# How to Successfully Apply Animal Studies in Experimental Allergic Encephalomyelitis to Research on Multiple Sclerosis

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In their Point of View entitled "Experimental Allergic Encephalomyelitis: A Misleading Model of Multiple Sclerosis," Sriram and Steiner[1] wrote, "The most disappointing aspect of EAE [experimental allergic encephalomyelitis] as a potential model for MS is its almost total inability to point toward a meaningful therapy or therapeutic approach for MS." Actually, EAE has led directly to the development of three therapies approved for use in multiple sclerosis (MS): glatiramer acetate, mitoxantrone, and natalizumab. Several new approaches to MS are in clinical trials based on positive indications in preclinical work relying on EAE. New clues to the pathogenesis of MS and new potential surrogate markers for MS are shown from research involving EAE when it is critically coupled with actual findings in MS. There are pitfalls in overreliance on the EAE model, or on any animal model for any human disease. Nevertheless, over the past 73 years, the EAE model has proved itself remarkably useful for aiding research on MS.

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Any discussion of the pros and cons of the animal models of multiple sclerosis (MS), collectively known as experimental allergic encephalomyelitis (EAE), must address our present state of knowledge about MS. MS is a complicated disease, the cause and pathogenesis of which are incompletely understood. Though we have made progress in therapy of MS, treatment is imperfect. Current therapies reduce the frequency of relapse, somewhere between 33 and 66%, and delay disease progression to a modest extent in relapsing-remitting and secondary progressive MS.[2] There is no single test we can run to determine whether someone has "MS," and there is no surrogate marker for us to measure to assess whether MS is worsening. Whether MS is actually a single disease or whether it is primarily or initially an "immune disease," "an infectious disease," "an inflammatory disease," or a "degenerative disease," or a combination of all of these types are all questions with answers that are currently unknown. A few genetic factors have been associated with MS, most prominently genes of the major histocompatibility complex (MHC).[3] A genetic basis for MS is clearly only part of the story because concordance in identical twins is less than even 50%. Many environmental factors have been associated with MS, although none can be considered definitively linked. Therefore, set in this context in which nearly all of the major questions about human MS remain unanswered, this critique addresses how our understanding of MS has been aided by studies on a collection of animal models known as EAE, first described almost three quarters of a century ago. Given all these uncertainties about MS, it is remarkable that studies on EAE have culminated thus far in three MS therapies and have led to a better understanding of the biology of MS. Clever applications of the EAE model will be a valuable tool for understanding the pathology of MS, for making better biomarkers for its diagnosis and prognosis, and for creating ever improved and safe therapies for this disease. To study a disease such as MS, without support from available animal models, is to unnecessarily create obstacles in a task that is complicated enough.

## A Brief History of Experimental Autoimmune Encephalomyelitis

In the 1930s, workers at Rockefeller University discovered an animal model, now known as EAE.[4] The first experiments were aimed at understanding episodes of paralysis that sometimes accompany vaccination. Three years ago, on the 70th anniversary of the first publication on EAE, we wrote in the *Journal of Experimental Medicine* of Rockefeller University:

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One of the most enduring models of human disease now celebrates the seventieth anniversary of its publication in *The Journal of Experimental Medicine.* Thomas Rivers, working at the Hospital of the Rockefeller Institute for Medical Research, along with his colleagues D.H. Sprunt and G.P. Berry, submitted the article entitled, "Observations on Attempts to Produce Disseminated Encephalomyelitis in Monkeys," on Feb. 21, 1933 (4). Rivers established this model to try to understand what caused neurological reactions to certain viral infections like smallpox and in some circumstances to vaccinations like rabies: the very first sentence of this landmark paper reads, "During convalescence from certain diseases notably smallpox, vaccinia and measles, and during or following vaccination against rabies, an occasional patient develops symptoms and signs referable to the central nervous system."[5]

Thus, the EAE model was initially constructed to understand acute disseminated encephalomyelitis, not MS. Acute paralysis was observed in the first reported models with inflammatory changes in the central nervous system. Later versions of more chronic EAE have been developed with pathology including demyelination and axonal damage and clinical events such as relapsing and remitting episodes of paralysis,[6] all of which are features common to MS. We must remember then that EAE is a collection of various models reflecting features of acute disseminated encephalomyelitis, as well as MS. The relation between acute disseminated encephalomyelitis and MS remains an enigma itself.

Many refinements and variations have been developed in the past 75 years. Even the name EAE has evolved from experimental allergic encephalomyelitis to experimental autoimmune encephalomyelitis. Researchers have developed numerous variations of EAE including models for optic neuritis,[7] relapsing-remitting MS,[8–10] and progressive MS.[9] Some of these models reflect certain aspects of the pathology seen in MS including axonal degeneration together with demyelination.[10] Researchers have constructed EAE models with essential genes, such as human leukocyte antigen (HLA) DR2 associated with susceptibility to MS, installed into mice as transgenes.[11,12] Others have devised forms of EAE in nonhuman primates such as the marmoset that reflect essential aspects of the pathology of MS with high fidelity.[13] Numerous mouse versions of EAE exist where important components of the immune system have been "knocked out" by homologous recombination.[14] Thus, there is no single model of EAE that we refer to in this critique, rather, it is the ensemble of EAE models, which have been reported in more than 5,000 publications since 1933.

### *Experimental Autoimmune Encephalomyelitis for the Development of Approved Therapies of Multiple Sclerosis: Three Case Studies*

Sriram and Steiner[1] wrote, "The most disappointing aspect of EAE as a potential model for MS is its almost total inability to point toward a meaningful therapy or therapeutic approach for MS." We take a position nearly diametrically opposite to that perspective: Indeed, six medications have received approval from the US Food and Drug Administration for treatment of MS, and three of them, glatiramer acetate, mitoxantrone and natalizumab, were developed after showing promise in EAE. Moreover, glatiramer acetate and natalizumab were invented after a set of logical and forward-looking experiments in the EAE model, which elucidated key targets in the pathogenesis of MS. Here, we review how experiments in EAE led to the development of three approved drugs for MS and how the model has been useful in helping us to understand the disease. Approved therapies that have been developed with the EAE model and new targets of interest developed using the EAE model are shown in the Figure.

Michael Sela and his colleagues Ruth Arnon and Dvora Teitelbaum[15–17] first conceived glatiramer acetate in the early 1970s. They made a series of random copolymers based on the molar ratios of four amino acids, glutamate, alanine, tyrosine, and lysine, that are present in myelin basic protein. Sela and McDevitt[18] had shown 5 years earlier that the antibody response to ordered copolymers of tyrosine and glutamate on a backbone of alanine and lysine was under strict genetic control linked to the MHC. McDevitt and Sela's work opened the field of the genetic control of the immune response. Their discovery that such control was linked to the MHC had widespread implications for immunology. More than just a coincidence, genes within the MHC are the most critical for imparting genetic susceptibility to MS. Moreover, the MHC HLA class I and class II gene products, HLA-A, -B, -DR, and -DQ, are the likely targets for glatiramer. Interactions of glatiramer with the MHC turned out to be critical in understanding its mechanism of action (see Fig).

In 1971, Sela and colleagues[15] showed that the random copolymer composed of glutamate, tyrosine, alanine, and lysine, termed *Copolymer 1,* was able to suppress the induction of acute EAE. They then showed that Copolymer 1 blocked relapsing EAE in the guinea pig and EAE in the nonhuman primate.[19–21] Initial clinical testing of glatiramer was undertaken in Jerusalem under the direction of Abramsky[22] in patients with MS and acute disseminated encephalomyelitis. Clinical testing of glatiramer by Bornstein and colleagues[23] showed that glatiramer was effective in reducing relapses in relapsing-remitting MS. A pivotal trial leading to FDA approval, under the leadership of Johnson,[24] showed that relapses were reduced by 29% in patients

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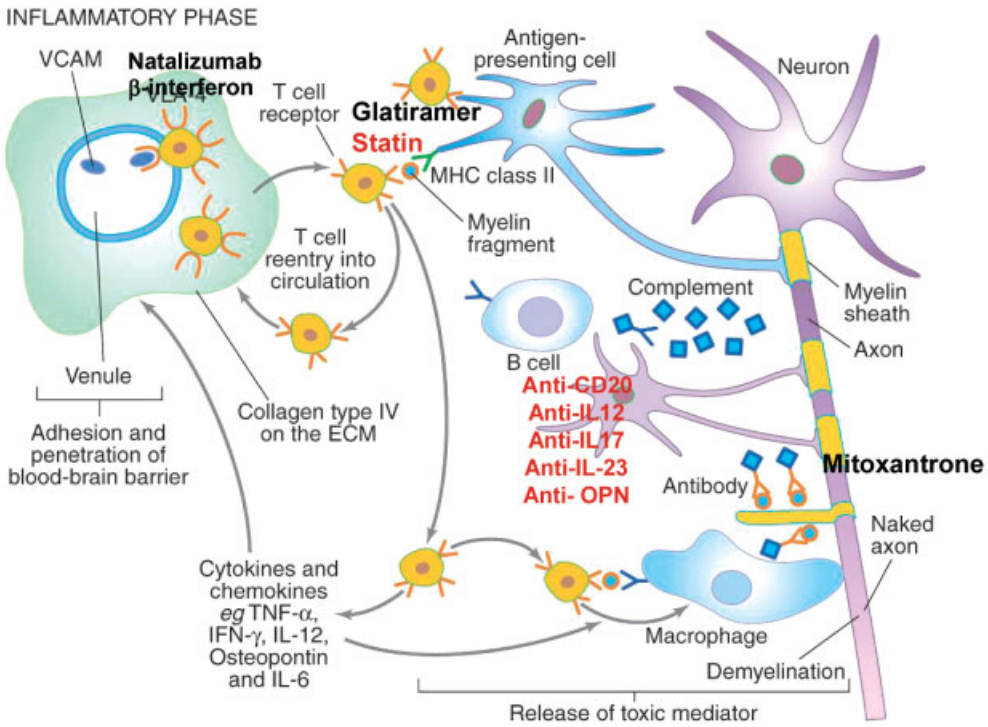


*Fig. General scheme for pathogenesis of MS. T and B cell homing to the central nervous system is followed by inflammation mediated by antibodies, complement and the toxic effect of cytokines. Medications approved for multiple sclerosis (MS) that arose from studies on experimental allergic encephalomyelitis (EAE) are shown in black. Promising therapies for MS elucidated from a creative interplay of work in MS and in EAE are shown in red. ECM = extracellular matrix; IFN = interferon; IL = interleukin; MHC = major histocompatibility complex; TNF = tumor necrosis factor; VCAM = vascular cell adhesion molecule.*

receiving daily injections. Glatiramer acetate, trademarked Copaxone from the original name Copolymer 1, was approved in 1996 for treatment of relapsing-remitting MS. Glatiramer acetate was thus first derived from a preclinical conception and invention in the acute EAE model, and was then taken through proof of concept in various acute and relapsing models of EAE. Success in the EAE model was followed by demonstration of clinical efficacy in relapsing-remitting MS. Glatiramer acetate currently is one of the most popular medications for treatment of relapsing-remitting MS, and more than 100,000 individuals with MS worldwide have received glatiramer acetate treatment.[25] It took a quarter of a century for the development of glatiramer from the publications of the first results in EAE to its approval for relapsing-remitting MS!

There are multiple mechanisms of action associated with glatiramer, and many of these mechanisms were first unveiled in the EAE model. Antigen-specific modulation of the immune response to myelin basic protein has been described.[21,26] Modulation of the immune response with glatiramer leads to deviation of cytokine production in response to myelin basic protein from so-called Th1 cytokines such as γ interferon to Th2 cytokine production.[26] Another mechanism of action centers on the random chemical structures inherent in this random copolymer that allow it to bind to molecular targets with a wide combinatorial array of peptides based on four amino acids. Glatiramer binds to MHC molecules derived from most genetic backgrounds.[27–29] With its capacity to bind to a broad array of MHC molecules, glatiramer could compete with many proteins for these critical molecules responsible for presentation of antigen to T lymphocytes.

Given its widespread binding to diverse HLA molecules, it is not surprising that glatiramer may have non-specific effects on the immune system. Glatiramer has been shown not only to block EAE, but is active in preventing models of inflammatory bowel disease and even amyotrophic lateral sclerosis.[30–32] This implies that the effect on MS may not even be specific for this disease, but that it may have a more general effect on immune modulation. Even though there are multiple potential mechanisms of action for glatiramer, and even though this drug may have potential uses in other diseases, the undisputed history of the development of glatiramer shows that it emanated from experiments on a treatment for EAE. One might reasonably conclude

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that without EAE, there would not have been a glatiramer for treatment of MS.

Mitoxantrone was developed for treatment of MS, after promising results published in the mid-1980s in reversing paralysis in the EAE model. The first article on mitoxantrone in EAE described mitoxantrone as a novel "anthracenedione that has shown antineoplastic activity against a variety of experimental tumors."[33] At the time, there was great interest in this class of drugs, because numerous cytotoxic agents, including azathioprine, had shown promise in MS.[34] Thus, mitoxantrone was tried in the EAE model in the rat. Ongoing paralysis was reversed, and the number and extent of perivascular lesions in brain was reduced after treatment with mitoxantrone. Given its promise in the EAE model, clinical development of mitoxantrone was taken forward in the clinic, leading to its approval for use in MS. FDA approval in 2000 for its use in secondary progressive MS and progressive or worsening relapsing-remitting MS was granted for reducing frequency of relapses and slowing clinical progression of disease. Success in the EAE model clearly spurred translation of this approach to the clinic in MS.[35,36]

Natalizumab is yet another example of a drug that was developed directly from work in the EAE model. In the early 1990s, immunologists had developed the working hypothesis that there were specific "molecular addresses" for lymphocyte homing to various organs. Some referred to this as the "Zip Code Hypothesis" for lymphocyte homing. In collaboration with Yednock and colleagues[37] at a small biotechnology company, Athena Neurosciences, the Steinman laboratory at Stanford determined the precise molecule involved in lymphocyte adhesion to inflamed brains taken from rats with EAE.[38] Using an assay where frozen sections were cut on brains with EAE, the researchers bound human and rodent lymphocytes to the inflamed EAE sections. Monoclonal antibodies were then applied to these sections to see whether this lymphocyte binding could be blocked, and by inference, what molecules were involved in the association of lymphocytes to inflamed brain tissue. More than 20 monoclonal antibodies to most of the adhesion molecules known at that time were tried on these sections of EAE brain, and only monoclonal antibodies binding α4 or β1 integrin molecules inhibited adhesion of lymphocytes to the inflamed blood vessels in the brain.[37]

We then proceeded to test whether a monoclonal antibody to α4β1 integrin could block paralysis induced by T-cell clones that recognized myelin basic protein. These clones caused clinical paralysis and brain inflammation in a classic acute EAE model. The antibody to α4β1 integrin inhibited the development of paralysis when given at a dose of 4 to 6.4mg/kg in the Lewis rat. An article published in 1992 in *Nature* stated:

> Previous work on alpha-4 beta-1-dependent cell adhesion has mainly involved studies with endothelium that has been grown and stimulated in culture. The in vitro section assay described here extends those observations by showing that alpha-4 beta-1 integrin is crucial for the adhesion of leukocytes to vessels that have been activated in vivo. Furthermore, in vivo administration of anti-alpha 4 integrin prevented paralysis associated with the pathogenic inflammation of EAE. Therapy based on inhibiting alpha-4 beta-1 integrin, or the ligand for this receptor on brain endothelium may prove effective in treating inflammatory disease in CNS.[37]

From these experiments in EAE, it was recognized that blockade of α4β1 integrin might be useful for MS. In 1995, pathologists demonstrated the vascular cell adhesion molecule-1, the binding partner for α4β1 integrin, was expressed in MS lesions. Over the next 10 years, clinical development of a humanized monoclonal antibody to α4β1 integrin showed that it had remarkable efficacy in blocking relapses of MS and even delaying disease progression.[39,40] Phase 3 studies showed that over a 2-year period, injection of 300mg of monoclonal α4β1 integrin reduced the relapse rate by two thirds. The dose was directly in the range shown to be effective in the experiments in EAE reported in 1992.[37] Indeed, the development of natalizumab was a tangible result of research in the EAE model.[38]

Natalizumab has had a bipolar existence: It was approved in November 2004 for use after 1 year of data were available in the 2-year phase 3 clinical trial. Within 3 months, three cases of Progressive Multifocal Leukoencephalopathy (PML) were observed, with 2 deaths. The drug was voluntarily withdrawn. Unfortunately, PML does not occur in the animal species used in the EAE model, and this usually fatal complication was neither observed nor could it have been even tested in any EAE model or any available animal model for that matter. Investigators searched to see whether blockade of α4β1 was associated with increased risk for infection to microbes such as cytomegalovirus and Borna virus. No increased risk for opportunistic infections with either of these viruses was observed. The EAE model has limitations, and it is not particularly useful for examining the issue of opportunistic infections, especially when the microbe in question has a species barrier.

It should also be mentioned that β interferons, the other major category of drugs approved for treatment of MS, have shown success when tested in EAE models.[41] However, the β interferons were not developed initially because they showed promise in EAE, but rather because of the interest in development of antiviral therapies for MS. Therefore, we do not count the development of β interferons for therapy of MS as a triumph for applications of research on EAE. We do, however, consider the development of glatiramer, mi-

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toxantrone, and natalizumab a direct consequence of research in the EAE model. So far, research on EAE has given three gifts of new therapies for treatment of MS.

## Problems and Promise of Using Experimental Autoimmune Encephalomyelitis for Development of Therapies for Multiple Sclerosis

There is a long list of drugs that have shown promise in EAE models that are now being taken forward into the clinic. Other approaches including an orally available sphingosine inhibitor,[42] statins,[43–46] an orally available carboxamide,[47,48] and a monoclonal antibody to IL-2 receptor[49–51] have shown great promise in phase 2 trials based first on success in the EAE model (see Fig).

Of course, there are numerous examples of drugs that are effective in EAE, only to fail when tested in MS. One conclusion from these negative studies is that EAE is a poor predictor of success in MS. One must, however, examine the process of drug development to realize that preclinical research in EAE is merely just "exploratory," whereas human clinical trails in MS undergo a rigorous "developmental" process, involving three phases of testing. Thus, once success is seen in EAE, one has to contend with issues such as formulation, dosing, and unforeseen toxicities when a drug is taken forward into clinical testing on humans. Given the high costs of clinical development of therapies in MS, one must make astute choices, usually on the first try, when translating preclinical results in EAE to clinical trials of MS. Each attempt at refining therapy in human clinical trials of MS is often prohibitively expensive, so second chances are undertaken only in rare circumstances. In the case of natalizumab, the dose of monoclonal antibody in which a successful outcome was achieved in EAE was precisely translated to success in human clinical trials. However, for other drugs, problems with selecting a correct dose and dose frequency have confounded development. The case of altered peptide ligands, known as APL, which have shown great promise in EAE, exemplifies this problem.

An APL from the region of myelin basic protein p83-99 showed promise in reducing relapse rates and reversing paralysis in preclinical studies of EAE.[52–55] A version of this APL, known as NBI 5788, was designed and taken into clinical testing in patients with relapsing-remitting MS. This APL had an alteration in the main contact residues with human T-cell receptors recognizing this epitope of myelin basic protein. When NBI 5788 was given at a dosage of 50mg/week subcutaneously, it was associated with exacerbations in three MS patients in an open-label trial.[56] However, dosages of 5mg/week of this drug were associated with reduction in gadolinium-enhancing lesions on magnetic resonance imaging, and there was no evidence of disease exacerbations.[57] However, weekly dosing, at 5, 20, and 50mg, led to allergic-type hypersensitivity reactions. The basis for these hypersensitivity reactions, seen in MS patients in phase 2 clinical trials, was then investigated in the EAE model. In this new model of EAE, it was discovered that self-peptides of the myelin sheath could trigger fatal anaphylactic reactions in mice. The implications of this finding (ie, that even self-peptides were allergic) raised new and challenging questions. "A new version of horror autotoxicus,"[58] first described a century ago by Paul Ehrlich, was discovered from understanding a problem in a clinical trial of an MS drug. When dosing of NBI 5788 in phase 2b trials on relapsing-remitting MS was reduced to 5mg once a month instead of once a week to attempt to mitigate these hypersensitivity reactions, both the desirable activity in reducing magnetic resonance activity and the undesirable hypersensitivity reactions seen with APL disappeared.

Continued development of APL in humans would require further financial investment if this particular approach is to be pursued with different dosing schedules. Negative studies in humans, on novel drugs such as the APL, which so far have failed to translate into an approved drug for MS, may not be a fault of the EAE model per se, but rather a reality of the huge expenses required for development of new drugs. When going from animal to human studies, revisions in dosing and formulation may be required that are too expensive and time consuming to pursue, given competing priorities. It is worth noting that the EAE model was used to help understand one of the clinical complications of this approach, allergic-like hypersensitivity reactions to self-constituents, seen with administration of the APL.[58]

Another area where the EAE model has been called into question has been its inefficiency in predicting how blockade of various cytokines would work in MS. In rheumatoid arthritis and Crohn's disease, we have seen the major triumph in therapy with the class of drugs known as tumor necrosis factor (TNF) blockers. This stunning advance in therapy led to the award of a Lasker Prize in Clinical Medicine to Profs Marc Feldmann and Taini Maini to honor their achievement for implementing this mode of therapy in rheumatoid arthritis and Crohn's disease. More than one million patients with rheumatoid arthritis and Crohn's disease have benefited from this approach.[59] However, TNF blockade has been associated with worsening of MS,[59–61] and a "black box" label has been placed on these drugs, warning against their use in MS.[59] Studies in EAE have been equivocal, where some published experiments have shown the virtues of TNF blockade with anti-TNF monoclonal antibodies and soluble TNF-receptor constructs[62–64]; in contrast, other pub-

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