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The Experimental Autoimmune Encephalomyelitis (EAE) Model: A Gateway to Successful Translation of Multiple Sclerosis Therapies

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ABSTRACT

Multiple sclerosis (MS) is a neuroinflammatory disorder that is characterized by demyelination, neurodegeneration, and immune dysregulation. The experimental autoimmune encephalomyelitis (EAE) model has helped to elucidate MS pathophysiology and test therapies. This review synthesizes current literature on the development, applications, and translational significance of EAE models in MS research. It discusses various EAE induction protocols, including active and passive immunization, and highlights advancements such as humanized mice and induced pluripotent stem cell (iPSC)-derived neuronal models. The review evaluates the role of EAE in identifying immune pathways, validating therapeutic agents like glatiramer acetate and natalizumab, and exploring precision medicine approaches through biomarker discovery. The EAE model replicated the key features of MS, including inflammation, demyelination, and axonal loss, facilitating therapy development. However, its predictive validity faces limitations, such as heterogeneity in disease induction, underrepresentation of chronic progression, and species differences. Innovations, such as humanized mouse models and iPSC-derived neurons, show promise in addressing these challenges. EAE research has advanced biomarker-based personalized treatments, although further validation is required. Despite its widespread use, EAE has limitations in terms of variability in disease induction, incomplete MS feature replication, species-specific responses, and clinical translation. Addressing these limitations remains crucial for therapeutic development, focusing on analyzing model limitations and strategies to overcome translational barriers. This review offers immunologists a comprehensive overview of EAE's contributions of EAE to MS research and its potential to inform the development of novel therapeutic approaches for this debilitating disease.

Keywords: Demyelination; Experimental autoimmune encephalomyelitis; Immunopathogenesis; Inflammation; Multiple sclerosis; Precision medicine; Translational research

INTRODUCTION

Multiple sclerosis (MS) is a complex, chronic demyelinating disorder of the central nervous system (CNS) that is characterized by an immune-mediated attack on the myelin sheath. This demyelination leads to

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the formation of lesions or plaques, primarily in the brain and spinal cord.[1] The pathophysiology of MS is influenced by intricate interactions between genetic predispositions and environmental factors, resulting in immune dysregulation and the destruction of oligodendrocytes, which are the cells responsible for myelin production.[1,2]

Clinically, MS does not present with a single set of symptoms; instead, it encompasses a diverse range of manifestations. These may include visual disturbances, sensory abnormalities, muscle dysfunctions, and cognitive impairments.[3,4] Symptoms can vary significantly among individuals and may evolve, often following a relapsing-remitting course that transitions into a progressive phase marked by cumulative disability.[5] The clinical spectrum of MS is broad, with initial symptoms often including paresthesias, weakness, and visual disturbances such as optic neuritis. As the disease advances, patients may experience more severe symptoms including spasticity, bladder dysfunction, and cognitive decline.[1]

The heterogeneity of MS presents significant diagnostic challenges and complicates treatment strategies for acute relapses and the long-term disability associated with the disease.[6] Despite more than five decades since the initial identification of the fundamental aspects of MS pathophysiology, therapeutic options remain limited. Current therapies predominantly focus on immunosuppressive treatments aimed at preventing relapses.[7] However, these interventions do not effectively target the neurodegenerative pathways that contribute long-term disability.[7,8] Disease modifying treatment for progressive forms of MS are lacking, and C57BL/6 induced mice mimics progressive MS, thereby providing a basis for drug discovery.[9,10]

Animal models are essential for advancing our understanding of MS and for developing effective therapeutic interventions. Experimental autoimmune encephalomyelitis (EAE) has become a fundamental tool for investigating the pathophysiology of MS. When induced in C57BL/6 mice using myelin oligodendrocyte glycoprotein (MOG) peptide 35–55, EAE closely mimics the inflammatory and neurodegenerative that are characteristic of MS. This model effectively mirrors the hallmark features of MS, including demyelination and axonal damage, while allowing researchers to explore potential therapeutic strategies aimed at alleviating these pathological changes.[11] The limitations of existing approaches highlight the urgent need for the development of new strategies that address both inflammation and neurodegeneration. There is a substantial gap in understanding the mechanisms underlying disease progression and in translating preclinical discoveries into effective clinical therapies.

Insights from EAE studies have been instrumental in identifying and validating Food and Drug Administration (FDA)-approved therapies, demonstrating the model's value in bridging preclinical research and clinical applications.[12] By elucidating the mechanisms underlying neuroinflammation and neurodegeneration, EAE continues to inform treatment strategies that address both acute relapses and long-term disability in MS patients.[9] This review examines how the EAE model has contributed to the understanding of MS, evaluates translational successes and limitations, and discusses emerging strategies—such as innovative preclinical models and biomarkers—aimed at enhancing therapeutic development and improving patient outcomes in MS.

Literature Search

We conducted a thorough literature search using the electronic databases PubMed, Web of Science, and Scopus between January 6th and January 7th, 2025. Only recently published articles in English were included. Main search terms used were combinationsof the following keywords: "Experimental autoimmune encephalomyelitis" OR "EAE" OR "Animal model" AND "Multiple sclerosis" OR "MS" AND "Therapy" OR "Treatment" AND "Translational research." Original articles and reviews that focused on EAE as an MS model were included. Articles from non-peer-reviewed sources and those written in languages other than English were also excluded.

Ethical Considerations Associated with Using EAE Models in Experimental Research

Several ethical considerations arise when EAE models are used in research. Ethical considerations in animal research emphasize the importance of adhering to legal and moral standards, including the 4Rs principles of reduction, refinement, replacement, and responsibility. Researchers must justify animal use with potential benefits outweighing harm to animals, design studies to minimize animal suffering (pain and distress), and ensure proper handling by trained individuals. Researchers should use the least invasive methods,

minimize the number of animals, and consider alternatives. Alternatives such as in vitro and in silico methods and invertebrate models are encouraged to reduce animal use. Transparency and accountability are crucial, along with strict adherence to guidelines and ethics committee approval. The potential for human benefit should be evident, with research aimed at understanding and treating human diseases such as MS. Responsible and ethical research practices are essential in EAE model studies. An ethical review by multidisciplinary committees ensures that the protocols align with these principles. Overall, while animal research has advanced biomedical science, ongoing efforts focus on enhancing ethical practices, developing alternatives, and fostering responsible animal welfare throughout the research process.[13]

The EAE Model: A Comprehensive Overview

The heterogeneity of MS poses significant challenges for both diagnosis and the development of effective treatment strategies.[6] Over the past five and a half decades, fundamental insights into MS pathophysiology have been established, and therapeutic advancements remain limited. Current therapies predominantly focus on immunosuppressive treatments aimed at reducing relapse rates.[7] However, these approaches fail to address the neurodegenerative pathways that contribute to long-term disability.[7,8] The limited efficacy of existing interventions to treat MS underscores the urgent need for innovative strategies that simultaneously target inflammation and neurodegeneration. Advancing our understanding of the mechanisms underlying disease progression is essential for the translation of preclinical discoveries into effective clinical therapies. The EAE model serves as a critical platform for bridging this gap and offers valuable insights into the complex interplay between immune-mediated inflammation and neurodegeneration.

History and Development of the EAE Model

Since its inception, the EAE has emerged as a pivotal model for studying MS and autoimmune neuroinflammation. The origins of EAE date back to 1933, when researchers successfully induced the condition using spinal cord homogenates, providing the first evidence of CNS self-reactivity.[14] A significant advancement came with Jules Freund's complete adjuvant (CFA) which facilitated disease induction with fewer immunizations, revolutionizing EAE research.[9,15]

Over time, EAE has undergone extensive refinement to replicate the clinical and pathological features of MS. Key discoveries identified specific myelin antigens, such as myelin basic protein (MBP) and proteolipid protein (PLP), as primary targets for immune-mediated damage.[16] The model's inherent heterogeneity, stemming from variations in mouse strains and immunization protocols, has resulted in diverse clinical manifestations and immunopathological profiles. These variations allow researchers to tailor their approaches to address specific aspects of MS pathogenesis.[9] As a result, EAE has become an indispensable tool for elucidating disease mechanisms and evaluating therapeutic interventions. Its ability to capture the complexities of MS has established its role in both fundamental research and translational studies, thereby driving the development of innovative treatment strategies.

EAE Model Variants and Their Characteristics

EAE encompasses a range of variants, each of which is characterized by distinct induction methods, patterns of disease progression, and pathophysiological features. Selecting the appropriate EAE model is crucial, as it significantly affects research outcomes related to MS therapies and the understanding of disease pathogenesis. The chosen model directly shapes interpretations of immune mechanisms involved in demyelination and neurodegeneration.[11,17,18]

The subtle differences between EAE models highlight the importance of aligning model selection with specific research objectives. Studies indicate that advancements in understanding of these distinctions can significantly enhance translational research efforts, ultimately aiding in the development of more effective MS treatments.[18] For instance, while both T helper 1 (TH1) and T helper 17 (TH17) cells can induce EAE with similar clinical features, the nature of CNS-infiltrating lymphocytes and their responses to immunomodulatory therapies differ between these forms.[19]

EAE models exhibit significant variability in their induction methods, immune responses, and the onset and severity of symptoms. Active EAE is induced by sensitizing subjects to specific myelin antigens, such as MOG, PLP, or MBP, typically administered alongside

CFA. In contrast, passive EAE involves the adoptive transfer of myelin-specific immune cells, such as $CD4^+$ T cells, derived from previously immunized animals.[17,20]

The autoimmune response in active EAE tends to be more severe and systemic, whereas passive EAE relies on the reintroduction of T cells that specifically target myelin antigens. The clinical manifestations of these models vary, ranging from gradual onset to acute presentation. Active EAE is often employed to screen potential therapeutic agents because of its reproducible disease progression, whereas passive EAE is a powerful tool for investigating immune responses and elucidating mechanisms of autoimmunity in MS research.[16,21,22] Table 1 depicts EAE variants and their clinical MS counterparts. The most widely utilized EAE models include the following.

MOG-EAE: MOG-induced EAE is one of the most widely used models for studying MS because of its reproducibility and relevance to relapsing forms of the disease. This model was established by immunizing mice with either MOG_{35-55} or full-length MOG_{1-125}. The MOG_{1-125} variant, in particular, exhibits distinct pharmacological responses, making it valuable for exploring both T-cell and B-cell contributions to disease progression.[18]

The MOG-EAE model typically leads to a chronic disease course characterized by significant motor deficits and demyelination.[7] It predominantly involving a TH17-dominated immune response, marked by the infiltration of $CD4^+$ T cells and production of inflammatory cytokines such as interleukin (IL)-17. Clinically, MOG-EAE manifests as acute episodes of paralysis, making it an effective model for examining relapsing forms of MS.[23] Furthermore, it serves as a key platform for evaluating therapies targeting T cell pathways, given its close resemblance to MS clinical symptoms.

***Chronic EAE in C57BL/6 Mice*:** This variant is induced in C57BL/6 mice by immunization with MOG_{35-55} or MOG_{1-125} emulsified in CFA, followed by administration of pertussis toxin. This model is particularly notable for its chronic progression and extensive neuropathological changes, which closely mimic those observed in human MS. Consequently, it provides an excellent platform for studying the long-term effects of therapeutic interventions and exploring the mechanisms underlying neurodegeneration.[9,11] Similar to MS, chronic EAE induces substantial neuroinflammatory responses. Compared to other models, chronic EAE exhibited more pronounced activation of glial cells and more extensive demyelination areas. It was shown that B lymphocytes were critically involved in this process, and have been implicated in mechanisms of neurodegeneration presented by EAE.[11,24]

Furthermore, CSF oligoclonal IgG bands, a hallmark of MS, are also found in many of The EAE models.[20,24] The EAE of mice C57BL/6 is chronic, similar to the progressive forms of MS in human patients. Other EAE models, including SJL/J mice, might develop a relapsing-remitting disease course, whereas C57BL/6 mice predominantly become affected with a chronic–progressive disease course.[9] Chronic EAE shows significant neuroinflammatory responses similar to those seen in MS. Unlike in other models, in chronic EAE, this is accompanied by the activation of glial cells and evidenced by extensive demyelination areas.[25] This is important because researchers can study the long-term effects of neurodegeneration and neuroinflammation that are typical of progressive MS.

PLP-EAE: The PLP-EAE model uses peptide fragments derived from PLP. and is characterized by a predominantly TH1-driven immune response with elevated production of interferon-gamma (IFNγ). Clinical manifestations often include progressive paralysis and more severe CNS damage than in MOG-EAE. The immune response in PLP-EAE is more heterogeneous, involving both T cells and B cells.[11] This model is particularly valuable for investigating humoral immune mechanisms implicated in MS.[16,26] In essence, PLP-EAE mimics chronic or progressive forms of MS. Certain models, especially those inducing sustained or worsening pathology, are viewed as models for secondary progressive MS or even primary progressive MS, although complete replication remains challenging.[16]

Zebrafish EAE Model: This model allows for rapid observation of disease progression and treatment effects, as zebrafish embryos develop quickly, enabling experiments to yield results within a week. This has significantly accelerated the drug discovery process. Moreover, the zebrafish EAE model supports high-throughput screening, allowing researchers to test multiple compounds simultaneously, thereby reducing the time and costs associated with traditional rodent models.[27,28]

Table 1. EAE Variants and their Clinical MS Counterparts

EAE Variant	Induction Method	Typical Clinical Course in EAE	MS Clinical Counterpart	Pathological Features	Advantages	Limitations	Refrences
Active EAE (MOG_{35-55} peptide)	Immunization with myelin oligodendrocyte glycoprotein peptide + adjuvant	Chronic-progressive or relapsing-remitting	RRMS, Chronic MS	CNS inflammation, demyelination, axonal loss, gliosis	Well-characterized, reproducible; mimics T-cell mediated pathology	Predominantly spinal cord lesions; limited cortical involvement	16,24
Adoptive Transfer EAE	Transfer of encephalitogenic T cells into naïve mice	Acute or relapsing-remitting	RRMS	Similar to active EAE; allows study of T-cell subsets	Enables study of specific T-cell populations	Requires donor animals; less variability control	16,24
$PLP_{139-151}$-induced EAE	Immunization with proteolipid protein peptide + adjuvant	Relapsing-remitting	RRMS	Brain and spinal cord lesions; demyelination	Models relapsing disease course	Strain-dependent susceptibility	32,33
Theiler's Murine Encephalomyelitis Virus (TMEV)-induced demyelination	Viral infection	Chronic-progressive	Progressive MS	Chronic CNS inflammation, demyelination, viral persistence	Models' viral triggers and chronic neurodegeneration	Viral model; less immunologically defined	24,34,35
Chronic-relapsing EAE (SJL mice)	Immunization with PLP peptide	Relapsing-remitting with spontaneous relapses	RRMS	Multifocal CNS lesions, demyelination	Models relapsing disease; spontaneous relapse	Limited to specific mouse strains	36
Non-human primate EAE	Immunization with myelin proteins	Variable; often progressive	MS (varied clinical forms)	Closer CNS anatomy and immune system to humans	Better translational relevance	Ethical and cost constraints	36

EAE:Experimental autoimmune encephalomyelitis; RRMS: Relapsing-remitting multiple sclerosis

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The zebrafish genome shares significant homology with that of humans, enhancing its translational potential. Additionally, zebrafish is cost-effective to maintain, enabling the inclusion of larger experimental cohorts without a proportional increase in costs.[28,29] Real-time imaging of physiological processes in zebrafish provides valuable insights into mechanisms of drug action, particularly in the contexts of demyelination and remyelination.[28,30,31] Recent validations of various MS treatments using the zebrafish EAE model further underscore its reliability.[27,31]

The zebrafish EAE model, which is useful for studying MS, has some limitations. This model may not fully replicate the complexity of human MS, as zebrafish lack certain anatomical structures present in humans, such as a distinct prefrontal cortex. Additionally, the zebrafish nervous system, which is similar to humans in many aspects, has some differences that could affect the translation of the results to human patients. For example, zebrafish possess a significantly more complex trace-aminergic system than humans. Furthermore, the developmental stage of zebrafish larvae used in the experiments is an important consideration, as their blood-brain barrier and other systems may still be developing, potentially affecting drug efficacy and toxicity assessments. These limitations highlight the need for careful interpretation of results and complementary studies in other models or human patients to ensure the translational relevance of findings from zebrafish EAE models.[29]

Immunological and Pathological Features of EAE

EAE is characterized by significant infiltration of immune cells into the CNS, leading to demyelination and axonal damage.[37,38] Upon induction, autoreactive $CD4^+$ T cells migrate from peripheral lymphoid organs into the CNS, where they interact with resident antigen-presenting cells that present myelin-derived antigens. This interaction is critical for T cell reactivation and the subsequent inflammatory response.[26] In addition to T cells, other immune cells, such as macrophages and B cells, infiltrate the CNS, further amplifying the inflammatory environment. The pathological features of EAE highlight the complex interplay between various immune components, each playing a role in disease progression. The initial migration of autoreactive T cells serves as a pivotal event, triggering a cascade of immune responses that ultimately leads to the characteristic lesions observed in EAE. Understanding these mechanisms is vital for developing targeted therapeutic strategies for conditions like MS, which shares many pathological and immunological similarities with EAE.[24]

Role of Pro-inflammatory Cytokines

Activated T cells, particularly TH1 cells, are pivotal in the production of pro-inflammatory cytokines, such as IFN-γ, TNF-α, and IL-17. These cytokines play critical roles in the pathogenesis of demyelination by directly damaging oligodendrocytes and exacerbating inflammation. The inflammatory response promotes the production of IL-1α, TNF-α, and C1q in reactive astrocytes and M1 microglial cells. This pro-inflammatory cytokine trio, in turn, activates these glial cells. The resulting damage leads to the destruction of oligodendrocytes. For instance, studies have demonstrated that IFN-γ enhances the expression of MHC molecules on oligodendrocytes, thereby increasing their susceptibility to immune-mediated attacks.[39] Furthermore, TNF-α has been shown to induce apoptosis in oligodendrocytes, disrupt their survival signals, and contribute to myelin loss.[40]

Inflammatory Mediators and Cellular Interactions

T cells rely on their interactions with glial cells to initiate demyelination. During the onset of demyelination, activated microglia and macrophages engage in phagocytosis of myelin debris, a process that can exacerbate inflammation. This activity establishes a self-perpetuating cycle of damage, characterized by the release of reactive oxygen species (ROS) and pro-inflammatory cytokines. Additionally, dysregulation of the signaling pathways within these phagocytes can hinder remyelination. For instance, in aged phagocytes, the accumulation of cholesterol may activate the inflammasome, resulting in chronic inflammation that obstructs recovery processes.[39]

The interplay between T and glial cells is crucial for understanding the mechanisms underlying neuroinflammatory conditions. Studies have shown that as demyelinating diseases progress, there is a significant increase in T-cell infiltration and glial cell activation, both of which contribute to immunological neurodegeneration.[40] The activation of glial cells plays a significant role in mediating the inflammatory response, making them a potential target for therapeutic interventions aimed at mitigating the effects of demyelination.[41]

Oligodendrocyte Dysfunction

The integrity of myelin is critically dependent on oligodendrocytes, which are essential for the maturation and maintenance of myelin sheaths. During demyelination, oligodendrocytes are disrupted by inflammatory signals, with the Notch signaling pathway emerging as a key mechanism regulating the proliferation and differentiation of oligodendrocyte precursor cells (OPCs).[42] Notably, the activation of the Notch pathway in OPCs inhibits their maturation into myelinating oligodendrocytes, thereby impairing remyelination under inflammatory conditions.[41] Similarly, while Wnt/β-catenin signaling promotes the proliferation of adult OPCs, it also suppresses their differentiation in the presence of inflammation.[40] These insights underscores the complex interplay between oligodendrocytes and inflammatory processes, highlighting potential therapeutic targets aimed at enhancing remyelination and restoring myelin integrity.

The EAE Model in MS Drug Development

Preclinical Efficacy of MS Therapies in EAE

The EAE model has been extensively utilized for preclinical evaluation of novel therapies for MS.[42-46] Numerous immunomodulatory agents have demonstrated efficacy in this model, providing a foundation for subsequent clinical trials.[46]

One notable example of an effective therapeutic intervention is the use of monoclonal antibodies that target specific immune pathways. For instance, anti-CD4 monoclonal antibodies have been shown to significantly reduce disease severity in EAE models by inhibiting T-cell activation and preventing their infiltration into the CNS. This finding underscores the value of the EAE model as a powerful tool for assessing the preclinical efficacy of emerging therapies designed to modulate immune responses.[11]

In addition to monoclonal antibodies, the pharmacological inhibition of small molecules has also demonstrated significant effectiveness in the EAE model. A recent study on a Janus kinase (JAK) inhibitor revealed substantial suppression of inflammatory cytokine production and alleviation of clinical symptoms in EAE mice.[45] This finding not only reinforces the validity of the EAE model, but also provides a robust framework for identifying and optimizing therapeutic candidates before their progression to human clinical trials.

Correlation between EAE Results and Clinical Trial Outcomes

A topic of considerable interest is the capacity of the EAE model to predict the clinical efficacy of MS therapies. Although many treatments demonstrate effectiveness in the EAE model and subsequently advance to clinical trials with some success, preclinical results do not always align with clinical outcomes.[9,47]

Despite the challenges associated with the EAE model, certain trends suggest a degree of predictive reliability for the therapeutic outcomes. Specifically, therapies that significantly reduce inflammatory markers and clinical scores in EAE models often correlate with favorable results in early phase clinical trials.[11] For instance, the compound berberine as demonstrated efficacy by inhibiting of TH17 cell differentiation and modulating of TH1 cell functions through pathways such as JAK/STAT and NF-κβ. This indicates the potential of targeting these pathways to mitigate inflammation in autoimmune diseases. Notably, drugs aimed at modulating TH1 and TH17 cells have consistently shown efficacy in both preclinical and clinical settings, reinforcing the translational potential of specific immune-modulatory approaches.[48]

However, unresolved questions persist, particularly concerning the heterogeneity of MS and the ability of EAE models to accurately replicate the complexities of human diseases. The genetic diversity among patients and the influence of environmental factors on outcomes underscore the need for further refinement of preclinical models to enhance their relevance and predictive accuracy.[9]

Limitations and Challenges of Using EAE in Drug Development

Although the EAE model is widely used in drug development, it has notable limitations that can impede its progress.

Phenotypic Variability in EAE Models

One significant challenge is the variability introduced by the different methods of EAE induction, which can result in inconsistent disease phenotypes across studies. This variability may obscure the true therapeutic effects of potential treatments.[11] While EAE effectively mimics certain aspects of MS pathology,[47] it does not entirely replicate all features of the human disease, particularly chronic progression, secondary

neurodegeneration, and the contributions of cytotoxic $CD8^+$ T cells. These limitations highlight gaps in the model's ability to capture the complexities of MS and underscore the need for complementary approaches to enhance drug development efforts.[9]

Species Differences Limiting Translation

Another significant challenge in utilizing the EAE model is translating preclinical results to patients, primarily because of species differences in immune responses between rodents and humans.[34,47,49] Key immune pathways often exhibit substantial divergence between these species, increasing the risk of unexpected adverse effects or lack of efficacy when treatments are applied clinically.[50] For instance, some therapies that have demonstrated success in preclinical EAE trials failed in human studies, either exacerbating disease severity or causing severe adverse events in patients. These outcomes underscore the limitations of the EAE model in fully predicting clinical success.[17] For example, several therapies that have shown promise in EAE have failed during human trials, raising concerns about the translational validity of this model. These discrepancies highlight the complexities of immune responses and inherent challenges in extrapolating findings from animal models to human diseases. Recognizing these limitations is essential for refining preclinical models and improving their predictive accuracy by addressing these gaps, researchers can enhance the translation of promising preclinical findings into effective clinical treatments for MS.[9,47]

Emerging Solutions: Humanized Models and Methodological Refinements

Despite its limitations, EAE remains a cornerstone in MS research and drug development; its limitations necessitate methodological refinement and complementary strategies. Recently, there has been a growing emphasis on employing humanized mouse models that incorporate human immune components, offering a more accurate characterization of disease mechanisms and bridging the gap between preclinical and clinical outcomes.[9,47]

The EAE model and its use for the preclinical evaluation of MS therapies are schematically shown in this figure. EAE induction modalities in laboratory animals are shown in the central panel with various stages of disease progression, including clinical symptoms, inflammatory cell infiltration, and demyelination in the central panel. Toward the bottom of this illustration, numerous therapeutic strategies initially experimented with in the EAE were translated into FDA-approved treatment modalities for MS. These therapies abolished EAE symptoms and characteristics of overexpression of inflammatory cytokines, and similar results were documented in clinical cases of MS, with promising results in clinical trials.

Successful Translation of EAE Findings to Clinical Therapies

The development of effective treatment strategies for MS has benefited significantly from the successful translation of findings from EAE to clinical therapies. Notable examples include natalizumab and glatiramer acetate, which demonstrate how insights from EAE studies can lead to substantial advancements in the treatment of MS. In addition to these therapies, several others have undergone this translational process, ultimately earning the FDA approval for use in patients with MS. These developments, as illustrated in Figure 1, highlight the crucial role of EAE as a preclinical model for shaping therapeutic options for MS.

Glatiramer Acetate (Copaxone): Insight into the actions of glatiramer acetate was gained from EAE models, which demonstrated its ability to modulate immune responses relevant to the pathophysiology of MS. Glatiramer acetate is a synthetic copolymer that mimics MBP and shifts the immune response to a Th2 phenotype, which is associated with reduced inflammation.[51,52] Clinical trials have shown that glatiramer acetate, especially when administered during the early phases of the disease, dramatically reduces the frequency of relapses and MRI-detected lesions in patients with relapsing-remitting MS (RRMS).[51] EAE models have provided critical insights into the mechanisms of action of numerous MS therapies. For example, studies on EAE revealed how glatiramer acetate induces a shift in T cell responses, promoting regulatory T cells (Tregs) while suppressing pro-inflammatory TH1 and TH17 responses, which are key drivers of MS pathology.[33]

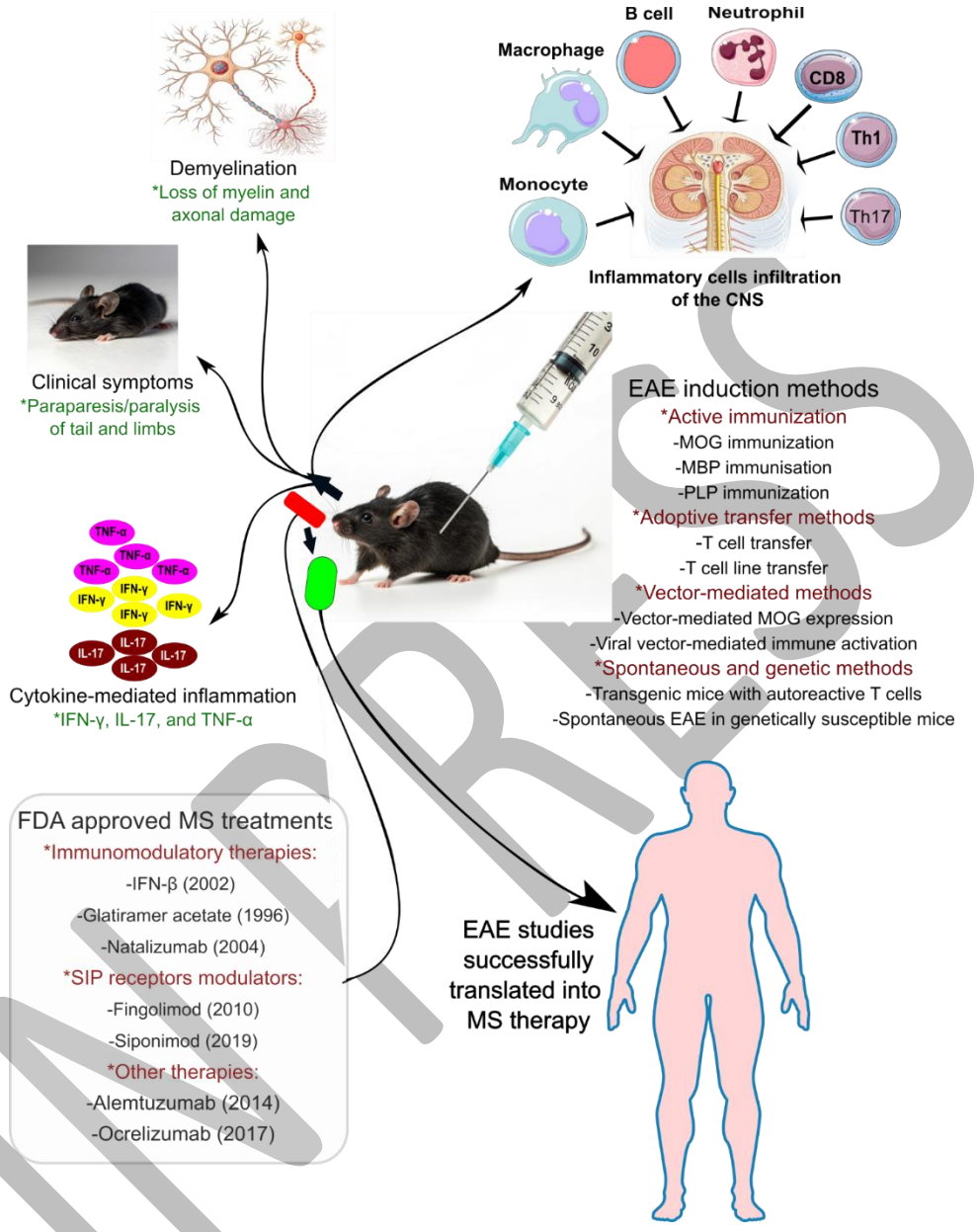

Figure. 1 EAE Model as a Translational Tool for MS Therapy Development.

Natalizumab: Research using EAE models suggests that natalizumab, a recombinant humanized monoclonal antibody targeting the α4 subunit of α4-β1 and α4-β7 integrins, plays a critical role in reducing leukocyte migration into the CNS. Natalizumab functions by blocking the α4 integrin-mediated migration of leukocytes across the blood-brain barrier, which is essential for preventing neuronal damage and mitigating CNS inflammation in MS.[51] This monoclonal antibody prevents lymphocyte infiltration into the CNS, leading to a marked decrease in relapse rates and slowing of disability progression in MS patients. Numerous studies have demonstrated significant reductions in relapse rates and MRI-detected disease activity, confirming the therapeutic efficacy of natalizumab.[33,51]

IFN-β: It is a type I interferon that modulates immune response by controlling inflammation and inhibiting the proliferation of T and B cells. IFN-β is a

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potent immunomodulatory agent with antiproliferative activity, expressed by immune cells, endothelial cells and fibroblasts.[47]

Studies using the EAE model have demonstrated that IFN-β induces the expression of interleukin-10 (IL-10) while downregulating the expression of TNF-α, granulocyte monocyte-colony stimulating factor (GM-CSF), and Fas ligand (FasL: CD95L) in T-helper cells. These effects block the expression of IL-1β in monocytes, collectively ameliorating the autoimmune response in the EAE model.[53]

Additionally, IFN-β downregulates the expression of adhesion molecules in endothelial cells, thereby limiting the influx of immune cells into the CNS. IFN-β has been approved as a first-line treatment for RRMS, where it significantly reduces relapse rates and slows disease progression.[54,55]

Fingolimod: Fingolimod is an oral therapy approved for RRMS. It functions as a sphingosine-1-phosphate (S1P) receptor modulator. Upon phosphorylation to fingolimod-phosphate, it binds to S1P receptors on lymphocytes, preventing their egress from lymphoid tissues into circulation. This process reduces the infiltration of autoreactive T cells into the CNS.[56,57]

Studies using the MOG-induced EAE model have demonstrated that fingolimod decreases EAE symptoms, as well as serum levels of IL-17 and immunoglobulin A.[18] Fingolimod also facilitates myelination by promoting oligodendrocyte lineage proliferation and differentiation through the activation of the sonic hedgehog signaling pathway, with significant upregulation of related signaling molecules.[58]

Additionally, fingolimod inhibits the proliferation and activation of myeloid cells within lymphoid organs and prevents the influx of B and T cells into the CNS, highlighting its immunomodulatory effects in EAE.[18,59] Clinical studies have documented a significant decline in disease score among RRMS patients treated with fingolimod, attributed to the sequestration of lymphocytes within lymphoid organs, which lowers their numbers in circulation and subsequently in the CNS.[60,61]

Siponimod: Similar to fingolimod but with greater specificity, siponimod is an S1P receptor modulator that selectively binds to S1P1 and S1P5 receptors. By blocking the egress of peripheral lymphocytes from lymphoid tissues, siponimod reduced CNS inflammation and modulated astrocyte immune responsiveness. It is approved for the treatment of secondary progressive MS (SPMS) to slow the disability progression in patients with active disease.[62,63]

Siponimod enhances remyelination by inducing growth factors that promote oligodendrocyte survival and differentiation.[62] Studies using the EAE model have indicated that siponimod upregulates IL-10 while downregulating IFN-γ, IL-17, and the RORγt transcription factor. These effects have also been observed in studies on MS.[32, 65]

Additionally, siponimod, like fingolimod, inhibits the NF-κβ and JAK/STAT signaling pathways, which are involved in the production of pro-inflammatory cytokines.[66] This dual pathway inhibition underscores the therapeutic potential of siponimod in modulating immune responses in MS.

Alemtuzumab: Alemtuzumab is a monoclonal antibody directed against CD52, a protein expressed in immune cells. Alemtuzumab effectively depleted CNS-infiltrating autoreactive T and B lymphocytes in EAE models induced by MBP and PLP Moreover, it has been shown to sustain prolongedmild symptoms in established murine EAE.[67]

Alemtuzumab rapidly depletes circulating lymphocytes, leading to immune system reconstitution and potentially influencing the progression of MS. It is approved for use in RRMS in patients with active illness, particularly when earlier therapies have proven unsuccessful.[68,69] Alemtuzumab induces an immunoregulatory environment through potentiation of FoxP3 expression in Tregs, increased memory B and T cells, and the expansion of immunoregulatory CD56 bright natural killer (NK) cells. Additionally, it facilitates immune cell reconstitution after therapy in MS patients.[70]

Ocrelizumab: Ocrelizumab is a humanized monoclonal antibody that targets the CD20 antigen predominantly expressed on B cells. It has been approved for use in RRMS and is the first therapy shown to be effective for primary progressive MS (PPMS). Ocrelizumab induces rapid depletion of circulating B cells, which correlates with a significant reduction in both relapse rates and progression of disability. Clinical trials have demonstrated its ability to reduce relapse frequency, alleviate disease activity as evidenced by MRI findings, and improve patient outcomes.[71] The efficacy and safety profile of ocrelizumab establishes it as a cornerstone therapy in the treatment landscape for MS, offering hope for improved management of this

challenging condition.[68,72] Ocrelizumab has also been shown to reverse the count of Th40 T cells in RRMS patients. Th40 cells are encephalitogenic autoreactive T cells characterized by the upregulated expression of pro-inflammatory cytokines such as IL-17, IL-6, IFN-γ and TNF-α.[73]

In EAE models, ocrelizumab therapy decreased the levels of IL-6, IL-17, and B-cell activating factor (BAFF), ensuring effective B cell depletion,[74] reducing the number of activated T cells, increasing the number of Tregs.[14] Moreover, it inhibiting the NF-κβ[75] and JAK/STAT[18] signaling pathways, effectively modulating inflammation in EAE mice.

These therapies represent substantial developments in MS therapy, with each targeting distinct aspects of the immune response. Although their efficacy has been demonstrated in both experimental (EAE) and clinical studies, further research is necessary to fully understand their long-term impact and potential side effects. Table 2 outlines the EAE-tested therapy that was successfully translated into FDA-approved human use.

Table 2. EAE-informed therapies for MS

Therapy	Mechanism of Action	EAE Model Findings	Clinical Trial Results	Current Clinical Status	References
Glatiramer acetate	Modulates immune response by mimicking myelin proteins	Ameliorated EAE symptoms and reduced inflammation	Approved for relapsing MS; reduces relapse rates	FDA-approved for RRMS	14,76-78
Natalizumab	Monoclonal antibody against α4-integrin	Reduced leukocyte infiltration into the central nervous system	Approved for relapsing MS; decreases disability progression	FDA-approved for RRMS	9,36,10,79
Fluoxetine	Antidepressant with neuroprotective effects	Partially ameliorated paralysis in EAE models	Showed trends towards reduced new enhancing lesions	Investigational/Off-label use	80-83
Riluzole	Glutamate release inhibitor	Reduced severity of inflammation and demyelination in EAE	Some evidence of slowing progression in progressive MS	Investigational/Off-label use	80,84,85
Fingolimod	Sphingosine-1-phosphate receptor (S1PR) modulator; inhibits lymphocyte egress from nodes	Prevented EAE development and reduced severity	Reduced relapse rate and MRI activity in RRMS; effective in pediatric MS	FDA-approved for RRMS and pediatric MS	56,57,61,.86
Siponimod	Selective S1PR1/5 modulator; inhibits lymphocyte egress, reduces meningeal lymphoid tissue	Reduced EAE severity, decreased meningeal ectopic lymphoid tissue formation	Reduced disability progression in active SPMS; favorable long-term safety profile	FDA-approved for active SPMS and RRM	62-64

EAE: experimental autoimmune encephalomyelitis; RRMS: relapsing-remitting multiple sclerosis

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Clinical Trial Design and EAE-Informed Endpoints

The design of clinical trials for MS therapies increasingly leverages insights from EAE studies to increase predictive validity and efficacy assessments. For example, endpoints derived from EAE models, such as clinical scores reflecting motor function and cognitive performance, are now employed in clinical trials.[35,45]

Endpoint Selection: Behavioral assessments in EAE models have informed the development of clinical trial endpoints such as the Expanded Disability Status Scale (EDSS) and relapse rates. These endpoints provide a comprehensive evaluation of treatment efficacy, extending beyond lesion reduction typically observed on MRI scans.[87]

Trial Design Innovations: EAE studies have also contributed to improving trial design by integrating biomarkers identified in preclinical research. For example, surrogate markers for treatment response, such as cytokine levels or immune cell population profiles, allow for more tailored patient selection and monitoring.[33,87]

The implementation of these strategies allows researchers to evaluate the therapeutic potential of new agents more effectively while refining existing treatments before progressing to full-scale clinical trials, all supported by robust preclinical data. Moreover, the transition from EAE models to clinical therapies underscores the critical role of animal models in understanding the basis of therapeutic interventions for MS. Case studies highlight the substantial advancements achieved through this translational research approach, with ongoing discoveries in the field continuing to refine the mechanisms of action and innovate clinical trial designs.

Future Directions and Emerging Trends

Advances in EAE Modeling: Humanized Mice and Beyond

Recent advances in EAE modeling, particularly the development of humanized mouse models, have greatly improved our knowledge of the immunopathogenesis of MS.[11,88,89] The B2m-NOG model, for instance, allows for the engraftment of human peripheral blood mononuclear cells (PBMCs), enabling researchers to investigate human immune responses in a controlled experimental setting. This model has replicated certain MS-like features, such as T cell infiltration and CNS inflammatory lesions, especially when PBMCs from MS patients with Epstein Barr virus reactivation are used.[90]

Humanized mouse models offer great potential for a variety of applications.[91] They can be employed to investigate patient-specific immune responses, evaluate the efficacy of novel therapeutics, and elucidate the roles of particular immune pathways in disease progression. However, ongoing refinements are necessary to address key limitations, including low monocyte engraftment and the absence of demyelination which are essential for accurately reflecting MS pathology.[92]

Several challenges in using PBMC-engrafted humanized mice for evaluating immune-related therapies have been encountered, including limited cellular diversity and variability among individual mice owing to the small number of transplanted PBMCs, which affects reproducibility. Additionally, these models lack the follicular dendritic cells necessary for affinity maturation, resulting in a less effective production of high-affinity antibodies. The potential development of GVHD, despite suppression measures, can compromise the longevity of immune responses, whereas the predominant memory phenotype of engrafted lymphocytes limits the assessment of responses involving naïve immune cells. Furthermore, fundamental differences between human and mouse immune systems pose inherent barriers to accurately mimicking human immunity, and these issues can lead to an overestimation of drug efficacy, complicating the translation of findings to clinical settings.[90] Another study reported that the model fails to fully recapitulate the complex interplay of immune cells and CNS tissue damage seen in MS. The absence of monocyte engraftment, demyelination, organized lymphoid structures, and antibody responses, coupled with the lack of clinical symptoms, limits the model's ability to comprehensively study MS pathogenesis and potential therapies.[92]

Integration of EAE with other Preclinical Models

The integration of EAE with other preclinical models represents a promising opportunity to improve translational research on MS. For example, combining EAE with induced pluripotent stem cell (iPSC)-derived neurons allows researchers to study neuronal responses to immunological challenges in human-relevant contexts. This approach provides critical insights into the interactions between immune and neuronal cells, shedding light on the mechanisms that lead to neurodegeneration in MS.[93]

Additionally, researchers are exploring the use of bioengineered particles to enhance the expansion of

myelin-specific Tregs in conjunction with EAE models.[94] This combinatorial strategy has demonstrated promising outcomes in EAE mice, including potential disease reversal and recovery. These findings highlight the possibility of developing effective therapeutic interventions for MS by integrating diverse preclinical approaches.

By utilizing preclinical models, scientists can deepen their understanding of the fundamental disease mechanisms and facilitate targeted therapies that address both the immunological and neurological aspects of MS. The innovative application of tolerogenic microparticles designed to promote Treg activity represents significant Progress in developing treatments against demyelination and associated disorders.[94]

EAE-informed Biomarkers and Precision Medicine Approaches

Studies utilizing EAE have been vital for identifying potential biomarkers to guide precision medicine strategies for MS. For instance, EAE research has highlighted cytokines and immune cell populations that correlate with disease activity and treatment responses. These biomarkers can be used to categorize patients according to their immunological profiles, enabling individualized treatment approaches designed for patient-specific disease mechanisms.[95]

Ongoing research is also focused on developing assays to monitor these biomarkers, allowing real-time tracking of disease progression and the efficacy of therapy. Precision medicine approaches hold great promise for improving patient outcomes by targeting therapies to specific immunological needs, rather than relying on a one-size-fits-all approach. Notably, these approaches have demonstrated the potential to differentiate drug responses in EAE models.[95,96]

The integration of EAE with other preclinical studies on MS presents exciting opportunities to advance our understanding of the disease. Advances in humanized models and innovative biomarker identification strategies are likely to result in more effective therapies and improved patient care.[65,94]

EAE and Biomarker Discovery

EAE serves as a pivotal preclinical model for identifying biomarkers relevant to MS. Recent proteomic and transcriptomic studies have revealed candidate biomarkers detectable in urine, blood, and CNS tissues that correlate with disease onset and progression in EAE models, including neurofilament light chain, glial fibrillary acidic protein, and cytokines such as IL-10 and IL-17.[97] These biomarkers not only reflect neurodegeneration and inflammation, but also offer translational potential for monitoring therapeutic efficacy. However, variability across EAE models and animal strains underscores the need for standardized approaches and validation in human cohorts. Integrating multi-omics data from EAE with clinical findings promises to enhance biomarker discovery, facilitating early diagnosis and personalized treatment strategies in MS.[98]

CONCLUSION

The EAE model remains a pivotal tool for investigating demyelination and assessing therapeutic interventions for MS. It accurately replicates critical aspects of disease pathogenesis, including inflammation, demyelination, and axonal degeneration, and has been instrumental in elucidating immune signaling pathways and cellular interactions. Moreover, EAE has been employed to evaluate disease-modifying therapies such as glatiramer acetate and natalizumab, both of which have shown clinical efficacy in reducing relapse rates and delaying the progression of disability. Despite its widespread application in MS drug development, the EAE model is not without limitations. Variability in induction methods can result in inconsistent phenotypes, and the role of cytotoxic $CD8^+$ T cells is often underestimated. Concerns regarding translational relevance arise from the use of rodent models due to interspecies differences in immune responses. Nonetheless, EAE remains a highly effective model for investigating neuroinflammation and immune-mediated injuries. With advancements in research, this model can be further refined through innovations such as the use of humanized mice and induced pluripotent stem cell-derived neurons, facilitating the analysis of human-specific immune responses and neuronal interactions. Additionally, the integration of omics technologies and organ-on-chip models will inspire further research. These developments hold the potential to enhance therapeutic approaches and drive the identification of biomarkers critical for precision medicine.

STATEMENT OF ETHICS

This study was approved by the International Campus Ethics Committee of the Tehran University of Medical Sciences (IR.TUMS.SPH.REC.1400.221).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Not applicable.

DATA AVAILABILITY

All available data were included.

AI ASSISTANCE DISCLOSURE

During the preparation of this work, the authors used [Perplexity and Paperpal] to [to improve language, style and readability]. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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