# Current and alternative therapeutic options for Multiple Sclerosis

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## Abstract

Multiple sclerosis (MS) is an autoimmune, chronic, inflammatory, and demyelinating disease that affects the central nervous system (CNS). It is characterized by recurrent multifocal attacks with symptoms of neurological distress. These include motor disorders such as muscle weakness, spasticity, impaired reflexes or sensory alterations such as vibration, thermalgesia, pain or symptoms with cerebellar character such as ataxia and tremors, among others. Due to the nature of the disease, treatment must target the symptoms, acute relapses and the modification of the disease. Different pharmacological treatments aim to inhibit or to modulate this response to avoid substantial motor and cognitive deterioration of the patient in the early stages and an individualized approach is therefore often required. This review updates current alternative treatments for MS at a preclinical and clinical level extracted from contemporary literature.

**Key words:** Multiple sclerosis, glatiramer acetate, neuroinflammation, autoimmune disease, alternative treatment.

## Resumen

La esclerosis múltiple (EM) es una enfermedad autoinmune, crónica, inflamatoria y desmielinizante que afecta al sistema nervioso central (SNC). Se caracteriza por ataques multifocales recurrentes con síntomas de malestar neurológico. Estos incluyen trastornos motores como debilidad muscular, espasticidad, reflejos perjudicados y alteraciones sensoriales como vibraciones, termoalgesia, dolor o síntomas de carácter cerebeloso como ataxia y temblores, entre otros. Debido a la naturaleza de la enfermedad, el tratamiento debe dirigirse a los síntomas, las recaídas agudas y la modificación de la enfermedad. Los diferentes tratamientos farmacológicos tienen como objetivo inhibir o modular esta respuesta para evitar un deterioro motor y cognitivo sustancial del paciente en las primeras etapas y, por lo tanto, a menudo se requiere un enfoque individualizado. Esta revisión actualiza los tratamientos alternativos actuales para la EM a nivel preclínico y clínico extraídos de la literatura contemporánea.

**Palabras clave:** *E*sclerosis múltiple, acetato de glatiramer, neuroinflamación, enfermedad autoinmune, tratamientos alternativos.

## Introduction

Multiple sclerosis (MS) is an autoimmune, chronic, inflammatory, and demyelinating disease that affects the central nervous system (CNS) (Blevins & Martin, 2003). It is characterized by recurrent multifocal attacks with symptoms of neurological distress, including motor disorders such as muscle weakness, spasticity, impaired reflexes or sensory conditions (such as vibrations), thermalgesia, pain or symptoms with cerebellar character (such as ataxia and tremors), with variable degrees of recovery (Porras-Betancourt et al., 2007).

In the MS there was an alteration mediated due to the abnormal presence of activated antigen-specific autoreactive TCD4 + lymphocyte clones that attack the myelin basic protein. This occurs in susceptible people without having identified what the factors are that trigger the response. The main data available is the plates of demyelination of the white matter of the CNS. These are well-defined areas with few cells and loss of myelin, relative preservation of axons and gliosis, with increased predilection for optic nerves, periventricular white matter, brainstem, cerebellum and spinal cord, which generally surround one or more blood vessels of medium caliber (Noseworthy et al., 2000).

Inflammatory cells are located around the blood vessels, with diffuse parenchymal infiltrates. These infiltrates vary in their components, depending on the state of the lesion activity, but generally contain lymphocytes and macrophages with products of the degradation of myelin in its interior. It is noted that this is more commonly found in lesions that are active. There are also areas of remyelination with precursor cells of oligodendrocytes; astrocytic infiltration producing areas of gliosis and axonal injury (Ferguson et al., 1997).

## ETIOLOGY

The etiology of the disease and its evolution remains unknown. There are however various etiological hypotheses. These mainly focus on the relationship between predisposing genetic factors and a person's interaction with the environment that generate effects capable of triggering an autoimmune response in the CNS (Lagumersindez et al., 2009). For example, it has been found that when comparing monozygotic twins to dizygotic twins, the former have been recorded to have a frequency of contracting the disease up to six times more than the latter due to pre-existing genetic factors. Furthermore, it has been shown that the risk of suffering from the disease among relatives affected with MS is less than 5%, this being between 20 and 40 times higher than in the rest of the population (Lagumersindez et al., 2009).

Among the observed environmental factors is a variety of infection, of which are typically viral in nature. Some of the proposed agents are measles virus, human herpes virus type 6 (HHV-6), Epstein Barr virus (EBV), and a variety of retroviruses. Viruses of the herpes family are notable candidates in the pathogenesis of MS due to their natural history of periodic reactivation and inactivation phases. However, at present no specific virus has been shown to be substantially responsible for the disease (Alonso et al., 2006).

Several theories try to explain the relationship these infections have with the onset of the disease. These are based mainly on microbial superantigens, holding that certain microbial peptides could have the ability to exacerbate a high number of lymphocytes, including autoreactive clones, which would pass to the CNS therefore triggering the disease. Another theory taken into consideration is that of molecular mimicry in which it is stated that during infection the activation of autoreactive TCD4 + lymphocytes as well as B lymphocytes occurs, through the antigenic determination pathway, the cross-reactivity between the generated antigens by the infectious pathogen and the autoantigens of the CNS would be the triggers of the autoimmune inflammatory reaction (De Andrés, 2003).

## PHYSIOPATHOLOGY

Different mechanisms have been proposed to explain the pathogenesis of progressive MS. The first postulates that brain damage is mediated by inflammatory processes similar to those present in remitting MS, but the inflammation varies in a way that it is no longer treatable by current therapeutic strategies (Frischer et al., 2009). In the second, MS begins as an inflammatory disease but over the years, it leads to neurodegeneration when presenting chronic inflammation, both being independent processes within the body (Cardona et al., 2006). Finally, the third suggests that MS has been immediately classified as a neurodegenerative disease and its progression can be modified or aggravated by the presence of inflammation in its early stages. (Cardona et al., 2006).

The physiopathology of progressive MS involves a variety of processes. These can present as stress oxidative, mitochondrial damage, inflammation, demyelinating plaque

formation, injury in diffuse tissue, activation of microglia and alteration of ion homeostasis axonal (that will be described later) (Lassman et al., 2012). Many components of innate and adaptive immunity induce demyelination, death of oligodendrocytes and axonal or neuronal damage in patients affected with MS, the including components are cytotoxic T cells and antibodies produced against neuronal or glial antigens. Additionally, the myelin sheaths are vulnerable to non-specific products secreted by activated macrophages and microglia that generate the destruction of tissues, such as cytotoxic cytokines, excitotoxins, and reactive oxygen (ROS) and nitrogen species (RNS) the cellular and molecular mechanisms of MS are represented on the figure 1. (Prineas et al., 1978; Kostic, M. et al., 2015).

Myelin destruction is widely considered to be mediated by self-reactive Th1 cells. These autoreactive cells secrete pro-inflammatory cytokines and chemokines, such as IL6, IL-12, IL-1β, IFNɣ and TNFα, which attract other cells in the immune system, such as B lymphocytes, monocytes, and neutrophils from the blood circulation. Monocytes differentiate into macrophages by action of the IFN-ɣ secreted by the Th1 cells polarizing them towards pro-inflammatory macrophages (M1). These M1 polarized macrophages secrete cytokines and ROS that promote apoptosis of oligodendrocytes and that together with RNS such as nitric oxide produce highly cytotoxic compounds such as peroxynitrite, which induces lipoperoxidation, thus, generating demyelination (Barthelmes et al, 2016). In the periphery of these lesions there is the activation of more B and lymphocytes in the lymph nodes, proliferating in the spleen and migrating through the circulation to the CNS (Barthelmes et al, 2016).

Mitochondrial dysfunction and oxidative stress generated by inflammatory processes, as well as the presence of divalent metal ions, catalyze the reaction through which OH is produced, these are highly toxic. According to Bolanos et al.(1997) and Smith et al. (1999) free radicals alter the normal enzymatic function of the mitochondria, in addition to generating modifications in proteins accelerating their degradation and interfering in the *de novo* synthesis of the components of

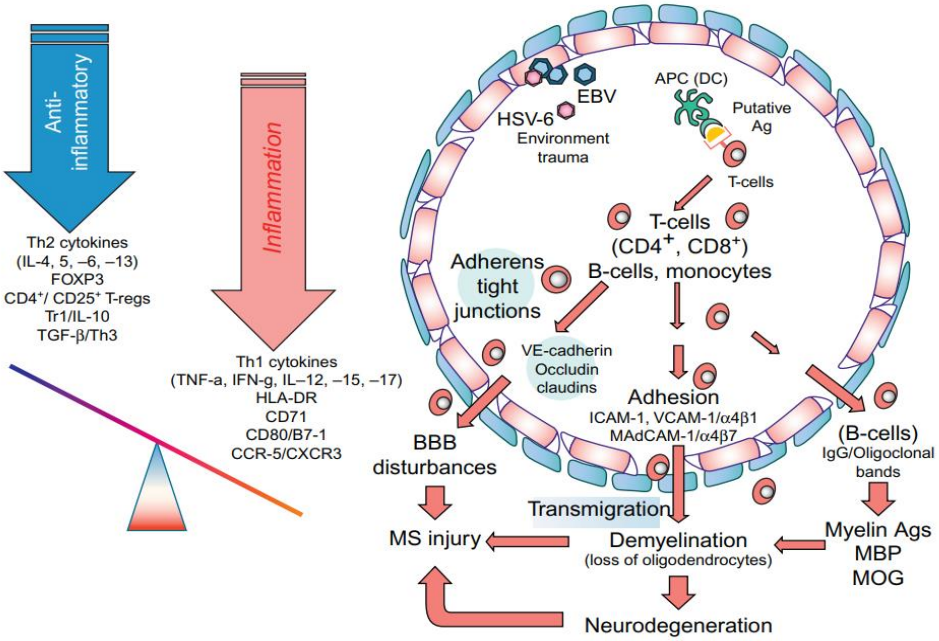


*Figure 1. Proposed MS etiology by Maghzi, A.-H. et. Al. (2011), after exposure to environmental antigens myelin-sensitized autoreactive leukocytes are activated via binding of the T-cell receptor to the antigens, which is conveyed to them by antigen-presenting cells. The activated leukocytes (T cells, B cells, and macrophages) cross the BBB through the disrupted cerebral endothelial tight and adherent junctions (by disintegrating junctional complexes containing VE-cadherin, occludin, claudin, and junctional adhesion molecules). The activated leukocytes also secrete several pro- and anti-inflammatory cytokines that play regulatory roles in polarization of the peripheral environment toward inflammatory or anti-inflammatory mechanisms. Once these cells gain access to the CNS environment, they identify more autoantigens and generate and release more cytokines and autoantibodies, causing loss of myelin/oligodendrocyte complex as well as neurodegeneration.*

the respiratory chain, which can lead to DNA damage. All these factors increase active lesions in MS. Oxidized lipids and DNA, as well as nitrotyrosine, are abundantly present in these. In the same way the presence of DNA and lipids in apoptotic oligodendrocytes and dystrophic axons contribute strongly to demyelination and neurodegeneration (Jomova et al., 2011).

The primary self-destructive mechanisms for the development of the pathophysiology of multiple sclerosis are: inflammation, demyelination, remyelination, neurodegeneration and formation of glial scars. These different processes are carried out in a focal or diffuse manner throughout the white matter and gray of the brain, as well as in the spinal cord (Haider et al., 2011).

1. Inflammation: inflammation is present in all stages of multiple sclerosis. Inflammatory lesions are present, consisting of perivascular and parenchymal infiltrates of neutrophils and macrophages, as well as CD8 +, CD4 + lymphocytes, and plasma or B cells or B lymphocytes (Lassman et al., 2012).

2. Alteration in sodium and potassium channels: Potassium channels are specialized structures within the membrane that separate neurons from the extracellular fluid. These channels open and close depending on electrical and chemical stimuli. When open they allow charged molecules, in this case potassium ions, to pass through them. In MS, the affected myelin exposes channels in the axon membrane that allow the loss of potassium ions, weakening the electrical current sent through the nerves, based on the physiology of the neuron and the action of the potential of action on myelinated and unmyelinated fibers (Álvarez Pinzón et al., 2018).

Sharma et al (2010) explain that in a myelinated fiber, only the areas of the axonal membrane corresponding to the nodes of Ranvier are in contact with the interstitial fluid. Virtually all sodium-potassium ion channels and pumps are concentrated in these areas. Therefore, action potentials can only be generated at the nodes and the nerve impulse then jumps from node to node, generating an acceleration in conduction. Due to the presence of a demyelinating inflammatory reaction, such as the characteristic in MS, the physiology of the action potential is affected, where potassium plays a very important role since when performing a stimulus on the affected myelin sheath, it does not free depolarization by loss of potassium ions is allowed. This weakens the electrical current sent through the nerves and it is there that the potassium channel must be blocked to allow a better nervous stimulus that accelerates driving and reduces the difficulty of walking in patients with the disease. The recovery of brain functions is done initially by the resolution of edema, changes in pH, and the decrease in inflammation. In the long term, treatment is continued by the recovery of Na + channels. The new myelin plaques that are produced, however, are not the same as the original ones in terms of their structure. Instead, they are produced with shorter internodes and finer myelin which causes the sequelae of the disease (Carretero et al., 2001).

3. Demyelination: The destruction of myelin is widely considered to be mediated by autoreactive Th1 cells. These autoreactive cells secrete pro-inflammatory cytokines and chemokines that attract other cells of the immune system such as B lymphocytes, monocytes, and neutrophils from the bloodstream. Monocytes differentiate into macrophages by means of IFN-Gamma secreted by Th1 cells, polarizing them towards a pro-inflammatory type macrophage (M1). These macrophages secrete cytokines and reactive oxygen species that promote apoptosis of oligodendrocytes, thus generating demyelination (Barthelmes et al., 2016).

In turn, the Th2 lymphocytes secrete anti-inflammatory cytokines such as interleukin-10 (IL-10) which generates the differentiation of B lymphocytes to plasma cells, secrete specific antibodies against the basic protein of myelin, promoting its degradation and generating in turn the degradation of axons and neurons giving rise to the characteristic lesions of MS. In the periphery of these lesions there is the activation of more B and T lymphocytes within their Th1 phenotype in the lymph nodes, proliferating in the spleen and migrating through the circulation to the CNS (Barthelmes et al., 2016).

Leukocyte extravasation from the bone marrow, spleen, and lymph nodes into the circulation and subsequently to the CNS

is a process that depends on several factors, including chemokine-mediated molecular interactions between leukocytes and endothelium and their receptors. The production of chemokines by various cell types can be induced during the immune response by secreted cytokines such as TNF-alpha, IFN-Gamma and interleukin-6 (IL-6), generating leukocyte migration to the site of inflammation (Barthelmes et al., 2016).

4. Free radical production: during inflammation, neutrophils and macrophages produce ROS and RNS through a series of reactions mediated by NADPH oxidase, an enzyme complex made up of four subunits that is active in neutrophils and other mononuclear phagocytes. Specifically, in neutrophils, the enzyme is induced by zymosan (a TLR2 agonist), subsequently phosphorylation of the regulatory subunit p47 takes place. The p91 subunit, a cytochrome, generates superoxide ($O^{2-}$), a free radical from oxygen ($O_2$) (Beutler, 2004).

The superoxide generated is converted to hydrogen peroxide through a reaction catalyzed by superoxide dismutase, this being a key reaction, since it allows the generation of other ROS. Among the products generated by hydrogen peroxide are halides, including hydrochloric acid, which is equivalent to the chlorine used daily. Hypochlorite can generate singlet oxygen - a high energy oxygen molecule which is extremely reactive with carbon double bonds and can react with superoxide to form the hydroxyl radical. RNS are also produced by a spontaneous reaction in which they use superoxide as a substrate, generating peroxynitrite. All the previously described molecules react with various molecular targets including lipids, proteins, and nucleic acids (Beutler, 2004).

In the specific case of MS, free radicals disrupt mitochondrial enzyme function, as well as modifying mitochondrial proteins and accelerating their degradation. Oxidative stress might also promote axonal tau phosphorylation, which has been detected in degenerating axons,94 and might explain the high levels of expression of molecules associated with endoplasmic reticulum stress (Cunnea, P., et. al., 2011). Oxidative injury is clearly associated with inflammation in RRMS lesions, but is also pronounced in progressive MS lesions, despite low levels of inflammation. These findings suggest that oxidative stress might additionally be driven by factors other than the inflammatory process in the progressive stage of MS. (Haider, L., et. al., 2011).

## DIAGNOSTIC CRITERIA

The diagnosis criteria of MS include a combination of clinical and paraclinical studies to help rule out other possible causes or injuries. The critical point to make an accurate diagnosis remains on the objective demonstration of dissemination of lesions in both time and space integrating magnetic resonance imaging (MRI) into the overall diagnosis scheme. The most well-known clinical diagnostic criteria are called McDonald's diagnostic criteria. This is where a flare is described as an episode of neurological disturbances suggestive of MS lasting more than 24 hours; these injuries can only be observed by performing a neurological examination (McDonald et al., 2001).

To be diagnosed with MS requires the existence of a T2 lesion in at least 2 of the following areas:

- Periventricular
- Juxtacortical
- Infratentorial
- Spinal cord

In patients with medullary or brainstem syndrome, symptomatic lesions are not part of the criteria and therefore should not be taken into consideration (Martinez-Altarriba et al., 2014).

MS is characterized by its great variability, whose symptoms and signs are determined by the area of the CNS affected by demyelinating lesions. According to the latest modifications of diagnosis made in 2013, it is possible to distinguish two clinical forms of presentation, relapsing-remitting disease (RRMS) and progressive disease (PMS) (Lublin et al., 2013).

About 85% of patients that present RRMS are characterized by the appearance of outbreaks of reversible neurological dysfunction that recur from time to time. Within this phenotype the isolated

neurological / demyelinating syndrome is included. This consists of a first acute episode affecting one or occasionally several areas and is the first clinical appearance form 80% of patients (Compston & Coles, 2008). Both forms can be active or not active (Lublin, 2014).

PMS is classified as primary progressive (PPMS), characterized by progressive accumulation of disability from the onset of the disease. Secondary progressive (SPMS), characterized by the progressive accumulation of disability after an initial course in outbreaks. Both forms are sub-classified, according to the level of disability in active / with progression, active / without progression, not active / with progression and not active / without progression (Lublin, 2014).

## PHARMACOLOGICAL APPROACH AND TREATMENT.

Since the disease was discovered a large number of treatment options have been proposed (Figure 2), however a curative therapy has yet to be developed. There is currently a wide range of disease course modifying therapies approved by the FDA as shown in Table 1. These treatments are supplemented with the symptomatic treatment of flare-ups and clinical manifestations of the illness. Disease modifying agents aim to alter the course of the disease, decreasing the outbreak rate and the appearance of new lesions on MRI, as well as the stabilization or improvement of disability. Until now its use was only approved in the early stages of the disease (Medina Heras 2019).

***Immunomodulators***

*Interferon-β*

Interferon-β represents the first class of disease modifying therapies (DMTs) for MS that has been shown to be effective in reducing the rate of flare-ups and resonance injuries. It induces a decrease of about 30% of the outbreak rate to the 2 years of treatment (IFNB MS Study Group, 2001). It presents a favorable safety profile, being necessary for monitoring of liver enzymes and leukocyte count. There is evidence of its intervention in the balance between pro-inflammatory and anti-inflammatory cytokines, as well as in reducing the number of inflammatory cells that pass through the Blood-brain barrier (BBB) (Mitsdoerffer & Kuchroo, 2009).

It is produced by various cell types including fibroblasts and macrophages. The mechanism of action of interferon beta is complex, involving effects at multiple levels of cellular function. It appears to directly increase expression and concentration of anti-inflammatory agents while down regulating the expression of proinflammatory cytokines exerting its biological effects by binding to specific receptors on the surface of human cells. This binding initiates a complex cascade of intracellular events that leads to the expression of numerous interferon-induced gene products and markers. These include MHC Class I, Mx protein, 2′/5′-oligoadenylate synthetase (OAS), β2-microglobulin, and neopterin (Madsen, 2017).

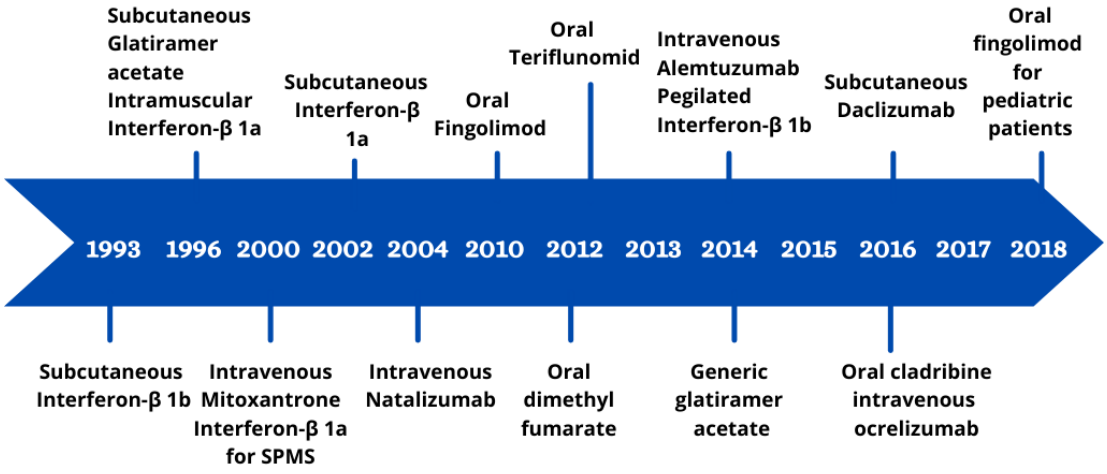


*Figure 2. Temporary evolution of Multiple Sclerosis Treatments Adapted from Medina Heras S. (2019)*

*Table 1. FDA approved disease modifying agents.*

| FDA approved Disease Modifying Agents (DMA's) | | | |
|---|---|---|---|
| **Pharmacological agent** | **Molecular mechanism** | **Year of approval** | **Reference** |
| Interferon | Effects at multiple levels of cellular function. It appears to directly increase expression and concentration of anti-inflammatory agents while down regulating the expression of proinflammatory cytokines | 1993 | Madsen, 2017 |
| Glatiramer acetate | Inhibits the activation of autoreactive T cells against MBP, clones with a Th1 phenotype that are exposed to it have shown a dose-dependent inhibition as well as an increase in IFN-$\gamma$ secretion, interfering with the activation of T cells | 1993 | Johnson et al., 1998 |
| Mitoxantrone | Inhibits type II topoisomerase and disruption of DNA synthesis and might stimulate microglial death | 2000 | Li, J.M. et. al., 2012 |
| Fingolimod | Acts on the sphingosine-1-phospate receptor, blocks the capacity of lymphocytes to egress from lymph nodes, reducing the number of lymphocytes into the peripheral circulation to start the inflammatory cascade associated with myelin destruction | September 2010 | English, C., & Aloi, J. J., 2015 |
| Teriflunomide | Inhibits dihydro-orotate dehydrogenase, an enzyme responsible for pyrimidine synthesis of nucleic acids. Halts the production of nucleic acids needed in the proliferation of activated lymphocytes and B cells involved in the inflammatory cascade responsible for myelin destruction | September 2012 | English, C., & Aloi, J. J., 2015 |
| Dimethyl-fumarate | Reported to activate the nuclear factor (erythroid-derived 2)-like 2 pathway that is involved in the cellular response to oxidative stress. Has protective properties for neurons and could further modulate immune response. | March 2013 | English, C., & Aloi, J. J., 2015 |

| | | | |
|---|---|---|---|
| Alemtuzumab | Humanized monoclonal antibody that targets CD52 on lymphocytes and monocytes. It readily depletes monocytes and B and T lymphocytes, leading to long lasting changes in adaptive immunity, and reduces the pathogenesis of inflammatory response in MS | November 2014 | English, C., & Aloi, J. J., 2015 |
| Natalizumab | Humanized monoclonal antibody directed against alpha 4-integrin, a cell adhesion molecule present in the surface of monocytes and lymphocytes, blocking their binding to VCAM1 on the endothelial surface. Because of their binding, it inhibits the migration of these cells through the blood-brain barrier (BBB) to CNS, reducing its inflammation | July 2015 | Sheremata et al., 2005 |
| Ocrelizumab | Humanized anti-CD20 monoclonal antibody through antibody-dependent cell mediated cytotoxicity, antibody-dependent cellular phagocytosis, complement-dependent cytotoxicity, and apoptosis | March 2017 | Syed Y. Y., 2018 |
| Cladribine | Selectively depletes lymphocytes and has a predilection for B lymphocytes. Aside from its pro-apoptotic effects, it promotes immune tolerance and reduces immune cell infiltration into the CNS | March 2019 | Jacobs, B. M., et. Al., 2018 |
| Siponimod | Selective S1PR1 and S1PR5 modulator which causes long-lasting internalization of the S1P receptor upon binding, causing a decrease in absolute lymphocyte count. | March 2019 | Goodman, A. D., Anadani, N., & Gerwitz, L., 2019 |

*Glatiramer acetate*

Cop-1 or glatiramer acetate is a synthetic polymer with an average molecular weight of 6.4 KD and an average length of 45 to 100 amino acids constituted of L-alanine, L-glutamic L-lysine, and L-tyrosine in a molar ratio of 4.2, 1.4, 3.4, and 1.0 (Bernard et al., 1992). The molecule was first synthesized in the 1960s at the Weizmann Institute of Sciences by Sela, Arnon, and Teitelbaum. Its discovery was accidental since this molecule was synthesized for the first time to study the interaction of myelin proteins and lipids that are capable of inducing experimental autoimmune encephalomyelitis (EAE). Cop-1 was synthesized in search of the immunogenic sequence of myelin basic protein (MBP), however, rather than inducing disease, it was found to induce a protective effect in a variety of species (Arnon, 1996).

The results of the first experiments showed that the presence of EAE could be reduced from 75% to 20% in the treated animals. Cop-1 has not shown specificity in the inhibition of EAE depending on the species, since this inhibition it has been

observed in guinea pigs, rabbits, mice, monkeys, and baboons. Subsequent studies showed that the suppressive effect of Cop-1 could be transferred by lymphoid cells from mice immunized with it in syngeneic receptors (Lando et al., 1979). At first it was proposed that immunological cross-reaction mechanisms could be involved in this suppressive phenomenon, this was demonstrated at a cellular level using lymphocyte proliferation and delayed hypersensitivity techniques (Teitelbaum et al., 1997). The humoral reactivity was later demonstrated in 1991 (Teitelbaum et al., 1991). Due to its high polarity and hydrophilic nature, Cop-1 does not cross the blood-brain barrier. It therefore exerts its greatest immunological effects in the periphery. The nature of the random composition of the amino acids and the relatively short length of the peptide allows its binding to the major histocompatibility complex class II (MHCII). This union suggests different mechanisms of action based on experimental evidence in the EAE model and MS patients treated with the drug (Teitelbaum et al., 1991).

The pharmacodynamics of Cop-1 have been evaluated through its immunomodulatory potential, based on its effect on specific cells of the immune system that respond to MBP and other possible myelin antigens. Four molecular mechanisms identified are: competition for the binding of Cop-1 versus MBP to molecules of the major histocompatibility complex (MHC), competition for binding of the Cop-1 complex MHC versus MBP / MHC complex to the receptor of T cell, activation and induction of tolerance of specific T cells against MBP and induction of regulatory Th2 cells (Johnson et al., 1998).

The drug inhibits the activation of autoreactive T cells against MBP, clones with a Th1 phenotype that are exposed to it have shown a dose-dependent inhibition as well as an increase in IFN-$\boldsymbol{\gamma}$ secretion, interfering with the activation of T cells, including those of an autoimmune nature (Gran et al., 2000). However, the effect of Cop-1 on the production of ROS and RNS is limited to the effect of the cytokines produced by Th2 lymphocytes (IL10, IL4 and TGFβ of the English transforming growth factor beta), for which the effect of this it is diminished on the demyelination process in its early stages (first 72 hours). In some models of spinal cord injury, the use of antioxidants has been used together with immunization with other modified neural peptides, observing a better motor recovery and neuronal preservation (Duthie et al., 1997), therefore the use of antioxidants with immunization with Cop-1 it could reduce the demyelination triggered and thereby preserve neural tissue and promote motor recovery.

*Natalizumab*

Natalizumab is a humanized monoclonal antibody administered intravenously that represents an important therapeutic option for the most aggressive forms of MS, natalizumab is directed against alpha 4-integrin, a cell adhesion molecule present in the surface of monocytes and lymphocytes, blocking their binding to VCAM1 on the endothelial surface (Sheremata et al., 2005). Because of their binding, it inhibits the migration of these cells through the BBB to CNS, reducing its inflammation. In clinical trials, it has shown natalizumab's effectiveness over 2 years, during which nearly half of early RRMS patients achieved no evidence of disease activity (NEDA). During year 2, nearly 75% of patients exhibited NEDA. Over 2 years, patients continued to experience significant cognitive and quality-of-life benefits. (Perumal, J. et. al., 2019).

The visible effects in clinical practice have resulted in a reduction of relapses, delay in disease progression and improvement on MRI in patients with RRMS (Fernández et el., 2015). In return, long-term exposure to Natalizumab increases the risk of progressive multifocal leukoencephalopathy (PML), an infection opportunistically caused by the John Cunningham virus or JC virus. This can reactivate in patients who are immunocompromised so patients taking natalizumab should undergo check-ups periodically (Rico Peñas, 2015).

*Mitoxantrone*

Mitoxantrone is a derivative of anthracycline that functions through inhibiting type II topoisomerase and disruption of DNA synthesis and might stimulate microglial death (Li, J.M. et. al., 2012). It is used in patients with recurrent-remitting or secondary progressive MS, with outbreaks that present

an increased clinical activity, and a treatment administrating conventional immunomodulators does not work. Mitoxantrone is effective in reducing progression of disability and outbreak rate, however its use has been considerably restricted due to its cardiotoxicity and risk of acute leukemia (Bustamante & Sánchez, 2015).

*Azathioprine*

Azathioprine is a derivative of mercaptopurine. Their primary effects are focused against cells whose division is high, causing the inhibition of both cellular and humoral immunity. As an MS treatment the efficacy of this drug has been studied in a retrospective meta-analysis, showing a slight reduction in the advance of the disease after 3 years of treatment, and a reduction in outbreaks (Fernández et el., 2015).

***Alternative Treatments***

*Plasmapheresis*

Therapeutic plasma exchange apheresis (PP) is an extracorporeal blood purification technique designed to remove substances with a large molecular weight. The utility of this procedure includes the removal of antibodies, alloantibodies, immune complexes, monoclonal proteins, toxins and cytokines, and it involves the replenishment of a specific plasma factor containing 5% albumin. It is performed using a commercial continuous flow cell separator with technology based on centrifugation or transmembrane filtration (Navarro-Martínez & Cauli, 2020).

This therapy is one of the most contemporary and has been tested by Faissner et al. (2016) in patients with the chronic progressive form of the disease and with concomitant immunosuppressive therapy in order to reduce the risk of rebound effects, showing restoration on the responsiveness to IFN$\beta$ therapy (Medenica et al., 1994) normalizing CD4+ counts, CD8+, HLADR antigen-bearing cells, NK, serum IFN, and the monocyte/macrophage cell population in PP responders. Unfortunately, the effect on IFN has proven to be transient, and lasts for 1 to 2 months even during the ongoing PP sessions. The beneficial effects of PP in MS patients range between 27–87% in clinical studies, and seems to depend on the number of PP sessions and the patient's characteristics.

*Diet modification*

Diet possibly plays an important role in MS as suggested by increased MS risk in populations with high meat and dairy consumption and suggestion of increased magnetic resonance imaging (MRI) disease activity with higher lipoproteins levels (Altowaijri et al., 2017). It is believed that diet may affect the disease by acting directly on enzymes, receptors, gene modification, vascular disease and microbiome modification, different kinds of diets have been suggested to improve the quality of life of MS patients, such as:

- Mediterranean Diet: extensively studied in cardiovascular disease, proven to have positive effects on vascular health in general. Since this diet has olive oil as its main source of fat it is suggested for MS due to the anti-inflammatory and free radical scavenger mediating a neuroprotective effect demonstrated in different animal models (Gardener et al., 2011).

- Paleolithic Diet: made popular by Dr. Terry Wahls, based on the concept that the human body is better equipped to handle a diet that might have been consumed by our ancestors (Wahls, 2014). It recommends a dietary pattern that includes a daily intake of three one-cup servings each of leafy green vegetables, sulfur containing vegetables, and intensely colored fruits and vegetables (FV). In addition, 6–12 ounces of meat and fish are recommended daily, as well as 16 ounces of fatty fish and 12 ounces of organ meat weekly. This specialized diet also excludes all dairy, gluten-containing grains, and eggs, and processed foods, added sugar, and trans-fat are to be avoided (Titcomb, T. J. et. al, 2020).

- Swank and Low-fat Diets: Dr. Roy Swank (1990, 2003) developed this diet based on the observation that MS prevalence increased in patients with high saturated fat intake. It limits the fat intake to 15 g per day and demonstrates reduced relapse rate, reduced disability progression, and improved overall survival (Wahls, T. et. al., 2018).

- McDougall Diet: it is a low-fat, plant-based diet that is based mainly on complex carbohydrates as the main

source of energy and was studied on brain MRI, clinical activity, fatigue, quality of life, metabolic biomarkers, adherence, and safety in people with relapsing-remitting MS. The diet group that was part of this experiment showed a significant reduction in fatigue, nevertheless, it did not show any effect on MS activity. (Yadav et al., 2016).

It is suggested that a combination of a good diet emphasizing intake of fruits, vegetables and legumes, and whole grains, low intakes of sugar, and red meat, and a healthy lifestyle are associated with a decrease in disability and symptoms in MS (Fitzgerald et al., 2017) as well as other holistic methods such as massages, acupuncture and meditation to improve fatigue in MS patients (Bisht et al., 2014).

*Vitamins*

The data regarding the relationship between antioxidant vitamins and MS is limited. It was found that low dietary intake of vitamin A, vitamin E and selenium but not of beta carotene or ascorbic acid, are associated to be a risk factor for the onset of the disease (Besler et al., 2002).

An investigation on the relevance of circulating carriers of vitamin D, vitamin D binding protein (DBP) and albumin in MS, it has been shown that the plasma level of DBP increases in patients during the remission phase (Rinaldi et al., 2015). This suggests a correlation between DBP and MS pathophysiology.

1,25(OH)D3, the active form of vitamin D, has a dual effect on the immune system by promoting the innate system response and suppressing the adaptive immune activity. Its effect is well characterized on T-helper cells due to the fact that their proliferation and cytokine production are under regulation of 1,25(OH)D, and has as suppressing effect on producing inflammatory cytokines mediated by type 1 T-helper (Th1) cells (R.F. Chun et al., 2014).

The maturation of human dendritic cells (DC) can also be regulated by 1,25(OH)D3 and the vitamin D receptor (VDR). Following exposure of differentiating human and mouse monocytes to 1,25(OH)D3, expression of molecules responsible for antigen capture is increased and DC differentiation and maturation is inhibited that leads to the insufficient stimulatory capacity of CD8+ T-cells specific antigen. Furthermore, the number of T-reg cells will be increased, which promotes IL-10 up-regulation from CD4+ T-cells and decreases the level of tumor necrosis factors (TNF) and in-terferons (IFN). Such molecules might influence suppression and interaction of DCs and T-cells in mice and humans (Khosravi-Largani et al., 2018).

Due to these results, it can be inferred that vitamin D supplementation can contribute to and delay the appearance of MS symptoms.

In another study, the effect of vitamin A on disease progression of 101 patients with MS was evaluated. The results show that vitamin A, administered as retinyl palmitate, suppresses the progression of upper limbs and cognitive disabilities and it seems to be valuable in suppressing neurodegenerative or inflammatory conditions of MS patients (Bitafran et al., 2015).

*Omega-3 fatty acid*

In MS patients, polyunsaturated fatty acid (PUFA) and antioxidant deficiencies along with a decrease on cellular antioxidant defense mechanisms have been observed, in EAE antioxidants and PUFA used as a treatment has shown a decrease in clinical signs of disease (Van Meeteren et al., 2005).

Omega-3 PUFAs are essential fatty acids, that means that mammals cannot synthesize them, but they are essential in the body to maintain health, so they are acids of fundamental intake in the human diet, found in large quantities in fish and shellfish, particularly oily fish, and in some plants such as flax seeds (Jelinek et al., 2013). There are three main Omega-3 acids. A-linolenic acid (ALA), which is the essential short-chain n-3 PUFA in the diet, and a precursor to the other two: eicosapentaenoic acid (EPA) and docosahexaenoic acid (ADH) (Layé et al., 2018).

In numerous studies, the effect of Omega-3 at the inflammatory level is evaluated, considering the variation in the levels of metalloproteinases of the matrix after its administration. In 2007, GM Liuzzi et

al. observed that treatment of microglia with Omega-3 PUFA, or dose-dependent fish oil, inhibited the amounts of matrix metalloproteinase-9 (MMP-9) produced by lipopolysaccharide-stimulated microglia, but not the levels of matrix metalloproteinase-2 (MMP-2).

L Shinto et al. conducted in 2009 a study in which the administration of Omega-3 through fish oil concentrate resulted in a significant 58% decrease in the levels of MMP-9 secreted in peripheral blood mononuclear cells (PBMC) after three months dietary supplementation while, on the other hand, there were no significant changes in terms of quality of life in patients.

On the other hand, many studies base their results on the expression levels of pro-inflammatory cytokines, such as IL or TNF, and nitric oxide (NO), directly related to T cell differentiation. The study carried out by W Kong et al. in 2011 is the first to show a significant decrease in the number of Th1 / Th17 encephalitogenic cells in the spleen and CNS of EAE mice which were fed with an ADH-enriched diet.

A recent clinical trial conducted by Kouchaki E., et. al. (2018) combined omega 3 fatty acid and vitamin D supplementation being randomized and placebo-controlled with Expanded Disability Status Scale (EDSS) score and inflammation as primary outcomes and oxidative stress biomarkers and metabolic profile as secondary outcomes showed a significant improvement in EDSS. In addition, cosupplementation resulted in a significant reduction in serum insulin, insulin resistance, and total/HDL-cholesterol, and a significant increase in insulin sensitivity and serum HDL-cholesterol concentrations proving also beneficial on overall metabolic status.

***Exogenous Antioxidants; Luteolin***

Flavonoids are chemical structures present in fruits, vegetables, seeds, nuts, etc. that have demonstrated antioxidant, anti-allergic, and anti-inflammatory activity (Fu et al., 2018).

Luteolin (LU) plays an important role by having antioxidant, anti-inflammatory, and neuroprotective properties in different diseases. LU has been found to modify the levels of enzymatic antioxidants and lipid peroxides, it also reduces the levels of glutathione peroxidase (GSH-px) and the inflammatory cytokine IL-12 in plasma or liver tissue in a liver cancer model in mice. Additionally, LU could activate erythroid-related nuclear factor 2 (Nrf2) - which is related to the expression of antioxidant genes in HepG2 cells of the hepatoma, and it has also been observed to prevent apoptosis induced by peroxidation by modulating signaling pathways associated with oxidative stress and apoptosis (Verbeek et al., 2004).

In particular, LU is a flavonoid that has shown inhibition of IL-3 secretion in basophils, as well as inhibition of the AP-1 pro-inflammatory pathway. Furthermore, it inhibits IgE-mediated secretion of histamine, leukotrienes, D2-type prostaglandins, and mast cell colony stimulating factor. Likewise, it reduces inflammation of the CNS by preventing the migration of monocytes through the BBB and can inhibit clinical symptoms of EAE, also inhibiting macrophages that phagocytose myelin, as well as the proliferation and activation of T cells. autoimmune or differentiation to a Th2 phenotype [66, 67].

Flavonoids can be beneficial in neurodegenerative diseases because they can cross the BBB and neutralize free radicals that are involved in the pathogenesis of MS and EAE. Unique subclasses of flavonoids, flavones and flavonols, inhibit human mast cell secretion of pro-inflammatory molecules in response to triggering allergies and IL-1. The antioxidant capacity of LU to inhibit mast cell and T cell activation presents a new approach to the treatment of MS (Jacobs et al., 1986).

***Epigallocatechin gallate (EGCG)***

Epigallocatechin gallate (EGCG) is one of the major components of green tea. Due to its activity, it is considered a powerful antioxidant because the rings of phenol located in its structure act as electron traps and free radical scavengers, inhibiting the formation of ROS and reducing the damage caused by oxidative stress (Chu C et al., 2017).

Closer examination of its antioxidant characteristics highlights the improvement of mitochondrial function. EGCG also improves insulin resistance mediated by lipid infusion,

related to increased expression of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase (Kim HS et al., 2014).

Studies have found that EGCG benefits mice with EAE disease progression, inflammatory process, and demyelination of the SNC30 (Meng et al., 2016). Furthermore, this neuroinflammatory activity has also recently been attributed to inhibition of the release of group 1 protein from high mobility responsible for mediating injury or inflammatory stimuli (HMGB1), induced by endotoxins (Meng et al., 2016).

Generally speaking, EGCG has a large potential to lead to healthy aging by improving inflammatory disorders, morphological and functional events that occur in the brain, increase learning capacity and reduce oxidative damage lesions in the brain (Atulkumar et al., 2016).

***Mesenchymal Stem Cells***

Stem cells are unspecialized cells with the ability to renew themselves for long periods without significant changes in their general properties, they have the ability to differentiate into various specialized cell types under certain physiological or experimental conditions (Wei, X. et.al., 2013).

Mesenchymal stem cells (MSCs) are non-hematopoietic adult stem cells with self-renewal ability, originating from the mesoderm, possess a multilineage differentiation capacity. They were isolated from bone marrow as colony forming unit fibroblast (CFU-F) by Friedenstein et al. in 1967 but they have also been isolated from other tissues such as adipose tissue, umbilical cord, fetal liver, muscle and lung. (Mansoor, S. R., Zabihi, E., & Ghasemi-Kasman, M., 2019).

The therapeutic potential of MSCs is directly associated with their differentiation capacity and paracrine effects. Furthermore, they are able to secrete different growth and trophic factors, cytokines, microRNAs, amongst others. In the specific context of treating MS, MSCs showed to be able to differentiate towards oligodendrocytes, by expressing oligodendrocyte progenitor cell (OPC) markers. A smaller percentage of cells also expressed myelin basic protein (MBP), which is a marker of mature oligodendrocyte. A very low percentage of cells also expressed the astrocyte marker glial fibrillary acidic protein (GFAP) (Ghasemi, N., 2018). The conditional medium (CM) included in the secretome derived from MSCs was able to enhance oligodendrogenesis in hippocampal neural stem cells and to support their integration into neuronal networks (Jadasz, J. J., et. al. 2018).

Several phase 1 and 2 clinical trials have been registered, their main aim was to evaluate the safety of MSCs transplantation, however some studies have also shown improvements. A phase 1 trial confirmed the suitability and safety of autologous bone marrow MSCs (BM-MSC) intravenous transplantation in RRMS and SPMS patients. At the 6-month follow-up no severe adverse events or demonstration of disease activation were recorded. Several of these studies have reported that the main side effects presented during treatment were minor, such as fever, headaches and some infections, mostly associated to the lumbar puncture or pain at the injected site in case of intravenous administration (Gugliandolo, A., Bramanti, P., & Mazzon, E. 2020).

Due to the nature of phase 1 and 2 clinical trials it's important to confirm the efficacy of the treatment on proper clinical trials, it is also necessary to take into consideration that each study used a different cohort of patients, different cell types, different cell numbers. For this reason, it is difficult to define the optimal treatment in terms of dosage, administration conditions and cell type.

## Conclusion

On behalf of the fact that several hypotheses suggest that the disease is the result of interactions between genetic factors and environmental agents, the research for preventive methods for MS has a high priority. Currently, various therapeutic strategies have focused on modulating the immune response through both pharmacological (glatiramer acetate, natalizumab, mitoxantrone, azathioprine) and non-pharmacological means (plasmapheresis, diet modification, vitamins, omega-3 fatty acid and exogenous antioxidants such as luteolin and epigallocatechin gallate) due to the lack of a

definitive cure. However, neither approach is successful in achieving substantial improvements in all MS patients due to the controversy generated by the choice of a functional and personalized treatment depending on the degree of inflammation present over time. Treating the right patient with the suitable drug soon in the initiation steps of the disease course, before disability would occur, could be accompanied with long-term positive results, a combination of these methods, in tandem with positive lifestyle habits, such as healthier eating, is recommended to increase the success of treatments and mitigate long-term symptoms of the chronic disease. There are other alternatives yet to be thoroughly explored, such as the transplantation of mesenchymal stem cells (MSC) considering their ability in differentiation, migration, immune-modulation and neuroregeneration; searching for strategies to restore myelination by targeting a specific transmembrane protein of nervous system, leucine-rich repeat and immunoglobulin domain containing 1 (LINGO-1) or even consider epigenetic therapy as described by Gholamzad, M., et. al. (2018).

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