

Experimental Autoimmune Encephalomyelitis Model for Discovery of New Therapy for Multiple Sclerosis

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Abstract: Experimental autoimmune encephalomyelitis (EAE) is the most widely accepted *in vivo* animal model of Multiple sclerosis (MS). EAE model are most suitable for analysis of immunogenetic elements (major histocompatibility complex) and for the study of histological features (inflammation, demyelination and degeneration) of the disease for screening of new treatments for MS. Some approved medications, Glatiramer acetate (GA), Natalizumab and Mitoxantron, were developed directly from studies in EAE. Several trials are ongoing in MS after success in EAE. The present review aimed to present and future treatment options for EAE model.

Key words: Experimental Autoimmune Encephalomyelitis • Multiple Sclerosis • Major Histocompatibility Complex • Demyelination • Degeneration

INTRODUCTION

Experimental autoimmune encephalomyelitis (EAE), an animal model of MS, is a T cell mediated autoimmune disease of the central nervous system (CNS) [1]. In MS and its animal model EAE, the immune system provokes the detrimental process via autoimmune inflammatory mechanism, leading to disseminated, which is the primary morphological hallmark of the disease. MS is a complex multifaceted disease with central role for axonal and neuronal pathology. Permanent clinical disability is revealed when a threshold of neuronal loss is exceeded and CNS compensatory resources are exhausted. Axonal and neuronal injury begins at early disease stages, both in the white and grey matter areas, supporting degenerative disease course. There are various aspects of axonal pathologies such as; axonal transaction, fragmentation, swelling, vacuolization, Wallerian-like degeneration, changes in neurofilament phosphorylation and in sodium channel distribution as well as transport deficits [2-4]. This model is based largely on experimental data coming from the animal model of rodent EAE, which focuses on the inflammatory stage of the disease [5].

The Clinical Course of the Disease 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 progressing 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 progressively 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 a month or so, relapses occur.

Table 1: Similarities between Experimental Autoimmune Encephalomyelitis and Multiple Sclerosis [6]:

Characteristic	Experimental Autoimmune Encephalomyelitis	Multiple Sclerosis
Genetic susceptibility	Strong association with MHC II Females more susceptible in certain strains	Strong association with MHC II Females more susceptible
Environmental triggers	Relapses with earlier infection; superantigens trigger	
Relapses	Association with earlier infection	
White matter pathology	Th1 T cells, B cells, CD ⁴⁺ and CD ⁸⁺ T cells, B cells and antibodies to myelin in lesions Clonal CD ⁴⁺ and CD ⁸⁺ T cells reactive to myelin components Macrophages, Microglia α 4 β 1 integrin, Complement	Th1 T cells, B cells, CD ⁴⁺ and CD ⁸⁺ T cells, B cells and antibodies to myelin in lesions, Clonal CD ⁴⁺ and CD ⁸⁺ T cells reactive to myelin components Macrophages, Microglia α 4 β 1 integrin, Complement
Grey matter pathology	Axonal degeneration	Axonal degeneration
Clinical presentation	Optic neuritis, myelitis, periventricular white matter Inflammation	Optic neuritis, myelitis, periventricular white matter Inflammation
Clinical forms	Relapsing remitting Progressive	Relapsing remitting Progressive

Table 2: EAE scoring system of immunized animals

EAE Score	Symptoms
0	No deficit
0.5	Partial loss of tail tone or slightly abnormal gait
1	Complete tail paralysis or both partial loss of tail tone and mild hind limb weakness
1.5	Complete tail paralysis and mild hind limb weakness
2	Tail paralysis with moderate hind limb weakness
2.5	No weight bearing on hind limbs but with some leg movement
3	Complete hind limb paralysis with no residual movement
3.5	Hind limb paralysis with mild weakness in forelimbs
4	Complete quadriplegia but with some movement of head
4.5	Moribund
5	Dead

In Chronic EAE with Delayed Onset: There is a weight loss and general weakness two weeks after the immunization with neurological symptoms starting in one or two months [7].

Various EAE Model for MS: A large number of EAE model exist, each parameter reflecting pathologies observed in CNS of MS patients. With different strains of mice, rats or other mammals and immunizing with different epitopes (peptides, whole proteins or complex mixtures like spinal cord homogenate) to achieve a range of pathological and temporal outcomes [8]. 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 MBP₁₋₃₇, MBP₁₋₁₁, MBP₁₋₉, MBP₈₃₋₉₉.
- Myelin oligodendrocyte glycoprotein (MOG) such as MOG₃₃₋₅₅ and MOG₅₅₋₇₅.
- Proteolipid protein (PLP) such as PLP₁₃₉₋₁₅₁.

- Myelin-associated oligodendrocytic basic protein and 2',3'-cyclic nucleotide 3'-phosphodiesterase.

Some of other myelin constituents such as neurofascin NF 155, other are expressed on myelin and axons such as the neuronal membrane protein neurofascin NF 186, the neuronal cytoskeletal protein neurofilament-M and the astrocyte-typical Ca²⁺ binding protein S100 β . Neurofascin NF 186 caused axonal injury without enhancing inflammation and demyelination in MOG-EAE. In contrast to MOG-EAE, contactin-2/TAG-1-specific T cells induced inflammatory lesions preferentially in the cerebral cortex and the spinal cord white and gray matter.

Induction of EAE Models in Different Species: The most common mode of EAE induction is based on the injection of an encephalitogenic peptide, mostly MOG₃₅₋₅₅ or PLP₁₃₉₋₁₅₁, which is emulsified in CFA containing mineral oil and *Mycobacterium tuberculosis* strains H37RA, followed by intraperitoneal injections of pertussis toxin. The resulting phenotype depends mainly on the antigen source and the genetic background of the animal species and the strains used. For example, PLP₁₃₉₋₁₅₁ induces a relapsing-remitting EAE in SJL mice, whereas MOG₃₅₋₅₅ triggers chronic-progressive EAE in C57BL/6 mice. Crossing of C57BL/6 mice, which over-express MOG-TCR and MOG specific B cells, resulted in a severe form of EAE with inflammatory lesions of optic nerves and spinal cord. MOG-TCR transgenic mice backcrossed to SJL/J background develop a relapsing-remitting form of EAE with episodes altering between optic nerve, cerebellum and spinal cord. All EAE models are directly accessible to investigation of the immune and nervous system, which interact during the pathogenesis of the disease and which are both targeted by established and experimental therapies [9]. Immunized animals are observe daily by

measuring their body weight, feed weight and assessing clinical signs of EAE, starting at 7 days post immunization [8].

Various forms of EAE have been induced depending on the immunizing neuroantigen and the rodent strain used. Lewis rat EAE showed a monophasic and self-remitting neuroinflammation in the CNS. Swiss-Jackson Laboratories (SJL) mice and Dark Agouti (DA) rats EAE showed a relapsing and remitting course of the disease. MOG induced EAE in the C57BL/6 mice showed a chronic progressive course and other EAE model induced in NOD and DBA/1 mice showed a chronic progressive and severe course of secondary progressive/primary progressive form of MS [10].

Limitations of EAE Models: EAE models are vital for studying general concepts as well as specific processes of autoimmunity. However rarely predict success in clinical trials. Nevertheless, their value is further challenged by understanding of the key pathogenetic mechanisms in EAE models and their failure due to the adverse effects. As with other murine disease models, including the NOD model of type 1 diabetes, it appears much easier to prevent, reverse or ameliorate EAE in mice than multiple sclerosis in man. Furthermore, since EAE almost always has to be induced, it cannot mimic a spontaneous disease. The most important component in the inducing adjuvant CFA is heat-inactivated *Mycobacterium tuberculosis*, which always induces a prominent CD⁴⁺ Th1 response by activating certain toll-like receptors. The time courses are very different. Since EAE develops over days in most models, they seem more similar to post-infectious acute demyelinating events. Indeed, the mice are rarely monitored for late

relapses and fatal adverse effects, such as those noted in marmosets. Nevertheless, the same treatment can have a different degree of efficacy or even opposite effects at different stages in EAE. In contrast, MS usually manifests insidiously over years, for example, relapsing-remitting and chronic forms of MS, by when antibodies and complement may also be more important than in most mouse models. Many aspects of pathology and immunology differ between MS and EAE. These differences are fundamental, as ongoing imbalances in immune regulation must be crucial for the progression of MS; such orders of complexity have not yet been recapitulated in EAE models. Immunological difference between mouse and human are summarized in Table 4 [12].

Approved Therapeutic Regimen for EAE: The goal of therapy of Multiple Sclerosis 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 but only very few therapeutics that are successful in pre-clinical EAE trials have shown similar efficacy in MS patients for example GA, mitoxantrone and natalizumab [12, 13]. The 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 [14].

Table 3: Commonly used rodent EAE models [11]

Model	Similarities to human disease	Differences from human disease	Further comments
Lewis rat Active EAE (CNS myelin, MBP, MOG, PLP)	T-cell inflammation and weak antibody response	Monophasic, little demyelination	Reliable model, commonly used for therapy studies. With guinea-pig MBP little Demyelination
Adoptive-transfer EAE (MBP, S-100, MOG, GFAP)	Marked T-cell inflammation. Topography of lesions	Monophasic, little demyelination	Homogeneous course, rapid onset. Differential recruitment of T cells or macrophages depending on autoantigen
Active EAE or AT-EAE co-transfer of anti-MOG antibodies	T-cell inflammation and demyelination	Only transient demyelination	Basic evidence for role of antibodies in demyelination
Congenetic Lewis, DA, BN strains Active EAE (recombinant MOG ₁₋₁₂₉)	Relapsing-remitting disorders, may completely mimic histopathology of multiple sclerosis and subtypes	No spontaneous disease	Chronic disease course, affection of the optic nerve, also axonal damage similar to multiple sclerosis
Murine EAE (SJL, C57BL/6, PL/J, Biozzi ABH) Active EAE (MBP, MOG, PLP and peptides)	Relapsing-remitting (SJL, Biozzi) and chronic-progressive (C57BL/6) disease courses with demyelination and axonal damage	No spontaneous disease	Pertussis toxin required for many strains, whilst it is often not needed for SJL and some Biozzi EAE models. Higher variability of disease incidence and course, often cytotoxic demyelination in C57BL/6. With rat MBP inflammatory vasculitis with little demyelination
Murine EAE in transgenic mice or knockout mice (mostly C57BL/6 background)	Specifically addresses role of defined immune molecules neurotrophic or Cytokines or neuroanatomical Tracts	Most results obtained with artificial permanent transgenic or knockouts	Extensive backcrossing (>10 times) on C57BL/6 background required. Future work with conditional (cre/loxP) or inducible (e.g. Tet-on) mutants

Table 4: Immunological difference between mouse and human relevant for testing Multiple Sclerosis therapeutics

	Mouse	Human
General	Inbred; homozygous Short lifespan: high fecundity Fixed diet; pathogen-free Clean environment	Outbred; heterozygous Long lifespan: low fecundity Varied diet; carriers of potential pathogens, e.g. EBV, JCV etc Open access to new infections
EAE and Multiple Sclerosis	May be monophasic, Mice tested while epitopes are spreading	Different subtypes usually relapsing, Epitopes must often have spread long before diagnosis
Induction	Usually with CFA	Spontaneous
Testing new therapeutics	Induction of EAE studied much more than ongoing disease, Only a few dozen mice tested	Ongoing disease Hundreds of MS patients; some side-effects are too rare to be seen in mice
Scrutiny	Less detailed	Detailed, would be missed in mice
Follow up	Often short-term only	Several years or life-long
Molecular differences in immune response		
T cell responses	Often stereotypical	Usually idiosyncratic, even to recurring epitopes
Lymphocytes in peripheral blood	75-90%	30-50%
CD ⁴⁺ expression	Lymphocytes	Lymphocytes, macrophages
CD ⁸⁺ expression	Lymphocytes, dendritic cell	Lymphocytes
IL-10 expression	Th2	Th1 and Th2
IFN- α response	No preferential Th1 differentiation	Promotes Th1 response
IL-4 and IFN- γ expression by Th	Exclusively one or the other	Sometimes both
CD28 expression	? 100% of CD ⁴⁺ and CD ⁸⁺ T cells	? 80% of CD ⁴⁺ T cells, 50% of CD ⁸⁺ T cells
MHC class II expression	Absent on T cells and endothelial cells	Present on T cells and endothelial cells
CD52 expression	Not found in mice	Lymphocytes
Glucocorticoid-sensitivity	High	Low and variable

Mitoxantrone, a synthetic anthracenedione derivative, is an immunomodulatory agent. Mechanism of action of mitoxantrone, is the macrophage-mediated suppression of B-cell, T-helper and T-cytotoxic lymphocyte function [15]. Natalizumab is $\alpha 4$ -integrin antagonist, the first in its class for the treatment of RRMS. Natalizumab interferes with the migration of immune cells into the CNS and bind to the $\alpha 4$ subunit of $\alpha 4 \beta 1$ -integrin and preventing leukocyte adhesion to endothelial vascular cell adhesion molecule-1 [16].

All these therapies are most powerful but none of them completely recover the demyelination and inflammation. Apart from these, 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 and lower respiratory tracts infections [17]. GA injections also cause systemic reaction characterized by chest tightness, flushing, anxiety, dyspnoea and palpitations [18].

Mitoxantrone can have serious and life-threatening side effects; cardiotoxicity has been reported in cancer and MS patients receiving mitoxantrone as an immunosuppressive chemotherapeutic agent. It is characterized by changes in electrocardiogram (ECG), asymptomatic decrease in measures of left ventricular ejection fraction (LVEF), or symptomatic congestive heart failure (CHF). The mechanism of cardiotoxicity is not completely understood, but it is associated with higher cumulative doses of the drug [19].

Future Therapies and New Strategies for EAE:

To overcome the difficulties like adverse reaction caused by existing ones like an increasing number of emerging therapies for MS are currently being tested in pre-clinical phases by making use of the EAE model. The most promising experimental therapies are minocycline, Fingolimod, Statin and oral administration of small molecular weight disease-modifying drugs and intravenous or subcutaneous application of Monoclonal Antibody (mAb) targeting cells or molecules crucial in the pathogenesis of the disease.

Fingolimod is an oral sphingosine-1-phosphate receptor (S1PR) modulator. After rapid phosphorylation, fingolimod-P acts as a superagonist of the sphingosine-1-phosphate-1 receptor on thymocytes and lymphocytes and potentially reducing trafficking of pathogenic cells into the CNS [20]. Fingolimod potently inhibits the MS animal model, EAE, but it is ineffective in mice with selective deficiency of the S1P₁, S1PR subtype on astrocytes despite normal expression in the immune compartment. S1PR modulation by fingolimod in both the immune system and CNS, producing a combination of beneficial anti-inflammatory and possibly neuroprotective or reparative effects, may contribute to its efficacy in MS [21].

Another promising approach is Minocycline that inhibits matrix metalloproteinases and thereby T cell transmigration and Peroxisome proliferator activated receptors (PPAR) are nuclear hormone receptors characterized by their ability to regulate adipocyte differentiation and gene transcription. PPAR- α agonists

Table 5: Some immunomodulatory approaches of multiple sclerosis and their development from EAE or *in vitro* studies to clinical application

Therapeutic	Prevents EAE	Reverses ongoing EAE	Efficacy in MS
Glatiramer Acetate	Yes (used with adjuvants)	Not tested, in Approval Package	Approved for relapsing remitting MS, reduces relapse rate by 30%
IFN- β	Yes	Yes	Approved for relapsing remitting MS, reduces relapse rate by 30%
Altered Peptide Ligands(APL) to Myeline Basic Protein(MBP)	Yes	Yes	Phase II b, reduced magnetic resonance activity in Phase II a at low doses, hypersensitivity after repeated injection at high dose, reports of exacerbation only at high dose.
Native peptide for MBP	Yes	Yes	Reduces anti-MBP (Myelin Basic Protein) antibody levels in spinal fluid. Now in Phase II-III.
Anti- $\alpha 4\beta 1$ integrin, Natalizumab	Yes	Yes	Approved but withdrawn after cases of PML (Progressive Multifocal Leukoencephalopathy) were seen.
Statins	Yes	Yes	Early clinical trial with simvastatin showed reduction of activity on Magnetic resonance scans.
Quinolines	Yes	Yes	Quinoline carboxamide reduced MR activity in Phase II but withdrawn due to cardiotoxicity in Phase III. Later generation versions of these compounds appear not to have this toxicity.
Tolerizing DNA vaccine	Yes	Yes	Phase I-II trial in progress.

include troglitazone, pioglitazone and rosiglitazone and were initially designed as antidiabetic drugs because of their insulin-sensitizing effects but also exert anti-inflammatory cytokine IL-4 effects and were shown to inhibit EAE [22, 23].

Gangliosides also show effect on the switching of Th1 to Th2 cytokine expression during the acute phase of EAE and after recovery from the disease. Gangliosides displays mild disease, with low expression of IFN- γ mRNA and high TGF- β mRNA expression and modulated the Th1 cells by the synthesis of cytokines shifting the profile to the Th2/Th3 phenotype [24]. 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 [25].

Dexamethasone is a glucocorticoid that inhibits both innate and adaptive immune responses through direct interaction with glucocorticoid receptors (GR) and lipid membranes. Dexamethasone has a variety of pro-apoptotic and anti inflammatory effects both in MS and EAE and modulating survival and various functions of leucocytes and endothelial cells. Dexamethasone inhibit lymphocyte proliferation and migration into the CNS, production of type 1 cytokines, expression of other proinflammatory molecules such as inducible cyclooxygenase-2 and prostaglandins, while enhancing secretion of types 2 or 3 types of cytokines, CD⁴⁺ T-cell apoptosis and the relative number of T regulatory cells in the circulation [10, 26].

Statins are cholesterol-lowering agents who are also used as anti-inflammatory and neuroprotective. Statins are able to affect several tissue functions and modulate specific signal transduction pathways that could account for statin pleiotropic effects [27, 28]. The primary mechanism by which statins induce immunomodulation is related to the competitive displacement of 3-hydroxy-3-methylglutaryl coenzyme A. The inhibition of the

HMG-Co A reductase enzyme blocks the formation of mevalonate. Other mechanisms were also reported such as decreasing migration of leukocytes across biological membranes and diminishing C-reactive protein. Furthermore, statins seem to have neuroprotective effects, which are of special significance in the treatment of MS [29]. Statins also prevent cell death by multiple mechanisms such as anti-excitotoxic effects, anti-apoptotic effects and delayed pre-conditioning against oxygen-glucose deprivation and promoted neurorepair in demyelination models of MS [30]. Statins have shown to prevent the disease onset and even paralysis when treatment is initiated after EAE induction. They has shown to target multiple key elements of immunological cascade that is believed to lead to inflammatory infiltrations and tissue damage in MS. Based on these findings the outcomes of a large placebo controlled trial testing statin in early MS is being awaited with high expectations [31].

Immunomodulatory Effect of Combination Therapy for Treatment of EAE: 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 [32]. 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 β interferon-1a, is an immunomodulating agent that activates adenosine monophosphate-activated protein kinase [33], similarly, combination therapy with suboptimal doses of Lovastatin and Rolipram is complementary in a synergistic/additive manner to provide neuroprotection and promote neurorepair after inflammatory CNS demyelination [34].

Another promising approach is the combination of atorvastatin and minocycline could reduce disease severity, in both the acute and chronic phases of the disease, along with attenuation of inflammation, demyelination and axonal loss [35] and combination of minocycline and prednisone also reduced inflammation and demyelination and improved magnetic resonance imaging outcomes and also prevented the reduction of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) mRNA expression in cerebral cortex of EAE mice [36]. 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 [37, 38].

CONCLUSION

EAE is used as an animal model of multiple sclerosis. Future of multiple sclerosis therapy looks bright, but increasingly complex. There are number of agents who are used for the therapeutic regimen of EAE through orally and parenterally and some are in different stages of investigation or awaiting approval by federal agencies. All of these medications have demonstrated partial efficacy along with different side effect profiles.

Neurodegeneration occurs through non-inflammatory mechanisms but depend upon prior inflammation. In these case, anti-inflammatory therapies are best prevent the progression of disability in early course of the disease, before the cascade of events that leads to axon degeneration has been irretrievably established. This raises the difficult prospect of exposing well non-disabled young adults to potentially toxic treatments; a decision

made all the more complex by the unpredictable nature of multiple sclerosis. Finally, these drugs continue to inform basic science, revealing aspects of the pathogenesis of multiple sclerosis and reminding us of the complexity of the human immune system.

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