death of the cell [63]. It is evident from the reports that both ROS and Ca^{2+} plays a vital role in determining oxidative stress-induced mitochondrial dysfunction leading to cell death. In addition to apoptosis, elevated levels of ROS lead to other cellular fates such as senescence [64], necroptosis [65] and autophagy [66].

Oxidative Damage in Multiple Sclerosis

In MS, multiple pathological effects such as myelin destruction, axonal degeneration and inflammation are due to oxidative stress [67]. It is suggested that regions in CNS which are characterized by perivascular inflammatory infiltrates, depicted more mitochondrial dysfunction, fragmentation and impaired trafficking than other regions in CNS [68]. Similarly, profound mitochondrial protein alterations and DNA deletions in neurons have been found in active MS lesions [69].

Active lesions have oligodendrocytes which possess increased levels of oxidized DNA whereas oxidized phospholipids preferred to accumulate in axons with disturbed transport. It is also revealed that higher the extent of inflammation more is the severity of oxidative damage [70].

CNS autopsies revealed that macrophage-derived ROS promotes mitochondrial dysfunction and focal axonal degeneration also in axons with intact myelin [71]. This theory has supporting evidence as revealed in the report stating that accumulation of Amyloid Precursor Protein (APP), a marker for acute axonal damage, occurs not only in active demyelinating but also in remyelinating and inactive demyelinated lesions [72].

In fact, CNS is highly susceptible to oxidative stress owing to numerous factors such as immense demand of energy and mitochondrial activity, limited cell renewal and huge amount of iron and poly unsaturated fatty acids. Thus, these features increase the vulnerability of CNS for particular neurodegenerative hallmarks related to oxidative stress. It includes changes in iron metabolism, impaired mitochondrial function, and increased oxidative damage, defects in ubiquitin-proteasome system, presence of abnormal, aggregated proteins, inflammation, and excitotoxicity [73].

Nevertheless, oxidative damage is not only regulating factor of MS within the CNS but it also directs the immune response perpetuating in the periphery. At the first stage, higher levels of ROS damage the brain endothelium by decreasing its electrical resistance thereby affecting its permeability [74]. Nitric oxide metabolites are found up regulated in CSF samples of MS patients and found to have correlation with relapses depicting a deleterious function of nitric oxide played in inflammatory BBB dysfunction [75]. Reports have suggested that interaction of monocytes with the brain endothelium causes ROS to facilitate the intrusion of leukocytes within the CNS [76]. Infiltrating leukocytes also produce huge quantities of ROS, thereby inducing myelin phagocytosis by activated microglia and macrophages [77].

The immune system has developed resistance mechanisms and is lesser sensitive to high ROS levels. Generating H_2O_2 and hypochlorous acid enables neutrophils and phagocytes to kill bacteria [78]. ROS signaling is also essential to target cell killing by neutrophils and cytotoxic T cells [79]. Further, T cell receptor activation induces intracellular ROS production [80]. Undoubtedly, ROS signaling is a major contributor in the organism's defense system, but if homeostasis is breached, a vicious circle that comprises inflammation and degeneration will initiate [81]. Similar to MS, excessive or sustained ROS levels are involved in the pathogenesis of other neurodegenerative disease [82]. Moreover, the long-standing free radical theory of ageing proposes that ROS are also heavily involved in this natural process and in age-associated diseases [83]. Therefore, therapeutic treatments for MS and other diseases should be aimed at restoring general homeostasis, including redox balance, in order to prevent physiological ROS signaling from being revert.

Targeting Inflammation and Oxidative Stress to Treat Multiple Sclerosis

Approved MS Therapies

The therapeutics for MS has to face three major challenges:

1. To prevent relapses and progressive course of disease;
2. To efficiently handle acute relapses and MS-related symptoms; and
3. Cure the adverse side effects of drugs.

Now-a-days, Corticosteroids such as methylprednisolone are used as immune suppressants to combat acute MS. Methylprednisolone instantly reduces CD4+ lymphocytes and exhibit short-term reduction in production of IFNγ and expression levels of chemokines [84]. The rapid effect of corticosteroid to transient tightening of BBB during and shortly after treatment has been correlated [85]. However, steroid treatment resolves the acute relapse faster but long-lasting effects of treatment have not been yet detected.

Currently, US Food and Drug Administration (FDA) have approved twelve disease-modifying therapies as treatment for MS. Among them, three are injectable medications: glatiramer acetate and interferon beta-1a and beta-1b; five medications include small oral molecules: teriflunomide, dimethyl fumarate, fingolimod, cladribine and siponimod; four medications include administration via infusion: alemtuzumab, ocrelizumab, mitoxantrone and natalizumab.

Interferon beta-1b was the first drug approved as therapeutic for MS in 1993, followed by interferon beta-1a and glatiramer acetate soon [86]. Since then, after MS diagnosis, first-line treatment used has been combination of interferon beta and glatiramer acetate. Interferon beta-1a and interferon beta-1b are typically cytokine derivatives that decrease the infiltration of T cell into the CNS that result in alleviated central inflammation [87].

Glatiramer acetate is a discrete-sized mixture of peptide composed of glutamic acid, lysine, alanine and tyrosine. These four amino acids are major constituents of myelin basic protein which is a central component of the myelin sheaths [86]. It is suggested that glatiramer acetate exhibits a shift from pro-inflammatory Th1 cells to anti-inflammatory Th2 cells and there is increment in number of regulatory T cells [88,89].

The second-line of treatment includes two disease-modifying drugs: natalizumab and fingolimod. These drugs are used in Relapsing-Remitting MS (RRMS). Natalizumab is the humanized Monoclonal Antibody (mab) acting against the cell adhesion molecule, α 4-integrin and blocks trafficking of immune cells over the blood-brain barrier into the CNS parenchyma. Whereas, Fingolimod is the modulator of sphingosine-1-phosphate receptor and sequesters lymphocytes in lymph nodes, thereby stopping them from contributing to an autoimmune reaction and transforms macrophages into an anti-inflammatory phenotype [87].

Mitoxantrone, teriflunomide and cladribine are smaller molecules that exhibit inhibition or suppression of replication of T cells and B cells and also inhibit rapidly dividing cells in MS patients [87]. Dimethyl Fumarate (DMF) is another smaller molecule which transforms different immune cell subsets to an anti-inflammatory state and promotes neuronal survival [90].

Alemtuzumab is another humanized monoclonal antibody which acts against CD52. The CD52 is a glycoprotein present on the surface of mature lymphocytes. Their interaction leads to a rapid and long-lasting depletion of mature T and B cells [91]. Currently, FDA has approved a drug namely, Ocrelizumab, which is a humanized anti-CD20 mab. It was the first drug approved for the primary progressive form of MS. Ocrelizumab targets B lymphocytes and kills the cells *via* Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) and to a lesser extent, Complement-Dependent Cytotoxicity (CDC) [92]. In 2019, FDA approved a follow-up product of fingolimod named as Siponimod. It is a modulator for sphingosine-1-phosphate receptor. It can be used for RRMS and SPMS [93].

Current Developments

Antioxidant Therapy: As described above, progress of neuro-degeneration during disease course of MS is a multiple factor based complex process. It involves number of mechanisms including but not limited to inflammation, oxidative stress, primary apoptosis,

mitochondriopathy, synaptopathy etc. As mentioned above, oxidative stress and inflammation are much interrelated and exhibit impact on each other. Therefore, it can be inferred that apart from using anti-inflammatory and immunomodulatory treatments, neutralizing free radicals can be a potential therapeutic approach to combat oxidative stress. For example, DMF has been reported to activate anti-oxidative pathways and to enhance expression of the transcription factor Nrf2. It also stabilizes the cell metabolism and consequential protection from oxidants and preserves integrity of myelin [94].

Antioxidant complementary therapies and their relevance for MS: Anti-oxidants protect the body against free radicals. They are divided into enzymatic and non-enzymatic substances. Enzymatic anti-oxidant substances include catalase, glutathione peroxidase, Glutathione Reductase (GR) and Superoxide Dismutase (SOD). Non-enzymatic anti-oxidant substances are classified into two classes; low molecular weight antioxidants such as melatonin, vitamins, glutathione as well as coenzyme Q and the antioxidant elements- ions [81,95].

Melatonin is a neurohormone and important antioxidant that also activates antioxidant enzymes such as SOD, catalase, and glutathione peroxidase [96]. Melatonin supplementation improved antioxidant defense in MS through up regulation of catalase, manganese superoxide dismutase and sirtuin 1 (SIRT1), an inhibitor of oxidative stress [97]. Melatonin exhibits both immune-modulatory and antioxidant activities. Supplementation of Coenzyme Q10 for 12 weeks resulted in elevated level of SOD and decline in activity of malondialdehyde A in a randomized small clinical trial with RRMS patients. It implies that coenzyme Q10 supplement increases antioxidant enzyme activity and decreases oxidative stress [98]. These results indicated that interfering with oxidative stress is a promising therapeutic strategy to treat MS, but might not be sufficient as a single treatment. The combination of antioxidant therapy with other immune-suppressive or immuno-modulatory therapies might be superior to current approved therapies.

Selective modulation of the immune System: It is reported that treatment of MS patients *via* nonselective TNF inhibitors failed in clinical trials [99]. The failure might be due to the pleiotropic actions of TNF. TNF are of two types; soluble (stnf) and transmembrane bound (tmtnf). TNF activates two receptors namely TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2). The soluble TNF/ TNFR1 signaling activates inflammation and tissue degeneration while transmembrane bound TNF / TNFR2 promotes immune suppression in addition to tissue homeostasis and neuroprotection [100]. Blocking all the effects of TNF can diminish the MS. However, complete blocking of TNF causes some side effects. However, the selective blocking of soluble TNF / TNFR1 signaling, leaving TNFR2 signaling functional can be used to combat MS. Soluble TNF inhibits the capacity of microglia to phagocytose and clear myelin debris [36]. Next step to inhibition of soluble TNF / TNFR1 signaling, and specific activation of TNFR2 has come as a novel therapy for MS. TNF via TNFR2 contributes in the proliferation of oligodendrocyte progenitors and remyelination [101]. It has also been revealed that selective agonism of TNFR2 rescues neurons from oxidative stress-induced cell death and excitotoxic cell death [101,102]. Activation of TNFR2 induces expression of anti-apoptotic and detoxifying proteins. The cytokine interleukin 6 (IL-6) plays a significant role as a downstream mediator in MS pathology [103].

Repolarization of microglial cells as a therapeutic target: Microglia are reported to promote remyelination through several processes such as expression of anti-inflammatory molecules, phagocytosis of debris, and repair of tissues [104]. It has been revealed that microglia differentiate into discrete phenotypes both during demyelination as well as remyelination [105]. The M1 type of microglia causes inflammation and oxidative stress-induced oligodendrocyte damage while M2 type of microglia functions in immune regulation and drive oligodendrocyte differentiation at the time of CNS remyelination. When remyelination begins, there is a switch from M1 type to M2, a dominant response occurs in microglia and peripherally derived macrophages [45]. Genetic depletion of microglia is resulted in inefficient clearance of myelin debris, and subsequently remyelinating process gets impaired [106]. Therefore, it is counterproductive to inhibit microglia and to stop their proinflammatory and tissue destructive activity. On the contrary,

inflammatory modulation of the lesion such as repolarization of M1 into M2 microglia, might offer a more hopeful therapeutic approach. A drug named as glatiramer acetate is an approved drug for MS. It exerts neuroprotective effects. It is believed to be mediated by activated M2 microglia [107]. Likewise, a soluble TNF inhibitor, xpro1595 increases the repair potential of microglia promoting neuroprotection and remyelination in demyelinated lesions [36,108]. Many other compounds that modulate microglia/macrophage polarization are presently at preclinical stage. Similarly, Forskolin is an adenylyl cyclase activator. It alleviates experimental autoimmune Encephalomyelitis (EAE) motor disease by causing suppression in the expression of CD86 whereas increasing polarization of M2 macrophage at the inflammation site [109]. Lenalidomide is the clinically approved immunomodulatory agent. It promotes polarization of M2 macrophage and regulates autoimmunity of CNS subsequently resulting in abolished progression of EAE [110].

Conclusion

MS is a multifactorial immune driven disease with a complex etiology. Numerous other mechanisms such as immune-independent de-myelination, neuronal cell death and oxidative stress also contribute in MS pathology. All the therapeutics that are approved for MS modulate the immune system and subsequently suppress adaptive autoimmunity. However, all the aspects of MS pathology such as sensory deficits are not covered by these therapeutics. These therapeutics often create unspecific immune modulation leading to severe side effects. Researchers are now focusing on selective immune modulation such as targeting microglia polarization or specific cytokines. It is reported that cytokines TNF and IL-6 known to be pro-inflammatory mediators and contribute to MS pathology, carve up neuro-protection. The neutralization of these cytokines causes detrimental effect in clinical trials of MS. In the same way, microglial cells possess a high plasticity and contribute towards neuro-degeneration, but also play vital role in regeneration of tissue. Therefore, superior therapeutic strategies must be designed by selectively targeting the inflammatory activity of these mediators.

A number of approved MS therapeutics cause decrease in oxidative stress. It is believed that this property of therapeutic agents contributes to their therapeutic activity. However, some of the therapeutic strategies that intervene with oxidative stress failed in clinical evaluation. Yet, the antioxidants may prove to be advantageous as co-treatments with anti-inflammatory reagents resulting in better clinical outcome. In all, it can be concluded that numerous novel potential therapeutic strategies that specifically target neuro-inflammatory components are presently under preclinical and clinical evaluation. Subsequently, it may progress towards the development of novel MS therapeutics with improved and secured activity.

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