Neuron, Vol. 24, 511–514, November, 1999, Copyright ©1999 by Cell Press

Minireview

Assessment of Animal Models for MS and Demyelinating Disease in the Design of Rational Therapy

Lawrence Steinman*
Department of Neurology and Neurological Sciences
Beckman Center for Molecular Medicine
Stanford University
Stanford, California 94305

Experimental autoimmune encephalomyelitis (EAE) is a useful model of acute demyelinating disease. Some forms of EAE reflect chronic demyelination with exacerbations and remissions characteristic of multiple sclerosis (MS). The chronic models of MS reflect many of the pathophysiologic steps in MS, including the role of certain adhesion molecules, the influence of T cells and antibodies reactive to components of the myelin sheath, the participation of metalloproteases in penetrating the blood–brain barrier, and the cytotoxic role of certain cytokines. One of the three therapies, approved in the United States, for treatment of multiple sclerosis was developed preclinically based on its success in treating various models of EAE. However, the role of certain cytokines in EAE is contradictory to what is seen when tried experimentally in MS. Recognition of the discrepancies between MS and its experimental models is critical in attempting to design rational therapies for demyelinating disease.

Pathologic Features of MS and Demyelinating Disease that Must Be Reflected in Animal Models

MS is an inflammatory demyelinating disease of CNS white matter. It is the major chronic disease of white matter, affecting ~350,000 individuals in North America and about twice that number in Europe. MS is much less common in India and Asia, probably reflecting a genetic predisposition to a certain genotype encoded by the major histocompatibility locus on chromosome 6, termed HLA (human leukocyte antigen). Genes in the HLA DRB region encode the highly polymorphic portion of the molecule that presents peptides to thymic-derived T lymphocytes in the immune system. Individuals with a particular HLA type (HLA DRB1*1501, formerly known as HLA DR2) are three to four times more susceptible to MS than individuals who have a different HLA type at this locus. The incidence of MS in northern Europe, where HLA DR2 is most common, is as high as 1 per 750 individuals. Females are affected twice as often as males (Steinman, 1996).

Approximately 85% of MS cases begin as relapsing remitting disease, with episodes of neurologic impairment involving major white matter tracts impairing vision, other sensory modalities, motor performance, or coordination. Episodes of relapse and remission often evolve over many years to a more chronic form of disability without clear cut exacerbations. These clinical scenarios become important in the development of animal models of MS. The most useful animal models would develop acute attacks with specific neurologic deficits like paralysis, and then the animals would develop chronic neurologic disease, with relapsing episodes of paralysis followed by periods of clinical remission and ultimately permanent deficits. Many animal models of MS feature only acute attacks and recovery, a situation that is comparable to syndromes of acute demyelinating disease, syndromes that are closely related to MS and may represent initial attacks of MS that are self-contained.

There are three acute demyelinating diseases affecting the CNS—acute optic neuritis (AON), acute transverse myelopathy (ATM), and acute disemminated encephalomyelitis (ADE)—that appear identical both clinically and pathologically to MS during periods of exacerbation. Indeed, most relapses of chronic MS resemble these three acute conditions clinically and pathologically. There is no way to distinguish ADE, AON, and ATM from a first episode of MS, and in about 25% of cases these acute demyelinating syndromes culminate in recurrent episodes of inflammation in brain, optic nerve, or spinal cord. Once the syndromes return, the illness is then called MS. The acute demyelinating attacks last days to weeks, often occurring following a viral infection. AON is manifest by a transient disturbance of vision. ATM is characterized by transient paralysis and sensory disturbances in the extremities, often with bowel and bladder dysfunction. ADE is distinguished by various motor and sensory disturbances and occasionally by convulsions.

One of the first known examples of ADE occurred when "paralytic accidents," referring to spinal cord inflammation leading to clinical paralysis, were noted after rabies immunization. This observation served as a basis for the development of models of acute experimental allergic encephalomyelitis (Stuart and Krikorian, 1928; Rivers et al., 1935; Kabat et al., 1947). The "paralytic accidents" were due to immunization against brain tissue, used for growing the attenuated rabies virus.

Once it was recognized that immunization against brain tissue could produce paralysis and demyelination in experimental animals, models of ADE were developed in monkeys at the Rockefeller Institute by Rivers. Injection of a mixture of brain material with complete Freund's adjuvant, an emulsion made from killed mycobacteria tuberculosis, which is useful in initiating a vigorous immune response, permitted the development of highly reproducible models of acute paralysis and white matter inflammation (Kabat et al., 1947). The efforts of Rivers, Freund, and Kabat resulted in the establishment of animal models of experimentally induced autoimmunity in the nervous system, termed experimental allergic encephalomyelitis (later to be interchanged with experimental autoimmune encephalomyelitis), which is abbreviated EAE. In the basic protocol for inducing EAE, experimental animals are immunized with either foreign or self proteins from the white matter of the nervous system, most usually myelin basic protein (MBP), proteolipid protein (PLP), or myelin oligodendroglial glycoprotein (MOG). The acute EAE models reflect many of the features of AON, ATM, and ADE. Unlike MS, these illnesses occur acutely and then resolve with varying

*E-mail: steinman@stanford.edu.

degrees of residual disease in the optic nerves, spinal cord, or white matter of the brain. MS, in contrast to these acute diseases, is chronic, lasting a lifetime, and is commonly manifest by episodes of inflammation in the white matter, leading to permanent disabilities. Multiple schemes for immunization, including innoculation with T cell clones specific for myelin proteins and the injection of various antigens with different combinations of adjuvants, have been used to produce chronic relapsing EAE. These animal models of MS incorporate most of the features described below.

In MS, evidence points to an immune response against components of the myelin sheath. A widely accepted view of the process of demyelination reveals that T cells, immunoglobulin, and complement play a role in pathogenesis. Adhesion molecules, cytokines, chemokines, HLA molecules, and metalloproteases are critical participants in the development of the inflammatory response in brain. Initially, circulating autoreactive myelin-specific $CD4^+$ Th1 cells are activated in the periphery by nonself antigens, likely to be of microbial origin. These microbial components resemble CNS myelin proteins, and this resemblance between self and nonself molecules is called molecular mimicry (Steinman, 1996; Conlon et al., 1999). These circulating T cells, which recognize molecules resembling myelin, are captured on vascular endothelium in the brain first by selectins and then by ligand–receptor interactions between integrin molecules. T cells migrate through the endothelium in response to chemotactic signals, and metalloproteinases or other matrix-degrading enzymes facilitate T cell penetration through the basement lamina. Microglial cells and astrocytes reactivate T cells locally in the CNS by presentation of myelin antigens complexed with major histocompatibility complex class II molecules. T cells stimulate macrophages by secretion of inflammatory cytokines, and macrophages in turn show increased phagocytic activity and release free oxygen radicals, nitric oxide metabolites, proteases, arachidonic acid derivatives, and complement components that damage myelin. Autoantibodies against myelin antigens, such as myelin basic protein and myelin oligodendroglial glycoprotein, also have an important role in demyelination (Warren et al., 1995; Wucherpfennig et al., 1997; Genain et al., 1999). In addition to myelin damage, there is clear evidence in MS for axonal loss and dysfunction (Trapp et al., 1998). Many of these gene products involved in this scenario for the pathogenesis of MS have been demonstrated at the sites of lesions by standard methods of immunohistochemistry, in situ hybridization, and reverse transcriptase–based PCR.

Experimental Autoimmune Encephalomyelitis

Chronic relapsing EAE reflects many of the features seen in MS (Table 1). Females, who are twice as susceptible to MS as males, are overwhelmingly more susceptible to EAE induction in mice. Susceptibility to EAE in mice is closely linked to loci in the major histocompatibility complex that are comparable with HLA in humans. T cell and antibody responses to various myelin proteins, like myelin basic protein, myelin oligodendroglia glycoprotein, and proteolipid protein, can be detected in the cerebrospinal fluid of animals with EAE. T cell receptor rearrangements indicative of T cells reactive to myelin can even be detected in demyelinating areas from brains of mice with EAE (Steinman, 1996). Similarly, antibody responses to MBP and to MOG can be detected at the site of vesiculating myelin in EAE brain (Warren et al., 1995; Genain et al., 1999). Immunization with various myelin proteins in suitable adjuvants or with T cell clones specific for certain peptides within these proteins can trigger chronic relapsing, demyelinating disease in rodents and nonhuman primates. In EAE, T cell immune responses spread to different portions of a given myelin protein and then spread to various myelin antigens, a phenomenon known as “antigen spreading.” Immunization with microbial sequences resembling myelin antigens can either induce clinical paralysis and demyelination or protect against disease in EAE (Conlon et al., 1999). This is often interpreted to mean that the microbe, toward which the immune response is directed, actually triggered the disease (Wucherpfennig et al., 1997). Finally, in chronic relapsing EAE, there is also evidence for axonal pathology (Raine and Cross, 1988).

Table 1. Comparisons between Multiple Sclerosis and EAE

	MS	EAE
Clinical Presentation		
Relapses and remissions	present	present
Paralysis	present	present
Ataxia	present	present
Visual impairment	present	present
Genetics		
MHC-linked susceptibility	yes	yes
Females more susceptible	yes	yes
Pathology in Lesions		
T cells reactive to myelin	present	present
Antibodies to myelin	present	present
α4 integrin, complement	present	present
TNFα, γIFN	present	present
Demyelination	present	present
Axonal dystrophy	present	present
Therapy		
γIFN, systemic	worsens	cures
anti-TNFα, systemic	worsens	cures
IL-4 transduced T cells	not done	cures
TNFα transduced T cells	not done	worsens
Copaxone	improves	cures
βIFN	improves	improves

Experimental Encephalomyelitis as a Preclinical Proving Ground for MS Therapeutics: Successes and Problems

Molecules that can be detected in inflammatory MS lesions—α4 integrin, matrix metalloproteases, and the cytokines interleukin-6 (IL-6), tumor necrosis factor α (TNFα), and γ interferon (γ-IFN)—as well as $CD4^+$ T cells reactive to myelin proteins, can also be demonstrated in inflamed brain and spinal cord in EAE. Treatments with antibodies to α4 integrin, to $CD4^+$ T cells reactive to myelin proteins, and to the cytokines IL-6 and TNFα have all reversed paralysis in various models of EAE. Likewise, administration of small molecule metalloprotease inhibitors and altered peptide ligands (APLs) for T cell receptors recognizing myelin antigens have also reversed EAE (Conlon et al., 1999). This has lead to clinical trials targeting α4 integrin, CD4, T cells reactive to myelin antigens, TNFα, and γ-IFN. Many of these approaches are being tested in phase II clinical trials, where dose and timing of administration of the therapeutic agent are ascertained. To date, the most promising

therapeutic approach to come out of the EAE model is Copaxone, a synthetic polymer analog of MBP, which has been successful in reducing the relapse rate in MS in clinical trials and is currently approved for treatment of MS (Arnon, 1996).

β interferon (β-IFN), approved for use in the United States and the EEU for treatment of relapsing MS, was originally tried as an antiviral compound for treatment of MS, a disease often linked to a viral etiology. After β-IFN was proved to be effective clinically in relapsing remitting MS, it was shown that recombinant β-IFN could ameliorate EAE, though severe relapses were seen when β-IFN was discontinued (Ruuls et al., 1996).

Immunologists have divided T cell responses into two categories, based on the types of cytokines T cells produce after stimulation: Th1 responses, commonly found in T cells from individuals with organ-specific autoimmune diseases like MS or rheumatoid arthritis, lead to production of γ-IFN and TNFα, while Th2 responses, commonly found in T cells from individuals with allergic conditions or parasitic infections, are characterized by the release of IL-4, IL-5, and IL-10. MS is considered a Th1-type autoimmune disease, as is EAE. However, EAE can be induced with Th2-type T cell clones (Lafaille et al., 1997), demonstrating that other cytokines can assume critical functions in producing the phenotype characteristic of EAE. Clearly, there is a great deal of redundancy in the immune response. Although experimental autoimmune encephalomyelitis and experimental allergic encephalomyelitis are used interchangeably, perhaps it is time to refer to disease caused via Th1 clones as "experimental autoimmune encephalomyelitis," while disease induced by Th2 clones could be referred to as "experimental allergic encephalomyelitis."

It is clear that two of the critical cytokines involved in the pathogenesis of MS are γ-IFN and TNFα. A clinical trial was performed in the 1980s to test the efficacy of γ-IFN in MS. Results showed that γ-IFN exacerbated disease, and the trial was halted while in progress (Panitch et al., 1987). The results implied that γ-IFN is pathogenic in MS. Paradoxically, in EAE systemic administration of γ-IFN protects from paralysis (Krakowski and Owens, 1996).

Another cytokine believed to play a key role in MS is TNFα. This is based on the observation that there is an increase in TNFα in the cerebrospinal fluid preceding and during relapses of disease (Sharief and Hentges, 1991). However, thus far in humans, treatments with monoclonal anti-TNF antibody and soluble TNF receptor have actually worsened MS (van Osten et al., 1996). The anti-TNF treatments that failed in MS have been successful in other diseases like rheumatoid arthritis and Crohn's Disease, where TNFα plays a critical role. Clinical trials with reagents that block TNFα in the periphery have exacerbated MS, but therapeutic trials with phosphodiesterase inhibitors and other approaches like altered peptide ligands that can downregulate TNFα within the CNS have not been reported as yet.

The discrepancies between the results with inhibition of TNFα in EAE versus MS are quite perplexing. TNFα and lymphotoxin (LT) are members of the TNF family of cytokines, and although they have different structures they both bind to the same receptors and produce similar biologic effects. Whether it is TNFα or LT that may be the key inflammatory mediator in EAE or MS is an open question. Some resolution of this issue has been addressed with mice in which TNF family members—either TNFα, LT, or both—are disrupted.

TNFα, LT, and other members of the TNF family may play a key role in the pathogenesis of oligodendroglial damage. In vitro, TNFα can damage myelin and injure oligodendrocytes in culture (Selmaj and Raine, 1988). In various models of EAE, blockade of TNFα with monoclonal antibodies or soluble TNFα receptor constructs can prevent or reverse disease. Therapy with altered peptide analogs of MBP, which downregulate the expression of TNFα, reverses EAE even after paralysis is apparent (Steinman, 1996). Downregulation of TNFα by phosphodiesterase IV inhibitors prevents and reverses EAE as well. Overexpression of TNFα in the CNS in transgenic mice leads to spontaneous inflammatory demyelinating disease (Conlon et al., 1999).

Targeted gene deletion in mice of LTα alone, with TNFα expression left intact, blocks the development of EAE (Conlon et al., 1999). This indicates, at least in rodent models of demyelination, that LTα may play the decisive role in demyelination, rather than TNFα. In contrast, in mice with the TNFα gene deleted, there is nevertheless intense inflammation in the CNS. Mice deficient in TNFα develop a more severe form of EAE. EAE in these TNFα knockout mice can be ameliorated by administering TNFα (Liu et al., 1998).

Thus, it is unclear from various EAE models whether TNFα is a pathologic player and should be inhibited, or whether it might indeed have protective effects. When myelin-specific T cells transduced with retroviruses carrying cytokines have been given to mice with EAE, IL-4-transduced T cells ameliorated EAE, while TNFα-transduced cells worsened disease. When targeting is more precise, using retrovirally transduced myelin-specific T cells that can home to the site of disease, a Th1 cytokine exacerbated and a Th2 cytokine ameliorated EAE (Dal Canto et al., 1999). The effects of cytokines and their inhibitors when given systemically, or when their genes have been disrupted globally, may be paradoxical, especially when interpreting results with potential therapeutics such as monoclonal antibodies or soluble receptors that are unable to penetrate to the site of disease across the blood–brain barrier.

Though EAE has many features that reflect what is known about the pathophysiology of MS, there are many discrepancies between the pathology of EAE and MS. Therefore, extrapolations must be made with caution when predicting what might happen in MS, based on results obtained in the EAE model. Results of ongoing clinical trials targeting α4 integrin with humanized antibodies, metalloproteases with small molecule inhibitors, and T cells reactive to myelin antigens with humanized antibodies and altered peptide ligands should allow us to judge the utility of EAE as a model to predict therapeutic efficacy for MS. So far, the development of the approved MS drug, Copaxone, based on its efficacy in EAE and the success of β-IFN in treating EAE must be balanced with the failures of anti-TNFα and γ-IFN in MS, after their successful use in various models of EAE. It is too early to judge whether amelioration of EAE will be a reliable predictor of success in treating MS.

Selected Reading

Arnon, R. (1996). Immunol. Lett. *50*, 1–15.

Conlon, P., Oksenberg, J.R., Zhang, J., and Steinman, L. (1999). Neurobiol. Dis. *6*, 149–166.

Dal Canto, R.A., Shaw, M.K., Nolan, G.P., Steinman, L., and Fathman, C.G. (1999). Clin. Immunol. *90*, 10–14.

Genain, C.P., Cannella, B., Hauser, S.L., and Raine, C.S. (1999). Nat. Med. *5*, 170–175.

Kabat, E.A., Wolf, A., and Bezer, A.L. (1947). J. Exp. Med. *85*, 117–129.

Krakowski, M., and Owens, T. (1996). Eur. J. Immunol. *26*, 1641–1646.

Lafaille, J.J., Keere, F.V., Hsu, A.L., Baron, J., Hass, W., Raine, C.S., and Tonegawa, S. (1997). J. Exp. Med. *186*, 307–312.

Liu, J., Marino, M.W., Wong, G., Grail, D., Dunn, A., Bettadapura, J., Slavin, A., Old, L., and Bernard, C.C.A. (1998). Nat. Med. *4*, 78–83.

Panitch, H.S., Hirsch, R.L., Schindler, J., and Johnson, K.P. (1987). Neurology *37*, 1097–1103.

Raine, C.S., and Cross, A.H. (1988). Lab. Invest. *60*, 714–725.

Rivers, T.M., and Schwentker, F.F. (1935). J. Exp. Med. *61*, 689–702.

Ruuls, S.R., de Labie, M.C., Weber, K.S., Botman, C.A., Groenestein, R., Dijkstra, C.D., Olsson, T., and van der Meide, P.H. (1996). J. Immunol. *157*, 5721–5731.

Selmaj, K.W., and Raine, C.S. (1988). Ann. Neurol. *23*, 339–346.

Sharief, M.K., and Hentges, R. (1991). N. Engl. J. Med. *325*, 467–472.

Steinman, L. (1996). Cell *85*, 299–302.

Stuart, G., and Krikorian, K.S. (1928). Ann. Trop. Med. Parasitol. *22*, 327–377.

Trapp, B.D., Peterson, J., and Ransohoff, R.M. (1998). N. Engl. J. Med. *228*, 278–285.

van Osten, B.W., Barkhof, F., Truyen, L., Boringa, J.B., Bertelsmann, F.W., von Blomberg, B.M., Woody, J.N., Hartung, H.P., and Polman, C.H. (1996). Neurology *47*, 1531–1534.

Warren, K.G., Catz, I., and Steinman, L. Proc. Natl. Acad. Sci. USA *92*, 11061–11065.

Wucherpfennig, K.W., Catz, I., Hausmann, S., Strominger, J.L., Steinman, L., and Warren, K.G. (1997). J. Clin. Invest. *100*, 1114–1122.