Experimental Allergic Encephalomyelitis: A Misleading Model of Multiple Sclerosis

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Despite many years of intensive research, multiple sclerosis (MS) defies understanding and treatment remains suboptimal. The prevailing hypothesis is that MS is immune mediated and that experimental allergic encephalomyelitis (EAE) is a suitable model to elucidate pathogenesis and devise therapy. This review examines critically the validity that EAE is an adequate and useful animal model of MS and finds credible evidence lacking. EAE represents more a model of acute central nervous system inflammation than the counterpart of MS. We propose to reconsider the utilization of EAE, especially when this model is used to define therapy. This will also force us to examine MS without the restraints imposed by EAE, as to what it is, rather than what it looks like.

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Although the cause and pathogenesis of multiple sclerosis (MS) are unknown, current prevailing hypothesis favors MS to represent an autoimmune disorder directed against nervous system antigens. [1–3] The basic concept proposes that exposure to environmental pathogens activates autoreactive T cells that recognize central nervous system (CNS) autoantigens, leading to inflammation and demyelination.[4–7] This belief is promoted by some similarities between MS and the various animal models of experimental allergic encephalitis (EAE).[8]

Since the initial experiments by Rivers, the stage was set for the use of experimental animal models to study CNS inflammation and demyelination.[9] Over the last 30 years, the number of EAE-cited publications in English has quadrupled; a Medline search identifies a total of 678 articles on EAE between the years 1970 and 1980, 1,860 articles between 1990 and 2000, and approximately 1,600 publications since 2001.

Besides the utilization of EAE to study MS, it has also been harnessed for developing therapeutic strategies for MS.[10–12] Indeed, the majority of the current therapies being planned for phase II and III trials in MS were first examined in EAE. Thus, EAE has become a central player in the arena of MS. Is it indeed a suitable and relevant research tool for MS? It has improved our understanding of acute inflammatory demyelinating syndromes, advanced our knowledge of the genetic susceptibility to autoimmunity, and helped uncover mechanisms of lymphocyte trafficking and the role of blood–brain barrier in CNS inflammation. We propose, however, that although EAE is a useful model of acute human CNS demyelination such as acute disseminated encephalomyelitis (ADEM), its contribution to the understanding of MS has been limited. We focus here on the lack of resemblance of the EAE model with MS and examine its shortcomings when attempting to extrapolate the findings from the model to the human disease.

Experimental Allergic Encephalomyelitis, The Prototypic Autoimmune Model

Myelin basic protein (MBP), proteolipoprotein (PLP), myelin oligodendrocyte glycoprotein (MOG), myelin-associated glycoprotein (MAG), and S-100 protein are the major known CNS antigens that elicit an immune response and cause paralytic disease in mice.[13,14] MOG-induced EAE differs from MBP/PLP-induced EAE in two major respects. Unlike MBP and PLP-induced EAE, demyelination in EAE induced by MOG is aggravated with concomitant administration of anti–MOG antibodies, suggesting a prominent role for a humoral response in the development of the inflammatory pathology.[15] Also, in some strains of mice, the immune response to MOG is restricted by $CD8^+$ rather than $CD4^+$ T cells.[16,17] Two additional myelin antigens, MAG and CNP-ase are considered to be potential autoantigens in MS because they can induce en-

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cephalitis in animals. Studies of the presence of immune response to MAG in MS patients are limited.[18] Although the immune response to CNP-ase in MS patients is as yet undetermined, cross-reactivity in response to heat shock proteins and CNP-ase has suggested a mechanism for induction of an autoimmune response.[19] S-100 is an astrocytic protein and immunization of rodents produces an encephalitic picture with only minimal demyelination.[20]

EAE is characteristically an acute monophasic illness (as compared with the chronic relapsing course of MS) making it more pertinent to ADEM. However, even the development of chronic relapsing models of EAE (CR-EAE) in rodents has not improved the relevance of EAE in view of the continued differences between the CR-EAE and MS.[21–23]

The Nature of the Inflammatory Response in Experimental Allergic Encephalomyelitis and Multiple Sclerosis

The ability of $CD4^+$ MBP-reactive T cells to induce paralytic signs in mice established the immunological basis of EAE. Immunohistochemical studies examining the phenotype of the inflammatory cells have shown the presence of T cells in both EAE and MS lesions.[24–27] However, $CD4^+$ T cells dominate the perivascular regions of the inflammatory focus in EAE induced by MBP and PLP. On the other hand, the pathology of the demyelinating lesions in MS span a spectrum between those that show prominent inflammation and demyelination to others that represent an oligodendrogliopathy with minimal inflammation of demyelinated regions.[28] In inflammatory MS lesions, the predominant cells are macrophages and $CD8^+$ T cells. $CD4^+$ T cells while present are infrequent.[29–31] Isolation of T cells from MS brains by micromanipulation, followed by targeted amplification of T-cell receptor (TCR) genes showed a restricted expansion of CD8 clones. Although CD4 clones were also isolated from MS brains, they did not show a restricted TCR expression pattern, suggesting they were not representative of a clonally expanded population.[32,33] In another study, overrepresentation of $CD8^+$ T cells was seen in spinal fluid of MS patients. These T cells, some of the memory phenotype, were stable over several months. In certain patients, the expansion of CD8 T cells involved a restricted TCR V gene expression pattern indicative of a clonal expansion.[34,35] In the brain parenchyma of MS patients, these $CD8^+$ T cells are present in close apposition to the myelin membranes, signifying that they may indeed play a role in tissue damage.[36] To further confound the issue, CD8-restricted T-cell reactivity to MOG is sufficient to induce EAE in mice, whereas there is little evidence of $CD8^+$ T cells reactive to MOG peptides in MS.[37,38] These findings question the relevance of the $CD4^+$ autoreactive T-cell repertoire in the periphery of EAE lesions to MS pathogenesis[39] (Table 1).

Is Multiple Sclerosis a Th1-Mediated Disease as Is Experimental Allergic Encephalomyelitis?

The separation of T-cell clones into two mutually exclusive cytokine secretion patterns, Th1 and Th2, evolved into dividing presumable inflammatory diseases as being either Th1 (characterized by secretion of interleukin [IL]–2, and γ-interferon) or Th2 (characterized by secretion of IL-4, IL-5, and IL-13) mediated.[40] Because Th1 cells are sufficient for adoptive transfer of EAE, and γ-interferon is seen in MS lesions, it was proposed that Th1 cells may be directly involved in both MS and EAE. However, this is not true in all EAE models and in every biological context: (1) MBP-reactive Th2 cell clones that secrete IL-4 and low levels of γ-interferon also cause EAE[41]; (2) γ-interferon–deficient mice developed EAE with greater severity after immunization with either MBP or MOG peptides[42]; (3) treatment of mice with γ-interferon caused attenuation of disease and treatment of mice with anti–γ-interferon antibodies induced worsening of EAE.[43]

A clinical study that reported worsening of the disease in patients receiving γ-interferon has supported the view that MS, like EAE, is a Th1-mediated autoimmune disease. However, a careful reading of the report raises several questions. Side effects such as fever, myalgias, and arthralgias were noted in virtually all five patients in the group of patients receiving high-dose γ-interferon which subsided with discontinuation of the drug.[44,45] All of the exacerbations involved the worsening of old symptoms, and at the completion of the study there was no residual defect in any of the seven patients with relapses. Whether corticosteroids were given to the patients with relapses is unclear, and the study was done at a time when magnetic resonance imaging (MRI) scans were not readily available. Thus, the transient neurological symptomatology induced by γ-interferon could merely represent clinical decompensation due to fever or the action of other cytokines ("pseudorelapses") and not necessarily the evolution of a new inflammatory process. In other studies, induction of γ-interferon has been observed after treatment of MS patients with intravenous immune globulin without any increase in the incidence of relapses. Also administration of poly-ICLC in secondary progressive MS, a known γ-interferon inducer, has not shown any adverse effect in MS patients.[46,47] Hence, assigning a central role for Th1 cytokines in MS, which therefore would serve as a major argument for the relevance of EAE to MS, seems unfounded.

Table 1. Immunopathology and Response to Therapy in EAE and MS

	EAE	MS
Pathology		
Location of demyelination	Predominantly, perivenous sleeves of myelin loss in spinal cord and brain	Demyelination not restricted to perivenous regions of white matter; extensive demyelination of cerebral cortex in the absence of inflammation is common
Location of lesions	Dependent on the autoantigen used for induction: inflammation dominates in lumbar regions in MBP and PLP EAE and brainstem in MOG EAE	Periventricular areas, cortical mantle, brainstem, optic nerves, and upper cervical cord; lesions are uncommon in thoracic and lumbar regions
Phenotype of cellular infiltrate	$CD4^+$ T cells (MBP and PLP EAE) activated macrophages and few $CD8^+$ T cells	Activated macrophages and $CD8^+$ T cells of a restricted clonotype
Cytokine predominance	TH1 bias in MBP and PLP EAE; TH2 bias worsens MOG EAE	Variable; no clear cytokine preponderance
CSF immunology	Antibodies to myelin antigens present in CSF	Antibodies to myelin antigens are infrequent in CSF and do not constitute the antigen specificity of oligoclonal bands
Effect of immunotherapies		
γ interferon	Depends on route of administration and can either worsen on ameliorate EAE	Worsening of inflammatory lesions unproven
β interferon	Variable; can worsen EAE if given after immunization	Decreases relapse rate: effect on progression modest
Anti–TNF antibody	Reverses EAE	Worsens MS
Anti–VLA-4 antibody	Reverses EAE	Decreases relapses; effect on progression not known
Anti–CD4 antibodies	Cures EAE	No evidence of clinical efficacy on relapses or progression

EAE = experimental allergic encephalomyelitis; MS = multiple sclerosis; MBP = myelin basic protein; PLP = proteolipoprotein; MOG = myelin oligodendrocyte glycoprotein; CSF = cerebrospinal fluid.

Fundamental Differences in the Pathology between Multiple Sclerosis and Experimental Allergic Encephalomyelitis

In a manner analogous to that seen in EAE, the inflammatory response in MS is thought to be mediated by the trafficking to the CNS of autoreactive T cells (see Table 1). Such a mechanism, sometimes referred to as the "outside to inside hypothesis," was recently challenged by work by Barnett and Prineas[48] and supported by other studies.[24,48–50] They noted the occurrence of oligodendrocyte death as the very early and perhaps the initial event in the pathology of the plaque, even before development of inflammation. These observations are by no means novel.[51] Evidence of early noninflammatory changes in the CNS has also been suggested by imaging studies but not confirmed histologically.[52–57] However, normal-appearing white matter on MRI may still contain microscopic evidence of inflammation. This and other observations may interpret the inflammation in MS as one of the following: (1) an epi-phenomenon that follows areas where the loss of myelin is large, such as in the vicinity of large fiber tracts or (2) an attempt to fight a damaging process that initiates oligodendrocyte death. Under such a scenario, MS (unlike EAE) is not a disease that is mediated by the entry of T cells from the periphery but is caused by direct death and destruction of nervous system structures including, to a large extent, the destruction of myelin. Although the neurological consequences of inflammation, induced by the destruction of the oligodendrocyte-myelin unit cannot be ignored and may contribute to morbidity, this concept will foretell that long-term reduction in the inflammatory response (with the use of either antiinflammatory or immunosuppressive therapies) is unlikely to alter the natural course of the disease.

The former view that MS is exclusively a white matter disease was challenged and proved wrong by histological and imaging studies. Indeed, axonal damage and neuronal loss are common features of MS and may be a direct consequence of inflammation or because loss of trophic factors necessary to maintain the integrity of the neural-axonal unit. Histological and MRI studies have shown significant cortical and axonal damage in MS that is not seen in EAE.[58–60] Whatever the mechanism, demyelination in the cortical gray matter mantle extends from the pial surface to the gray white junction and spreads laterally over several contiguous gyrii.[58–60] Most importantly, these areas of myelin loss lack an inflammatory response. Similarly, large regions of the spinal cord around the central canal showed loss of myelin with decrease of neuronal structures.

Pitfalls in Extension of Immunotherapies from Experimental Allergic Encephalomyelitis to Multiple Sclerosis

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 (Table 2). The spectrum of agents and approaches that showed promising results in EAE is immense and range from turmeric (used in Asian cooking) and Padma-28 (exotic natural drug found in health food stores) to modern genetic manipulation of the immune system with cytokines and antigen. Nevertheless, when applied to the human "counterpart," most, but not all, of these therapies proved disappointing.[61,62] Glatiramer acetate represents the only drug currently in use whose application in a clinical setting was first proved useful in EAE.[63] Glatiramer acetate is modestly effective in reducing relapses but has not prevented the progression of MS.[64]

The reasons for this failure are not only, as shown here, that MS and EAE differ quite substantially, but also that even from the larger, more comprehensive picture, most of the evidence suggests that the EAE models do not reflect the pathology of a progressive disorder as MS. Moreover, the various EAE models are dissimilar in their pathology and immunology to such an extent that it is unclear why one EAE model will be better served than another.

In clinical studies aimed at inducing antigen-specific tolerance to a potential encephalitogenic autoantigen such as MBP, either worsening of disease was noted or there was no change in the clinical course.[65,66] Induction of oral tolerance in trials aimed to prove this point were also a disappointment.[67] Likewise, there was no beneficial effect of anti-CD4 antibody therapy on the progression of MS despite profound decrease of $CD4^+$ T cells in peripheral blood.[68,69] Equally, examination of the therapeutic approach of switching from a Th1 to a Th2 profile in MS patients might prove a dangerous experiment, because pathological studies of brains of patients with MS show a Th2 response (presence of antibodies and complement) in the most destructive of lesions and glatiramer acetate induced a Th2 profile only after prolonged *in vitro* cultures of lymphocytes.[31,70,71] All this puts into question the hope that an immunosuppressive and/or antiinflammatory drug are likely to have a significant impact on MS.[72] The reports of worsening of MS after bone marrow transplantation may become disheartening proof.[73,74]

Conclusions

He who would distinguish the true from the false, must have an adequate idea of what is true and false.—Baruch Spinoza 1632–1677

The arguments we have presented should lead to several conclusions: EAE is a disorder that differs im-

Table 2. Agents Successful in Treating EAE

Antibodies to T-cell surface antigens	CD3,CD4,T-cellreceptor,CD2,IL-2R,IL-2R,CD24,CD40LCD28
Antibodies directed to antigen-presenting cells	MHC class II antigens, CD40, B7-1 and B7-2, Fc receptor blockade
Antibodies to NK cells	Anti–NK cell antibody, α-Gal ceramide
Antibodies to adhesion molecules	VLA-4, ICAM-1, LFA-1
Antibodies to cytokines	IL-2, IL-6, IL-12, IL-15, TNF-α, IL-1, IL-23
Antibodies to chemokines	Anti–MIP-1—α Rantes
Antiinflammatory cytokines	IL-4, IL-10, TGF-β, IFN-β, IFN-α, ?γ-IFN
Antagonists of signaling molecules	Tyrphostins (inhibitors of JAK-Stat activation), lysofyline, inhibitors of MAP kinase pathway, inhibitors of NF-κB activation, Inhibitors of iNOS activation, amsamycin, cholera toxin, AMPA antagonists, glutamate receptor antagonists, IL-1 receptor antagonists
Activation of nuclear receptors	PPAR-γ retinoic acid
Hormones	Estrogen, progesterone, vitamin D, DHEA, leptin antagonists
Antibiotics	Minocycline, rapamycin
Antimetabolites and immunosuppressants	FK-506, cyclosporin, dyspergualin, corticosteroids, azathioprine, cyclophosphamide, mycophenolate, bone marrow transplantation
Gene therapies	Targeted delivery of IL-4, IL-10
Inhibitors of enzymes	HOMG coreductase inhibitors (statins), COX-2 inhibitors
Peptides/proteins	Oral myelin proteins, glatiramer acetate, myelin peptides (iv)
Food supplements	Essential fatty acid, omega 3 fatty acid, curcumin, padma-28, fish oil
Small organic molecules	Linomide, silica, sodium phenyl acetate, copper chelators (*N*-acetylcysteine aminde), laquinamod, piperazylbutroxide, uric acid, dermatan sulphate, amionoguanidine, cuprizone, roliprim, H-2 receptor antagonists, indoleamine 2-3 deoxygenase, FTY-270, pentoxyfyline
Miscellaneous	Incomplete Fruend's adjuvant, BCG vaccination, Helminthic infections

AMPA = alpha-amino hydroxy methyl propionic acid; BCG = Bacille Calmette Guerin; DHEA = dehydro epi androsterone; EAE = experimental allergic encephalomyelitis; HMG = hydroxymethyl glutaryl coreductase; IFN = interferon; IL = interleukin; iNOS = inducible nitric oxide synthase; MAP = microtubule-associated protein; MHC = major histocompatibity complex; MIP = macrophage inflammatory protein; TGF-β = transforming growth factor-β.

munologically and pathologically between species, according, in part, to the type of antigen used to induce it and the species in which the model is tested. None of the EAE models represent MS and they therefore are imprecise methods to elucidate either the pathogenesis or to develop therapeutic strategies in MS. In addition, EAE is not a valuable vehicle to examine therapies: the inability to apply the therapeutic successes of our findings from the EAE model to the human condition is one of the arguments against the autoimmune hypothesis for the pathogenesis of MS.

We propose a much more careful use of EAE, especially when this model is utilized to define therapy. There are more than 100 compounds of proven efficacy in EAE, and we believe that it is pointless to add any more to this list (see Table 2). It may also be important not to extrapolate successful therapies from other dysimmune conditions in the hope that MS may represent a variation on the theme of a common disease mechanism.

We therefore are forced to examine MS without the restraints of EAE, as to what it is, rather than what it looks like. It would be interesting to ask the question of how one could approach the disease if animal models were unavailable, and the only recourse would be to examine the clues offered by our patients and from relevant genetic, imaging, and epidemiological studies in humans. We believe that the current available pathologic as well as radiological data would argue favorably in examining issues outside of the "autoimmune hypothesis" as central elements in the disease process.

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