MULTIPLE SCLEROSIS: AN INSIGHT INTO EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE)

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ABSTRACT

Multiple sclerosis (MS) is a demyelinating disease of the CNS that results in motor, sensory, and cognitive impairment. MS is driven by myelin-specific auto-reactive T cells that infiltrate the CNS and mediate an inflammatory response that results in demyelination and axonal degradation. It eventually leads to the patches of nerve scarring, known as ‘sclerosis,’ which gave this its name, multiple sclerosis. Experimental autoimmune encephalomyelitis (EAE) is currently the most commonly used and well-characterized animal model with clinical and pathological characteristics highly relevant to MS. The majority of current therapies for MS have been first examined and validated in EAE animal models. US food and drug administration (USFDA) has approved many therapies such as interferon-β, glatiramer acetate (GA), natalizumab and mitoxantrone for the treatment of various forms of MS like PRMS, RRMS and SPMS. Despite the tremendous advances in medicine, introduction of immunomodulators and anti-inflammatory agents, the MS is one of the most difficult conditions to treat permanently and successfully.

KEYWORDS: Multiple sclerosis; EAE; demyelination; autoimmune.

AN OVERVIEW OF MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is an inflammatory disease of the CNS characterized by a demyelination of axons with inflammatory plaques leading to a deficiency or complete loss in nerve impulse transmission. Actually MS is an autoimmune disease involving multifocal...
areas of brain lesions which contain demyelinated axons due to the initiation of an inflammatory response associated with the recruitment of lymphocytes auto reactive to myelin proteins. The inflammation causes patches of damage called plaques and the pattern of plaque formation will be different in each individual, although it characteristically occurs in the white matter of the CNS. \[1,2,3\]

MS is one of the most common disabling neurological diseases in young adults. It is more prevalent in Caucasians of northern European ancestry. Despite numerous studies and experimental trials a complete understanding of the pathogenesis of MS still remains unclear. The disease course is unpredictable and life-long. The etiology of the disease seems to be dependent on the combination of genetic and environmental factors which play an important role in the process of demyelination. Evidence from a variety of studies also indicates that certain people are genetically predisposed to MS and several studies have suggested viral or microbial infections as a contributor to the disease. \[4\]

The CNS pathology of MS is characterized by the breakdown of the BBB followed by inflammation and neuronal damage. Immune cells are believed to enter the CNS through the disrupted BBB. \[5,6,7\] The myelin sheath and the axons are the primary target of the inflammation. As disease progresses, new myelin antigens are presented by APCs (epitope spreading), leading to subsequent activation of newly infiltrated T cells. \[8\]

Many of the typical symptoms of MS affect walking, balance, coordination, bladder function, etc. All of these functions depend on the cables connecting the brain to the limbs or bladder via the spinal cord. Nevertheless, the different parts of the cortex are connected by white matter, so some subtle intellectual impairment is consistent with demyelination and axonal damage. Loss of muscular control can also result from motor axon damage (i.e. breakage, transaction and degeneration). Although remyelination can occur in early or acute MS, this process of recovery is limited in the more progressive forms of the disease when repair of the damaged myelin sheath will become impossible. \[9,10\]

**ANIMAL MODELS OF MS**

MS is a major disabling disease of the CNS, which has been described for over two hundred years, yet it is still enigmatic and inadequately controlled. \[11\] As the CNS cannot easily be sampled, to gain ideas about disease mechanisms, a number of models have been developed. These include myelin mutants, chemically-induced lesions, viral and autoimmune models all
of which show some evidence of demyelination, a pathological hallmark of MS.[12] These models have largely been used to study mechanisms of de/remyelination and are currently used as pre-clinical drug screening tools for MS.

A number of viruses, including Semliki Forest Virus and Theiler’s Murine Encephalomyelitis virus (TMEV), have been found to induce disease by neurotrophic infection of the CNS, specifically oligodendrocytes. Whilst some viral strains may be cytopathic to the oligodendrocytes, in many instances virally-infected cells are attacked by T cell and humoral responses, leading to demyelinating disease. TMEV has notably been used to demonstrate mechanisms by which autoimmunity may develop following a viral infection. This paradigm is consistent with the aetiology of MS, where viral molecular mimicry and determinant spread, where damage from infection may stimulate subsequent autoimmunity may contribute to the generation of an auto aggressive immune response.

Much extensive research on the autoimmune disease MS has been performed mainly on its animal model known as experimental autoimmune encephalomyelitis (EAE). EAE animal models have been extensively used to investigate potential therapeutics for MS. The origin of the model is traced to the development of the rabies vaccine when it caused encephalomyopathy in a small percentage of humans who received the vaccine. It was later determined that these vaccines contained myelin antigens which triggered an immune response targeting the myelin of the vaccine recipient.[13]

**EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE)**

EAE is a demyelinating and T cell mediated autoimmune disease of the CNS. It is one of the best studied models for human autoimmune diseases, giving insight into a broad array of immunological and histopathological mechanism, so it serves as the animal model for MS. In MS and its animal model EAE, the immune system provokes the detrimental process via autoimmune inflammatory mechanism, leading to activation of cascade induction and proliferation of proinflammatory cytokines which is the primary morphological hallmark of the disease.[14,15]

EAE in mice represents an acute paralytic disease mediated by CD4\(^+\) T cells. A critical step occurs in the development of brain inflammation is the adhesion of leukocytes on endothelial cells and their subsequent migration through the BBB into the CNS tissue.[16,17] Within CNS, both local and infiltrating APCs present MHC class II associated myelin peptides in the
context of costimulatory molecules. The infiltrating myelin-specific T cells are then reactivated, initiating a cascade of events including the secretion of chemokines that recruit predominantly macrophages to the sites of T-cell activation. They encounter their antigen myelin and start to release cytokines and chemokines, which are responsible for the initiation and perpetuation of inflammation.\cite{18,19}

The inflammation of the brain causes the recruitment of T cells, macrophages and cytokines such as TNF-\(\alpha\) and IL-1\cite{20} and the activation of microglia. This neuroinflammatory response results in infiltration of T cells, B cells and macrophages, and focal plaques of demyelination in the CNS that are similar to the pathology seen in MS. Based on these correlations, EAE has received the most attention as a model of MS and is routinely used in testing the therapeutic strategies for MS.\cite{21}

**Direct and Adoptive transfer models of EAE**

EAE mouse models are broadly classified into two broad categories as direct EAE and adoptive transfer. Adoptive transfer of encephalitogenic T cells and active immunization with myelin antigen are known as passive and active ways of inducing EAE. In active immunization, susceptible animals of different species can be immunized with variety of neuroantigens (encephalitogenic peptides).\cite{22,23}

Direct EAE (actively induced EAE) consists of an induction phase and an effector phase. The induction phase of the disease involves the priming of myelin epitope–specific CD4\(^+\) T cells following immunization with myelin proteins or peptides in complete Freund’s adjuvant (CFA). The effector phase consists of multiple stages:\cite{1} migration of activated myelin-specific T cells to the CNS, which involves extravasations of the T cells across the tight endothelial junctions comprising the blood-brain barrier;\cite{2} elaboration of chemokines and cytokines by the myelin specific T cells, which induce the influx of peripheral mononuclear phagocytes into the CNS parenchyma;\cite{3} activation of peripheral monocytes/macrophages and CNS-resident microglial cells by TH cell–derived cytokines; and\cite{4} demyelination of CNS axonal tracts by the phagocytic activity of activated mononuclear cells and by the inflammatory and cytotoxic effects of cytokines (e.g., IFN-\(\gamma\), IL-17, TNF-\(\alpha\) and NO) released from activated CD4\(^+\) T cells and monocytes.\cite{24}

In the adoptive-transfer (AT) model of EAE, disease is induced by the peripheral introduction of a preactivated population of myelin epitope–specific CD4\(^+\) T cells to a naive mouse i.e. it
is induced by the transfer of *in-vitro* encephalitogenic T cell from mice immunized with these encephalitogenic neuroantigens (myelin proteins). Development of EAE by this method usually results in more severe disease, with higher incidence and a more accelerated and synchronous disease course. EAE can also be reproducibly induced by the adoptive transfer of long-term T cell lines or clones specific for PLP139-151, PLP178-191, MBP84-104, or MOG35-55.

**Clinical Presentation of EAE**

EAE is neither multiple sclerosis, nor is it a single disease in a single species, but nature of its different forms resemble various stages and types of MS very closely in a large number of ways. EAE assumes a variable disease course that progressively results in escalating degree of ascending paralysis. Paralysis usually begins with a weakened tail that gradually followed by hind limb paralysis and rarely front limb paralysis. The resulting paralysis is debilitating, but not painful and most animals show some degree of recovery even from advanced stages of EAE. EAE disease progression can be monitored with a scoring system that can differ between investigators but always starts with the normal condition and ends when the mice become moribund. Since EAE is a variable disease in a sense that there is no reliable way to predict whether an animal will recover or not. As a result, close monitoring is needed in this animal model.\(^{[25]}\)

The clinical course of the EAE typically consists of four different types:

- acute fatal EAE
- chronic progressive EAE
- chronic relapsing EAE
- chronic EAE with delayed onset

In acute fatal EAE, there is an abrupt weight loss, weakness of hind limbs, altered gait of animals, rapidly progresses to paralysis of the involved extremities, incontinence and impaired respiration which lead to animal’s death shortly after the immunization. In chronic progressive EAE, the disease develops slowly but becomes progressive within two weeks. In chronic relapsing EAE, the animals suffer from acute disease with variable intensities. The signs would either be mild consisting of weak hind limbs, altered gait and incontinence or severe paraplegia of hind legs. After a complete recovery which lasts for few days or so, relapses occur. In chronic EAE with delayed onset, there is a weight loss and general
weakness occur in two weeks after the immunization with ascending neurological symptoms starting in one or two weeks.\textsuperscript{[26]}

In this each mouse is graded daily and assigned a score ranging from 0 to 5. Scoring is based on clinical manifestations according to the following parameters: 0: no disease; 0.5: loss of tonicity of the distal portion of the tail; 1: total loss of tail tonicity; 2: motor incoordination and hind limb weakness; 3: hind limb paralysis and fore limb weakness (partial paralysis); 4: complete hind limb and fore limb paralysis; 5: moribund.

**Various Actively induced EAE Models**

EAE can be induced in a variety of ways and generate different models. The choice of the peptide together with the animal species/strain and method of induction will determine the type of disease one wants to mimic.\textsuperscript{[27]} EAE has been induced in a number of different animal species including mice, rats, guinea pigs, rabbits, macaques, rhesus monkeys and marmosets. For various reasons including the number of immunological tools, the availability, lifespan and fecundity of the animals and the resemblance of the induced disease to MS, mice and rats are the most commonly used species. Due to the importance of the animal model of EAE in understanding mechanisms involved in multiple sclerosis, many different approaches have been performed to get the model.

With different strains of mice, rats, or other mammals and immunizing with different epitopes (peptides, whole proteins or complex mixtures like spinal cord homogenate-SCH), a range of pathological and temporal outcomes related to EAE can be achieved. Various proteins as a whole or in some parts that make up myelin surrounds nerve cells, induces an autoimmune response in the animals.

In 1970's and 1980's, EAE was readily generated with whole homogenate of spinal cord or brain white matter in guinea pigs, rats, rabbits and monkeys, but with many difficulties in mice. Developing sophisticated methods, researchers finally came up with the advantage of using peptides instead of whole brain and spinal cord homogenates. Among the most commonly employed are myelin basic protein (MBP, a major component of myelin), proteolipid protein (PLP, a major component of myelin) and myelin oligodendrocyte glycoprotein (MOG, found on the outer surface of the oligodendrocytes). Others include S100β and glial fibrillary acidic protein.
Various antigenic myelin proteins are obtained by stepwise reduction of the complexity of the antigenic material from crude brain tissue. Following are some examples of antigenic myelin proteins:

- Myelin basic protein (MBP) such as MBP, 1-37 MBP, MBP 1-11, 1-9, 83-99.
- Myelin oligodendrocyte glycoprotein (MOG) such as MOG and MOG 33-55, 55-75.
- Proteolipid protein (PLP) such as PLP139-151.
- Myelin-associated oligodendrocytic basic protein and 2’, 3’-cyclic nucleotide 3´-phosphodiesterase.

The immunization of susceptible animals with CNS antigens give rise to a spectrum of inflammatory disorder collectively like acute EAE in lewis rat by immunization with MBP, chronic EAE in C57BL/6 mice by MOG33-55, chronic relapsing in biozzi mouse by MOG/PLP/SCH and relapsing remitting form in SJL/J mice by PLP (29). So depending upon the type of EAE that is required, the protein would be selected.

A variety of well-characterized rodent and primate models are now available (refer table 1) that reproduce specific aspects of the immunopathology of the human disease.

**Table 1: Commonly used rodent EAE models**

<table>
<thead>
<tr>
<th>Model</th>
<th>Similarities to human disease</th>
<th>Differences from human disease</th>
<th>Further comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lewis rat Active EAE (CNS myelin, MBP, MOG, PLP)</td>
<td>T-cell inflammation and weak antibody response</td>
<td>Monophasic, little demyelination</td>
<td>Reliable model, commonly used for therapy studies. With guinea-pig MBP little demyelination</td>
</tr>
<tr>
<td>Adoptive-transfer EAE (MBP, S-100, MOG, GFAP)</td>
<td>Marked T-cell inflammation. Topography of lesions</td>
<td>Monophasic, little demyelination</td>
<td>Homogeneous course, rapid onset. Differential recruitment of T cells/ macrophages depending on autoantigen</td>
</tr>
<tr>
<td>Active EAE or AT-EAE + co-transfer of anti-MOG antibodies</td>
<td>T-cell inflammation and demyelination</td>
<td>Only transient demyelination</td>
<td>Basic evidence for role of antibodies in demyelination</td>
</tr>
<tr>
<td>Congenic Lewis, DA, BN strains Active EAE (recombinant MOG aa 1–125)</td>
<td>Relapsing–remitting disorders, completely mimic histopathology of multiple sclerosis and subtypes</td>
<td>No spontaneous disease</td>
<td>Chronic disease course, affection of the optic nerve, also axonal damage similar to multiple sclerosis</td>
</tr>
<tr>
<td>Murine EAE (SJL, C57BL/6, PL/J, Biozzi ABH) Active EAE (MBP, MOG, PLP and peptides)</td>
<td>Relapsing–remitting (SJL, Biozzi) And chronic-progressive (C57BL/6) disease courses with demyelination and axonal damage</td>
<td>No spontaneous disease</td>
<td>Pertussis (toxin) required for many strains, it is often not needed for SJL and some Biozzi EAE models. High variability of disease incidence and course, often</td>
</tr>
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</table>
Evaluation of many different mouse strains which are commonly used in the study of autoimmune diseases gives a brief idea of sex differences in the disease course of EAE. Females SJL immunized with two different peptides of myelin proteolipid protein (PLP) and myelin oligodendrocytes glycoproteins (MOG) as well as female ASW and NZW mice show a greater incidence of EAE than males. However, male B10.PL and PL/J mice represent more severe disease than females. C57BL/6 or NOD strains shows no sex difference but a periodicity is observed in the clinical scores of females that may reflect the hormonal fluctuations in the murine estrus cycle.[31]

**Pathology of EAE**

MS is pathologically and pathogenetically heterogeneous and has been divided according to clinical and pathological features into four main subtypes (classical, acute, neuromyelitis optica and concentric sclerosis) with further subdivision of plaque types on the basis of a combination of morphological and immunohistochemical findings.[32]

Plaques of demyelination of varied size and shape can involve cerebral cortex and subcortical white and grey matter, cerebellar white matter, brain stem and spinal cord. Periventricular white matter, optic pathways and spinal cord are often extensively affected. Several schemes have been proposed for subdividing plaques according to disease activity, stage and presumed pathogenesis. A simple practical approach relies on a combination of a myelin stain (such as luxol fast blue (LFB)/cresyl violet or solochrome cyanin), a macrophage marker (e.g., antibody to CD68) and a stain for axons (e.g., Palmgen silver impregnation or immunohistochemistry for neurofilament proteins) to subdivide plaques into the following:

- **Active plaques**, containing perivascular infiltrates of lymphocytes and macrophages and large numbers of lipid-laden macrophages distributed throughout the plaque parenchyma; the macrophages contain myelin debris, which can be observed with appropriate stains.
- **Inactive plaques**, which are hypocellular and densely gliotic, and in which silver impregnation or immunohistochemistry for neurofilament proteins usually shows a reduced density of axons.

- **Chronic active plaques**, which are centrally hypocellular and densely gliotic but include a peripheral zone that is densely infiltrated by lipid-laden macrophages, within some of which myelin debris can usually be seen.

- **Shadow plaques**, which are sharply circumscribed regions of reduced but not absent myelin staining, due to the presence of thinly remyelinated axons.

Astrocytic gliosis in plaques is usually evident in sections stained with haematoxylin and eosin (H and E), but can be observed more obviously by immunolabelling the astrocytes—for example with antibody to glial fibrillary acidic protein. Other features strongly suggestive of MS include plaques of variable size and shape; markedly asymmetrical cerebral, cerebellar or brain stem involvement; and plaques in the cerebral cortex (especially in the subpial region), deep cerebral grey matter or spinal cord.

On H and E staining in EAE induced C57BL/6 mice showed a typical EAE lesion with massive perivascular infiltration and edema in brain and spinal cord. A severe inflammation characterized by leptomeningeal region cell infiltration and extensive perivascular cuffing of the brain and a significant neuronal death and demyelination found in spinal cords.\(^{33,34}\)

The histopathology of cervicothoracic region of spinal cord from the MOG induced EAE in C57BL/6 mice by H and E staining was characterized by inflammatory infiltration and vacuolation, gliosis, necrosis and myelin debris typical of wallerian degeneration. The entire segment of the white matter was affected by inflammatory infiltration and widespread vacuolation. A heavy inflammatory cellular infiltration is perceived in the meninges and the superficial spinal cord. On staining with LFB mild to moderate multifocal Wallerian degeneration, demyelination and necrosis are observed in the gray and white matter of spinal cord. In the white matter loss of focal myelin, multiple vacuoles (digestion chambers, asterisks) and myelin debris are also appeared.\(^{35}\)

Lewis rats immunised with myelin basic protein (MBP) emulsified with CFA showed a typical lesions, characterised by a massive perivascular infiltration of predominantly mononuclear cells, in both the brain and spinal cord.\(^{36}\) In addition, there was congestion of blood vessels. Initial analysis of brain and cervical spinal cord sections suggested that the inflammation intensity was similar between the acute and recovery phases. However,
morphometric analysis indicated that inflammatory infiltrates were significantly higher in the brain sections of acute-phase EAE rats and spinal cord infiltrates were also more intense, although this difference was not significant.

**CURRENT THERAPEUTIC APPROACHES FOR MS**

Only few therapeutics that were successful in pre-clinical EAE trials have shown similar efficacy in MS. The goal of therapy of MS is to produce a period of stabilization of symptoms and interrupt the progress of disease. There is no current treatment that can alter neurological damage. Now a day’s only few established RRMS therapies are available. These have been developed in the murine models which show relapsing remitting form (SJL mice and Dark Agouti (DA) rats) examples are glatiramer acetate (GA), interferon-β (IFN- β), mitoxantrone and natalizumab. These immunomodulators were thus considered first line options in strategies to modify the disease course of MS.

GA is a random polymer consisting of repeated sequences of the four amino acids glutamic acid, lysine, alanine and tyrosine that occur in MBP in a specific molar ratio. It was primarily called copolymer 1 and tested first for its encephalitogenic potency and subsequently for its influence on guinea pig EAE. Mechanism of action of GA, is the modification and killing of APCs, including generation of regulatory T cells and turning the polyclonal CD8+T cell response into an oligoclonal one.\[37\]

IFN- β, a cytokine is the first drug approved for MS. It exerts a wide variety of effects on the immune system. It inhibits both leukocyte proliferation and antigen presentation; it increases the production of anti-inflammatory cytokines and inhibits T-cell migration across the BBB (Billiau et al., 2004). Although widely used in MS, its long-term effectiveness and side-effects are still uncertain.\[38\]

Mitoxantrone has first been proven to be a powerful immunosuppressive drug in EAE and it is now a second-line component of escalating RRMS therapy. Its mechanism of action relies most probably on cytotoxic effects on lymphocytes and induction of apoptosis of APC such as macrophages and dendritic cells.\[39\]

Natalizumab is a monoclonal antibody (mAb) that inhibits the transmigration of immune cells into the inflamed parenchyma of lymphatic organs and the CNS. It binds to a4b1-integrin (CD49dCD29, very late activation antigen-4) on lymphocytes and blocks the interaction with
the integrin ligand CD106 (vascular cell adhesion molecule-1) on endothelial cells thereby
being effective in preventing EAE.\textsuperscript{[40]}

All these therapies are most powerful but they also produce serious side effects like the most
common adverse reaction caused by natalizumab is headache and fatigue. Other common
adverse reactions are: arthralgia, urinary tract infection, lower respiratory tracts
infections.\textsuperscript{[41,42]}

GA injection also causes systemic reaction characterized by chest tightness, flushing, anxiety,
dyspnea, and palpitations and have been responsible for the majority of withdrawals from
treatment (6.5 and 3.5\%, respectively). Daily subcutaneous injections have not yet been
reported as a reason for failed compliance with treatment. Lipoatrophy at the injection site
has been reported.\textsuperscript{[43]}

Mitoxantrone can have serious and life-threatening side effects; cardiotoxicity has been
reported in cancer and MS patients receiving mitoxantrone as immunosuppressive
chemotherapeutic agent. The most common adverse reactions reported by INF-\(\beta\) were
‘headache’, ‘flu-like symptoms’, gait disturbance, dystonia, ‘depression’ (suicide attempt)
and ‘MS aggravation’.

FUTURE THERAPEUTICS (UNDER PRECLINICAL/CLINICAL TRIALS)
To overcome the difficulties like adverse reaction caused by existing ones an increasing
number of emerging therapies for MS are currently being tested in pre-clinical phases by
making use of EAE models. The most promising experimental therapies are minocycline,
fingolimod, statins, cytotoxic agents, oral administration of small molecular weight disease-
modifying drugs and intravenous or subcutaneous application of monoclonal antibodies
(mAb) targeting cells or molecules crucial in the pathogenesis of the disease (refer table 2).

The mechanism of action of fingolimod is through the modulation of sphingosine-1-
phosphate (S1P) receptors in lymphoid and neural tissues. Sphingosine-based phospholipids
are abundant structural components of cell membranes and have chemoattractive function for
lymphoid cells. Resting T-cell and B-cells express high levels of S1P receptor (subtype 1)
and lymphocyte egress from lymph nodes and thymus is dependent on this receptor function.
Fingolimod-phosphate (which forms after in vivo phosphorylation of fingolimod) is a
structural analogue of S1P and after binding to lymphocytes S1P receptors results in their
internalization and renders lymphocytes insensitive to S1P signalling, thereby trapping them in secondary lymphoid organs. Fingolimod administration produces a rapid, reversible decrease in circulating lymphocytes, but does not result in immunosuppression. So, it does not impair T and B cell activation, proliferation and effective function. S1PR modulation by fingolimod in both the immune system and CNS, producing a combination of anti-inflammatory and possibly neuroprotective or reparative effects, may contribute to its efficacy in MS.

Another promising approach are minocycline that inhibits matrix metalloproteinases and thereby T cell transmigration and peroxisome proliferator-activated receptor (PPAR)-α agonists increase the anti-inflammatory cytokine IL-4 have shown good result in the treatment of EAE.\cite{44}

Stem cell transplantation can also be used to target EAE. However, mesenchymal stem cells can modulate the T cell function, decrease IL-17 via IL-23 secretion and neural stem cells can down-regulate the inflammation and stimulate the endogenous brain repair system.\cite{45,46}

Statins the inhibitors of 3-hydroxy-3-methylglutaryl-coenzymeA (HMG-CoA) reductase prevents geranyl-geranylation of RhoA GTPase and its tethering to the Membrane and thereby has proven to inhibit T cell activation and infiltration into the CNS. Based on these observations statins could be utilized as a treatment option for CNS autoimmune disease. The well tolerated oral statin has exerted the pleiotropic immunomodulatory effects in various animal models of human autoimmune disease. The immunomodulatory effects of statins, which encompass modification of endothelial function, plaque stability, thrombus formation and inflammatory pathways, are widely referred to as ‘pleiotropic effects’. These pleiotropic effects indicated that the therapeutic potential of statin might extend beyond CH lowering and CVD to other inflammatory disorders or conditions such as transplantation, MS, RA and chronic kidney disease. By modulation of post translation protein prenylation statins alter the immune function in EAE. Statins exhibited the immune response from Th1 to a protective Th2 response in EAE.\cite{47,48,49}

Atorvastatin treatment found to reduce secretion of APC derived cytokines that are involved in the differentiation of T cells into pro-inflammatory Th1 cells hence; it promotes the differentiation of Th0 cells into Th2 cells. Atorvastatin induced STAT6 phosphorylation and secretion of Th2 cytokines (interleukin (IL)-4, IL-5 and IL-10) and transforming growth
factor (TGF)-β. Conversely, STAT4 phosphorylation is inhibited and secretion of Th1 cytokines (IL-2, IL-12, interferon (IFN)-γ and tumour necrosis factor (TNF-α) is suppressed.\textsuperscript{[50]}

Combinational therapy might be useful in a different mode of action providing an additive or synergistic effect without overlapping toxicities. GA treatment appears to preferentially cause a Th2 deviation of T-cells that are specific for CNS auto-antigens. GA expressed immunomodulatory activity on APC, promoting the secretion of anti-inflammatory cytokines, and inhibiting the secretion of pro-inflammatory cytokines and on myelin-reactive lymphocytes.

Combination therapy of GA and epigallocatechin-3-gallate (EGCG) synergistically reduced neuronal cell death and promoted axonal outgrowth of primary neurons. These effects could be translated into the EAE model in which diminished clinical disease severity was associated with reduced CNS inflammation in a synergistic manner. These results strengthen the prospects of EGCG as an adjunct and well tolerated therapy for neuroinflammatory diseases and underscore the importance of evaluating combined anti-degenerative and anti-inflammatory treatments.\textsuperscript{[51]}

Combination of GA and atorvastatin indeed synergistically ameliorated CNS autoimmunity in the EAE mouse model. Similarly, the combination therapy of atorvastatin/lovastatin and 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside and the combination of atorvastatin with high dose of interferon-1α, is an immunomodulating agent that activates adenosine monophosphate-activated protein kinase. Similarly, combination therapy of Lovastatin and Rolipram is complementary in synergistic/additive manner to provide neuroprotection and promote neurorepair after inflammatory CNS demyelination.\textsuperscript{[52]}

Another promising approach is the combination of atorvastatin and minocycline could reduce disease severity, in both the acute and chronic phases of disease, along with attenuation of inflammation, demyelination and axonal loss and combination of minocycline and prednisone also reduced inflammation and demyelination and also prevented the reduction brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) mRNA expression in cerebral cortex of EAE mice.\textsuperscript{[53]} Combination therapy of methylprednisolone and erythropoietin also reduces the CNS inflammation and neurodegeneration and GA and Salirasib (Ras inhibitor
farnesylthiosalicylic acid) efficiently ameliorate EAE. The combination of these drugs decreases the cellular infiltration in the CNS and inflammation-associated neurodegeneration.

Table 2: Treatment approaches for MS

<table>
<thead>
<tr>
<th>Treatment approach</th>
<th>Status of development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glatiramer acetate</td>
<td>Approved for RRMS</td>
</tr>
<tr>
<td>Anti-a4 integrin</td>
<td>Taken off the market</td>
</tr>
<tr>
<td>Anti-CD4</td>
<td>Halted in phase II</td>
</tr>
<tr>
<td>CTLA-4-Ig</td>
<td>In phase III</td>
</tr>
<tr>
<td>IFN-b</td>
<td>Approved for RRMS</td>
</tr>
<tr>
<td>Natalizumab</td>
<td>Approved for RRMS</td>
</tr>
<tr>
<td>PPARg agonists</td>
<td>In phase II in combination with GA</td>
</tr>
<tr>
<td>Minocycline</td>
<td>In phase II in combination with GA</td>
</tr>
<tr>
<td>Statins</td>
<td>In phase III</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>Approved for RRMS</td>
</tr>
<tr>
<td>Laquinimod</td>
<td>In phase II</td>
</tr>
<tr>
<td>FTY720/SP-1 agonist</td>
<td>In phase III</td>
</tr>
<tr>
<td>Sulphasalazine</td>
<td>In phase III</td>
</tr>
<tr>
<td>Haematopoietic stem cell transplant</td>
<td>In phase III</td>
</tr>
<tr>
<td>IVIG</td>
<td>In phase II</td>
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REFERENCES


