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The experimental autoimmune encephalomyelitis (EAE) model of MS: utility for understanding disease pathophysiology and treatment

ANDREW P. ROBINSON[1,†], **CHRISTOPHER T. HARP**[1,†], **AVERTANO NORONHA**[2], and **STEPHEN D. MILLER**[1,*]

[1]Department of Microbiology-Immunology and Interdepartmental Immunobiology Center, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

[2]Department of Neurology, University of Chicago, Chicago, IL, USA

Abstract

While no single model can exactly recapitulate all aspects of multiple sclerosis (MS), animal models are essential in understanding the induction and pathogenesis of the disease and to develop therapeutic strategies that limit disease progression and eventually lead to effective treatments for the human disease. Several different models of MS exist, but by far the best understood and most commonly used is the rodent model of experimental autoimmune encephalomyelitis (EAE). This model is typically induced by either active immunization with myelin-derived proteins or peptides in adjuvant or by passive transfer of activated myelin-specific CD4+ T lymphocytes. Mouse models are most frequently used because of the inbred genotype of laboratory mice, their rapid breeding capacity, the ease of genetic manipulation, and availability of transgenic and knockout mice to facilitate mechanistic studies. Although not all therapeutic strategies for MS have been developed in EAE, all of the current US Food and Drug Administration (FDA)-approved immunomodulatory drugs are effective to some degree in treating EAE, a strong indicator that EAE is an extremely useful model to study potential treatments for MS. Several therapies, such as glatiramer acetate (GA: Copaxone), and natalizumab (Tysabri), were tested first in the mouse model of EAE and then went on to clinical trials. Here we discuss the usefulness of the EAE model in understanding basic disease pathophysiology and developing treatments for MS as well as the potential drawbacks of this model.

Keywords

Experimental autoimmune encephalomyelitis; immunotherapy; regulatory T-cells; epitope spreading; Th1/Th17; immune tolerance

INTRODUCTION

Mouse models of EAE

Experimental autoimmune encephalomyelitis (EAE) is a T-helper (Th) cell-mediated autoimmune disease characterized by T-cell and monocyte infiltration in the central nervous system (CNS) associated with local inflammation. The autoimmune molecular target(s)

*Correspondence to: Stephen D. Miller, Ph.D., Northwestern University Feinberg School of Medicine, 6-713 Tarry Building, 303 E. Chicago Ave., Chicago, IL 60611, USA. Tel: +1-312-503-7674, Fax: +1-312-503-1154, s-d-miller@northwestern.edu.
†A.P.R. and C.T.H. contributed equally to this review

identified and utilized have been proteins expressed by myelin-producing oligodendrocytes in the CNS. The result is primary demyelination of axonal tracks, impaired axonal conduction in the CNS, and progressive hind-limb paralysis. EAE is commonly employed as a model for multiple sclerosis (MS) and as such has been a powerful tool for studying disease pathogenesis as well as potential therapeutic interventions. There are currently many pathophysiologic forms of EAE with varying patterns of clinical presentation depending on the animal species and strain, priming protein/peptide, and route of immunization employed. Thus different models have been used to study disease development and specific histopathologic characteristics with relevance to MS, and to dissect mechanisms of potential therapeutic interventions.

EAE in the mouse was first induced over 60 years ago by active immunization with spinal cord homogenates (Olitsky and Yager, 1949). Extensive research has led to the discovery of numerous encephalitogenic peptides, and mice remain the most commonly employed animal species, in part due to the wide availability of transgenic and knockout mice available for targeted mechanistic studies. In the SJL (H-2^s) mouse, EAE can be actively induced by immunization with CNS homogenate, proteolipid protein (PLP), myelin basic protein (MBP), or encephalitogenic epitopes of PLP ($PLP_{139–151}$, $PLP_{178–191}$), myelin oligodendrocyte protein ($MOG_{92–106}$), or MBP ($MBP_{84–104}$) in an emulsion with complete Freund's adjuvant (CFA). The disease follows a predictable clinical course, characterized by a prodromal period of 10–15 days followed by ascending paralysis beginning in the tail and hind limbs and progressing to the fore-limbs concurrent with weight loss. In SJL mice the disease is characterized by a relapsing-remitting course of paralysis, allowing for mechanistic studies or immunomodulatory strategies in a relapsing autoimmune disease setting. $MOG_{35–55}$ is a potent encephalitogen in C57BL/6 (H-2^b) mice, which presents clinically in the form of a chronic progressive disease course. EAE can be induced in other mouse strains, e.g., PL/J and B10.PL (H-2^u), but is normally acute and rectifying. Strain and immunizing antigen variations continue to be explored for atypical manifestations (inflammation, mononuclear cell (MNC) infiltration, and clinical presentation) pertinent to the heterogeneous forms of MS presentation and pathology. For example, a novel clinical form of disease was recently reported displaying a relapsing-remitting course that developed into a chronic progressive phenotype with lesions in the brain as well as the spinal cord (Levy et al., 2010). This model may be particularly relevant to the most prevalent form of MS exhibiting relapsing-remitting followed by secondary progressive disease.

Method of immunization can be manipulated as well, allowing for more targeted studies of immunopathologic mechanisms. Whereas active EAE studies can be confounded by the robust immune response to the adjuvant itself, EAE can also be induced by adoptive transfer, whereby T cells are isolated from myelin peptide/protein-primed donors, stimulated *in vitro* with an encephalitogenic peptide, and resulting blast cells injected intravenously (i.v.) or intraperitoneally (i.p.) into naïve or immunodeficient recipient mice. This method allows for *in vitro* manipulation of the encephalitogenic T-cell population and disease induction with a fairly homogeneous population of antigen-specific T cells. Adoptive transfer of disease using T-cell receptor (TCR) transgenic mice allows for the study of myelin antigen-specific T cells (e.g., C57BL/6 2D2 $MOG_{35–55}$-specific or SJL/J 5B6 $PLP_{139–151}$-specific). "Humanized" mice expressing human TCRs specific for myelin epitopes presented by human major histocompatibility complex (MHC) class II molecules associated with genetic susceptibility to MS are also commercially available (e.g., a TCR specific for human $MBP_{84–102}$ bound to human leukocyte antigen (HLA)-DR2). Finally the adoptive transfer model is ideal for localizing T-cell populations *in vivo* throughout disease, as transferred cells can be labeled *in vitro* with fluorescent protein/dye or derived from congenic mice, allowing for *in vivo* tracking of encephalitogenic T-cell populations.

Rat models of EAE

Although the mouse model of EAE is the most commonly utilized animal model for MS, rat EAE has provided significant insight into the pathology of MS as well. In the rat model (usually the Lewis rat or Dark Agouti (DA) strains) of EAE, induced with either MBP or one of its encephalitogenic epitopes, the disease consists of inflammatory MNC infiltration into the spinal cord cerebellum and brainstem, but not the cortex. MBP-induced EAE in the Lewis rat model results in acute paralysis that recovers in 5–7 days (Swanborg, 2001). There is very limited demyelination, and rats remain resistant to the development of EAE with subsequent immunizations with MBP (Swanborg, 2001). Acute EAE can be passively induced in rat models of EAE with *in vitro* MBP reactivated CD4+ T cells (Swanborg, 2001). MBP-induced EAE in the rat model is less often utilized because demyelination is not a prominent feature of the disease. The paralytic episodes that occur during rat EAE are thought to be the result of blood–brain barrier breakdown, inflammation, and edema, but not from demyelination (Paterson et al., 1987). Interestingly, Lewis rats can be tolerized by immunization with MBP in incomplete Freund's adjuvant (IFA) prior to immunization with MBP in CFA, while DA rats are susceptible to the development of EAE with an MBP immunization in IFA alone (Swanborg, 2001). Additionally, unlike Lewis rats (Malotky et al., 1994), DA rats are not tolerizable by MBP-coupled splenocytes (Lenz et al., 1999). In contrast to MBP-induced EAE in the Lewis rat, which has limited demyelination and where clinical symptoms are very acute and mediated by CD4+ T cells, induction of EAE with recombinant MOG protein is completely dependent on demyelinating antibodies (Adelmann et al., 1995). EAE can also be induced in the Brown-Norway strain with recombinant MOG in CFA, and is also highly dependent on the demyelinating antibody response (Stefferl et al., 1999). These models have given the field insight into the significant deviation in pathologic responses based on the immunizing antigen and rodent species used.

INSIGHTS INTO MS PATHOGENESIS

Defining distinct pathogenic T-cell subsets

Since EAE is initiated by immunization with autoantigens presented to MHC class II-restricted CD4+ Th cells, the model is ideally suited to study Th-cell development, effector functions, and downstream T-cell-mediated signaling *in vivo*. According to the classic paradigm, MHC class II-restricted CD4+ Th cells were phenotypically classified as Th1 or Th2 cells based on cytokine production and transcription factor expression (Mosmann et al., 1986). Th1 cells characteristically secrete interferon (IFN)-γ and interleukin (IL)-2, are driven by antigen-presenting cell (APC)-derived IL-12 and IL-18, and mediate cell-mediated immunity against intracellular pathogens. Th2 cells differentiate in response to IL-4 and produce IL-4, IL-5, and IL-13 and promote clearance of extracellular parasites and humoral immunity. In the context of EAE, Th1 cells were originally found to have a pathogenic role through CD4+T-cell infiltration and production of IFN-γ in the CNS (Sriram et al., 1982). Additionally, disruption of T-bet expression, a transcription factor required for Th1-effector function, renders mice completely resistant to EAE (Bettelli et al., 2004). Conversely, Th2 cells have been associated with immunoregulation, alleviation of inflammation, and amelioration of clinical disease (O'Garra et al., 1997). Evidence for the dichotomous model has been found in human disease (e.g., elevated levels of IL-12 were detected in the CNS of MS patients (Comabella et al., 1998)), and this formed the basis for a long-standing theory of Th1-mediated auto-immunity underlying the pathogenesis of MS. Despite becoming the dominant theory, there were inconsistencies between the model and MS. For example, the distinction between Th1 and Th2 effector functions in humans is not as distinct as in mice, as demonstrated by the fact that human Th1 as well as Th2 cells are capable of producing anti-inflammatory IL-10 (Del Prete et al., 1993). More recently, the Th1/Th2 paradigm has been revised and expanded, based once again on discoveries in the EAE model. A CD4+ T-

cell population, driven by IL-23 and producing IL-17 (Th17), was shown to be required for EAE induction (Becher et al., 2003). The phenotype is distinct from Th1/Th2 as commitment to the Th17 lineage is antagonized by Th1 (IFN-γ) and Th2 (IL-4) cytokines, and Th17 cells secrete a unique proinflammatory cytokine signature, including IL-17A, IL-17 F, IL-22, and IL-21 (Langrish et al., 2005). IL-23 was shown to be the key driving factor as terminal differentiation of Th17 cells into mature effector cells requires IL-23 (Aggarwal et al., 2003). The evidence for a pathogenic role in autoimmune-mediated demyelination has been extensively documented. Mice deficient in IL-23 are completely resistant to EAE (Cua et al., 2003), and adoptively transferred IL-23-treated CD4+ T cells robustly infiltrate the CNS and are more encephalitogenic than cells treated with IL-12 (Langrish et al., 2005). Studies in human MS patients have largely corroborated findings in EAE of a critical role for Th17 cells in disease pathogenesis. Polyclonal stimulation *in vitro* of CD4+ T cells isolated from MS patients produced significantly more IL-17 compared to healthy controls with no differences in IFN-γ (Vaknin-Dembinsky et al., 2006). Examining T-cell-polarizing cytokine production, monocyte-derived dendritic cells from MS patients produced greater amounts of IL-23 compared to healthy controls with no differences in IL-12 (Vaknin-Dembinsky et al., 2006). Blood MNCs expressing IL-17 mRNA are more numerous than in healthy controls, and, within patients, IL-17-expressing MNCs are greater in the cerebrospinal fluid (CSF) than blood (Matusevicius et al., 1999). Further, IL-17 expression in MNCs directly correlates with disease exacerbation (Matusevicius et al., 1999).

Despite the current intense focus on Th17 cells as the pathogenic T-cell subset underlying MS, the Th1 paradigm should not be blindly discarded. Th1 and Th17 cells are both present in the CNS of EAE mice, though the proportion differs among mouse strains (Langrish et al., 2005; Korn et al., 2007). Likewise, mRNA transcripts associated with Th1 and Th17 cell subsets were both increased in active lesions from postmortem MS brains compared to controls without CNS pathology (Lock et al., 2002). Interestingly, cytokines produced by Th17 cells (IL-17A, IL-17 F, and IL-22) are not required for EAE, just as prototypical Th1 cytokines (IL-12, tumor necrosis factor (TNF)-α, and IFN-γ) are not essential (Willenborg et al., 1996; Frei et al., 1997; Becher et al., 2002; Kebir et al., 2007). EAE pathogenesis, or specific facets thereof, appears to be driven by both Th1 and Th17 cells to some degree, though the initiating subset has yet to be definitively defined. Indeed, there are subtle differences between Th1 and Th17-mediated auto-immune demyelination. Adoptive transfer of Th1 or Th17 *in vitro*-driven CD4+ T cells produces clinical disease with differing infiltrating leukocyte populations, lesion localization, and inflammation patterns (Kroenke et al., 2008; Stromnes et al., 2008). A possible explanation for the varied effects may be subset-specific migration based on differing chemokine receptor expression. Th1 cells characteristically express CXCR3 and CCR5, and while $CXCR3^{-/-}$ mice demonstrate exacerbated disease, $CCR5^{-/-}$ mice show normal EAE development (Tran et al., 2000; Liu et al., 2006a). Th17 cells highly express CCR6, and the ligand CCL20 is constitutively expressed in epithelial cells in the choroid plexus (Reboldi et al., 2009). CCR6 was upregulated in the CNS in EAE, and Th17 cells were reduced in the CNS of $CCR6^{-/-}$ EAE mice, accumulating at the choroid plexus epithelium (Reboldi et al., 2009). This suggests that chemokine upregulation in part mediates T-cell subset-specific migration into the CNS and disease severity.

The exact cooperative nature of T-cell subsets and the precise sequence of events in disease maintenance are only now being elucidated, largely in part through EAE studies, and may vary depending on the mouse strain employed. Evidence suggests IFN-γ may control localization of inflammation and efficient Th17 cell infiltration, while IL-23 is required for Th17 cell pathogenic effector functions and development of clinical disease (Cua et al., 2003; Lees et al., 2008; McGeachy et al., 2009). This is further complicated, though, by the

notion that terminally differentiated Th1/Th17 cells in the CNS of EAE mice may be capable of trans-differentiation. Indeed, there is evidence for a unidirectional plasticity between Th17 and Th1 cells (Luger et al., 2008; Lee et al., 2009). Currently, the exact contributions of Th1 and Th17 cells to MS pathogenesis remain to be teased out, although the discovery of potential mechanisms has relied heavily on EAE models, as have manipulations aiming towards therapies.

Regulatory T-cell maintenance of self-tolerance

No inbred mouse strain spontaneously develops EAE, though transgenic mice exclusively expressing TCRs specific for myelin epitopes bred on an immunocompetent background develop spontaneous EAE at a low and variable rate (Lafaille et al., 1994). Interestingly, bred on an immunocompromised background, these mice develop lethal EAE that can be prevented with transfer of WT CD4+ T cells (Olivares-Villagomez et al., 1998). This finding provides strong evidence for the existence of a naturally occurring suppressive immune cell, that has since been named the regulatory T cell (Treg). The Treg population, characterized by expression of CD25 and the transcription factor fork-head box p3 (Foxp3), is nonpathogenic and has suppressive effects on CD4+ CD25− T-cell stimulation (Qiao et al., 2007). The EAE model has since proved critical for defining a role for Treg in autoimmunity. Induced Treg contribute to self-tolerance in EAE by suppressing antigen-specific encephalitogenic T-cell proliferation and function (Zhang et al., 2010). A definitive mechanism underlying Treg-mediated immunosuppression has not been elucidated, though a role has been shown for local cytokine production, such as transforming growth factor-β, and cell surface molecule binding, such as CTLA-4 binding to costimulatory CD80/86 on effector T cells (Liu et al., 2006b; Zhang et al., 2010). In healthy humans a population of myelin-specific T cells has been identified in peripheral blood, suggesting that MS pathogenesis may be related to dysfunction of a Treg population rather than *de novo* generation of a self-reactive effector T-cell population (Burns et al., 1999). A growing body of evidence in mice and humans in fact supports a role for suppressive properties of CD4+ T cells expressing CD25 in maintaining self-tolerance (Hori et al., 2003). In MS patients, decreased Treg function has been directly linked to autoimmunity (Viglietta et al., 2004). Specifically, CD4+ CD25+ T cells from peripheral blood of MS patients showed impaired suppression of antigen-specific T-cell proliferation induced with MOG or MBP (Haas et al., 2005; Kumar et al., 2006). Functionally defining the Treg population will be critical as differences have also been associated with clinical subtypes of MS. While there was no difference in the number of CD4+ CD25+ Treg cells between relapsing-remitting and chronic progressive MS patients, suppressive function was impaired in relapsing patients compared to chronic progressive patients and controls (Venken et al., 2006). EAE studies will be critical to define the precise functions and mechanism of suppression by Treg and are already providing new avenues for therapeutic design.

Epitope spreading

One of the significant advantages in utilizing the EAE model is the ability to assess the emergence of T- and B-cell specificities to autoantigens temporally in response to an initiating epitope. In fact, the phenomenon of autoimmune epitope spreading was first described in the context of EAE (Lehmann et al., 1992). Since that time, EAE studies involving multiple strains and initiating epitopes have identified a predictable cascade of epitope spreading (Vanderlugt et al., 1998). Evidence that epitope spreading was at least partially responsible for relapse in the R-EAE model came from studies indicating that T cells specific for a spread epitope ($PLP_{178–191}$) derived from mice immunized with $PLP_{139–151}$ could transfer disease (McRae et al., 1995). An analogous epitope spreading can occur in the B-cell compartment during the course of some models of EAE (Bischof et al., 2004), but specific epitopes are difficult to map due to the fact that the B-cell receptor

(BCR) can recognize both linear and conformational protein epitopes that may also depend on specific posttranslational modifications. The identification of the invariant T-cell epitope spreading cascade in EAE has been useful in identifying that the CNS is the location where epitope spreading takes place (McMahon et al., 2005).

Although direct evidence for the occurrence of epitope spreading in MS is sparse, at least one study demonstrated sustained reactivity, focusing, and evolution of T-cell responses to serial PLP epitopes in patients with isolated monosymptomatic demyelinating syndromes who converted to clinically definite MS (Tuohy et al., 1997).

Multifaceted T-cell populations

Though the antigen and adjuvant combinations used in most EAE models initiate a CD4+ T-cell-mediated disease, CD8+ T cells have been implicated in MS pathology. Indeed, CD8+ T cells are found in MS lesions, often in high numbers, throughout the brain parenchyma and show preferential clonal expansion (Booss et al., 1983; McCallum et al., 1987; Babbe et al., 2000). This is not to say that the EAE model cannot be used as a platform to study the role of CD8+ T cells in disease pathogenesis. It has been reported that adoptive transfer of myelin-specific CD8+ T cells can induce robust EAE with active CD8+ T-cell infiltration in the CNS (Huseby et al., 2001; Sun et al., 2001). Interestingly, symptoms were observed that are characteristic of MS but absent in CD4-mediated EAE, including ataxia, spasticity, loss of motor coordination, and a preponderance of demyelinated lesions in the brain rather than the spinal cord (Huseby et al., 2001). Others however have reported that adoptive transfer of activated myelin-specific CD8+ T cells is incapable of inducing disease in naive mice (York et al., 2010). In humanized mice immunized with $PLP_{45–53}$, HLA-A restricted CD8 + T cells specific for PLP induced clinical disease with an early CNS infiltration of CD8+ T cells (Friese et al., 2008).

Interestingly, others have found a regulatory role for CD8+ T cells in EAE, protecting against clinical disease (Jiang et al., 1992; Koh et al., 1992; York et al., 2010). Antibody-mediated depletion of CD8+ T cells prior to EAE induction exacerbated clinical disease (Montero et al., 2004). Additionally, transgenic mice lacking functional CD8+ T cells and MHC class I display enhanced tissue destruction in the CNS after induction of EAE (Linker et al., 2005). In human disease the suppressor function of CD8+ T cells and a potential defect in the population in MS patients was demonstrated *in vitro* with CD8+ T cells isolated from peripheral blood (Antel et al., 1986). CD8+ T-cell suppressor function was also found to correlate inversely with clinical disease. CD8+ T-cell clones recognizing myelin-specific CD4+ T cells isolated from MS patients decreased more during exacerbations than remissions or controls (Correale and Villa, 2008). The precise nature of CD8+ pathogenic versus suppressor T cells in MS and animal models remains a topic of debate and is certainly complicated by a lack of distinctive cellular markers for subsets of CD8+ T cells. Nonetheless the EAE model may prove critical for dissecting this distinction in CD8+ T cells.

B cells and autoantibodies: another multifaceted cell type

Although EAE initiation involves priming a strong T-cell-mediated immune response, there is a crucial role for humoral immunity in disease pathogenesis. From EAE studies, a hallmark feature of MS has been defined, namely the production of oligoclonal immunoglobulins in the CSF of patients. Studies in animal models, though, suggest a more complex function for B cells than antibody production, including antigen-driven B-cell effector functions, stimulation of T cells through antigen presentation, and contribution to tertiary lymphoid structure formation in the CNS (McLaughlin and Wucherpfennig, 2008). Intrathecal synthesis of antibodies, specifically oligoclonal IgG, is common in MS patients

(Kabat et al., 1950; Bollengier et al., 1976). Though precise antigenic targets have yet to be identified, there is a direct correlation between IgG levels in CSF and MS disease progression (Villar et al., 2002). In MS postmortem tissue, antibodies specific for myelin and axonal proteins have been identified in lesions, even though they are not constituents of the oligoclonal IgG bands (Warren and Catz, 1993; Zhang et al., 2005). From these initial findings, the EAE model has been integral to investigating the role of antibodies against CNS constituents for a role in disease pathogenesis. The MOG antibody, 8-18C5, which recognizes a specific conformational epitope, exacerbates disease when administered to EAE mice (Linington et al., 1988). Though an attractive candidate for antibody-mediated pathogenesis in MS, studies of anti-MOG antibodies in patients have produced mixed results. One report found that antibodies to MOG are present in patients with clinically isolated syndrome (CIS) and correlate with an increased risk of progression to MS (Berger et al., 2003). Other groups found no association with progression from CIS to MS in a larger cohort of patients (Kuhle et al., 2007). While some studies found increased antibodies to MOG in the serum and CSF of MS patients compared to controls (Kennel De March et al., 2003), others found no difference between levels in MS and other neurologic diseases (Mantegazza et al., 2004). The detection of anti-MOG antibodies in CSF and serum varies widely between studies and may reflect variation between patients or methodology in the studies. Other antibodies against myelin proteins have been detected in serum and CSF of MS patients, though again, the frequency between studies has varied widely (Hafler et al., 2005). This may in fact highlight the heterogeneity of human disease classified clinically as MS and call for more rigorous analysis of antibody responses in specific subtypes of MS patients as well as EAE. Interestingly, a subset of MS patients mount an antibody response against the extracellular domain of the protein neurofascin, isoforms of which are expressed at the nodes of Ranvier on axons as well as oligodendrocytes (Mathey et al., 2007). Administration of neurofascin antibodies to EAE mice exacerbated disease and promoted complement deposition at the nodes of Ranvier (Mathey et al., 2007). In an *ex vivo* CNS culture system, these antibodies were shown histologically to bind axons at the nodes of Ranvier and inhibit axonal conduction via complement-mediated signaling (Mathey et al., 2007). These EAE and culture studies suggest a novel mechanism for antibody-mediated pathogenesis in MS, acting directly on the axons of neurons rather than the oligodendrocytes.

B cells are also potent APCs and, as a result, B-cell–T-cell interactions can modulate expression of costimulatory molecules, cytokines, and effector function of T cells. B cells can capture antigen through the BCR, degrade protein and load on to MHC class II molecules, and consequently upregulate costimulatory molecules for T-cell activation (McLaughlin and Wucherpfennig, 2008). Antigen presentation by B cells can be quite robust in autoimmune demyelination, as evidenced by the fact that the immunodominant epitope of MBP co-localizes with a T-cell epitope (Wucherpfennig et al., 1997). Binding of myelin antigens to the BCR may thus promote presentation to T cells. In EAE, B cells are required for development of disease following immunization with whole MOG protein, but not MOG peptides (Svensson et al., 2002). This suggests that potent B-cell processing and MHC class II presentation of antigen may be critical for MS development, as large fragments of myelin containing intact protein are common in MS lesions.

Functionally, ectopic germinal centers consisting of B cells and follicular dendritic cells (FDC) have been demonstrated in the CNS of relapsing-remitting EAE mice (Magliozzi et al., 2004). These tertiary lymphoid structures suggest that B cells having migrated to the CNS can: (1) present antigen-fostering T-cell effector function; and (2) differentiate into memory B cells or plasma-blasts at the site of inflammation. Ectopic follicles have been detected in the meninges of patients with secondary progressive MS and may exacerbate disease (Serafini et al., 2004). Differentiated B cells may also modulate MS through their

production of T-cell instructive cytokines. While B cells may drive differentiation of pathogenic Th1/Th17 cells, there is also evidence that B-cell-produced cytokines can promote anti-inflammatory Th2 cells (Pistoia, 1997). Evidence thus suggests a suppressive role for B cells as B cells produce the regulatory cytokine IL-10, which can alleviate EAE (Fillatreau et al., 2002; Matsushita et al., 2008). Conversely, B-cell-derived cytokine production can actively promote the organization of ectopic germinal centers and maturation of FDCs, serving as a self-sustaining loop promoting disease pathogenesis (Gommerman and Browning, 2003). EAE will be crucial for dissecting the pathogenic and regulatory mechanisms of B-cell antibody production and effector function. The role of B cells in MS and potential therapeutic strategies will likely ensue.

Axonal and neuronal pathology

As with immune cell infiltration and function, the mechanism and extent of axonal loss in the CNS of EAE mice vary widely across animal strain and method of immunization. Widespread axonal injury occurs in many EAE models closely localized with demyelinating lesions and infiltrating Th1 and Th17 cells (Aranami and Yamamura, 2008). Thus EAE may be considered a useful model for testing therapeutic strategies aimed at halting CD4+ T-cell-mediated axonal damage in MS lesions. Although neurons do not express MHC class II, intracellular neurofilaments released from necrotic neurons may exacerbate inflammation and disease. Indeed, neurofilaments can be readily detected in the CSF of MS patients (Teunissen et al., 2005). In MOG-induced EAE, Th17 cells were recently shown to mediate direct axonal injury without engagement of the TCR (Siffrin et al., 2010). Th17 cells visualized in the brainstem of EAE mice by two-photon microscopy effectively transected axons and induced Ca^{2+} fluctuations which can mediate neuronal death (Siffrin et al., 2010). A study in which Biozzi mice were immunized with neurofilaments led to robust EAE with more severe axonal damage and gray-matter lesions compared to MOG-induced EAE (Huizinga et al., 2008). CD8+ T-cell-mediated cytotoxicity may also contribute directly to axonal injury, although electrically active neurons do not typically express MHC class I (Neumann et al., 1995). MHC class I expression can be induced in electrically silenced neurons by exposure to proinflammatory IFN-γ (Neumann et al., 1995).

The role of autoantibodies in mediating axonal injury in MS has been controversial, though one study demonstrated that antibodies can directly contribute to axonal injury and clinical disease (Mathey et al., 2007). While relative preservation of axons in MS is not uncommon, permanent neurologic deficit has been associated more closely with axonal loss than demyelination (Kornek and Lassmann, 1999). Acute axonal injury in MS is most prominent in active demyelinating lesions, although a low level of acute injury continues to occur in chronic inactive lesions and in normal-appearing white matter (Kornek et al., 2000; Kuhlmann et al., 2002; Kutzelnigg et al., 2005). Thus, there are two patterns of axonal injury: acute injury directly correlating with active demyelinating lesions, and diffuse injury which is more closely associated with inflammation occurring throughout the brain and spinal cord. In some patients axonal injury appears to be more closely tied to inflammation rather than demyelination, as decreases in inflammation in late-stage disease are reflected in decreased axonal injury in normal-appearing white matter as well as demyelinating lesions (Frischer et al., 2009). Inflammation has been shown to inhibit axonal transport and is closely associated with activated macrophages and microglia in the CNS, which express a variety of proinflammatory molecules (Aboul-Enein et al., 2006). Within the lesion small-caliber axons are more vulnerable to injury than thick ones, probably owing to the energy demands relative to mitochondrial mass (Shintaku et al., 1988).

A number of insights suggest that mitochondrial dysfunction and ensuing energy failure contribute to the pathogenesis of EAE through the production of nitric oxide free radicals (Redford et al., 1997; Smith et al., 2001). Furthermore, mitochondrial dysfunction correlates

with cortical lesions in a subset of MS patients (Dutta et al., 2006). Although EAE is a CD4+ T-cell-mediated disease directed against myelin components, it has lent much insight into the downstream effects of oligodendrocyte damage that correlate closely with clinical symptoms. Additionally EAE has recently been employed to study direct T-cell and B-cell effects on axonal pathology. This direct interaction between the autoimmune component and neurologic pathology in MS highlights not only the complexity of the disease but also the unique suitability of the EAE model for understanding pathology and designing therapeutic strategies.

USE OF THE EAE MODEL IN THE DEVELOPMENT OF IMMUNOMODULATORY THERAPIES IN MS

Although there are differences between the pathophysiology of EAE and the human disease MS, EAE has become a very powerful and often utilized animal model in the development therapies in the treatment of MS. Because of the inflammatory nature of EAE and the clear autoimmune contribution to the disease, EAE has been most useful in determining the efficacy of immunomodulatory treatments. Notwithstanding, EAE is also useful in assessing treatments that directly affect the CNS tissue. Here we review currently FDA-approved and likely to be approved therapies for MS as well as recent clinical trial failures and the contribution and relevance of EAE to the development of these therapies.

Inhibition of leukocyte trafficking (natalizumab and FTY720)

Natalizumab (trade name: Tysabri, Elan & Biogen Idec) is a monoclonal antibody against the α4β1 integrin otherwise known as VLA-4. Anti-VLA-4 monoclonal antibody therapy is one of the most successful MS therapies that have come from initial studies in the EAE model. The adhesion of activated lymphocytes to EAE brain blood vessels was found to be inhibited by a monoclonal antibody to VLA-4 (Yednock et al., 1992) and therefore was hypothesized to be an important target in leukocyte trafficking to the CNS of MS patients. VLA-4 is present on most circulating peripheral, tissue-resident, and lymphoid-resident lymphocytes, as well as weakly on monocytes (Hemler, 1990), making it an excellent target for immunotherapy in the context of MS. Natalizumab therapy was shown to be highly effective in the treatment relapses and lesions of relapsing-remitting MS (RRMS) (Miller et al., 2003) and is now an FDA-approved therapy. While it was withdrawn from the market briefly because a small subset of patients developed progressive multifocal leukoencephalopathy (PML), this drug is back on the market and continues to be first- and second-line monotherapy (Warnke et al., 2010). The development of PML is thought to result from diminished immune surveillance in the CNS and was thought to be associated with combination therapy, although early findings suggest that PML can develop even with natalizumab monotherapy (Warnke et al., 2010). While longitudinal studies have not been undertaken to assess the efficacy of natalizumab in preventing long-term disease progression, especially in progressive MS patients, its efficacy in limiting disease relapse and lesion development further emphasizes the need for models such as EAE that emphasize the inflammatory nature of the disease.

FTY720 (fingolimod, Novartis Pharmaceuticals) is the first FDA-approved, orally administered, immunomodulatory therapy approved for the treatment of RRMS. FTY720 acts by limiting egress of lymphocytes out of secondary lymphoid tissues, inhibiting the G protein-coupled sphingosine-1-phospate (S1P) receptors (Matloubian et al., 2004). S1P is present in the serum but largely absent from secondary lymphoid tissue. Recently activated lymphocytes are initially retained in the secondary lymphoid organs due to upregulation of CD69, which specifically inhibits S1P-1 receptor (Shiow et al., 2006). Activated lymphocytes eventually downregulate surface CD69 expression, which allows them to

respond to the S1P gradient and exit the secondary lymphoid tissue. FTY720 potently downregulates the expression of S1P-1 receptors, preventing them exiting from secondary lymphoid organs in response to the S1P gradient (Matloubian et al., 2004). FTY720 was demonstrated to be effective in almost completely preventing the development of MBP-induced EAE in Lewis rats (Fujino et al., 2003). Additional studies went on to demonstrate that FTY720 is effective in limiting the development of PLP-induced EAE in SJL mice (Webb et al., 2004). The most likely explanation for the effectiveness of this treatment is the inhibition of T-cell infiltration into the CNS by sequestration of T cells in secondary lymphoid tissue (Kataoka et al., 2005), but may also have an effect on promoting myelin repair via its effects on glial cells (Balatoni et al., 2007; Foster et al., 2007, 2009). These results demonstrate the usefulness of the EAE models in assessing therapeutic targets, and additionally allow for the identification of an additional unexplored mechanism by which a specific therapy can have an effect, such as the pluripotent effects of FTY720 on lymphocytes as well as resident CNS cells.

Antigen-specific immunomodulation

Soon after it became clear that EAE was a disease mediated by proinflammatory CD4+ T cells, experimental treatment strategies largely focused on global suppression of this response. However, the ultimate goal in the immunomodulation of MS would be to eliminate the immune response targeting CNS autoantigens while keeping the larger immune response to pathogenic insults intact. In this way EAE has been extremely useful in developing therapies that target an antigen-specific response. The administration of soluble myelin-derived peptides or proteins, whether mucosally (Bitar and Whitacre, 1988; Higgins and Weiner, 1988; Whitacre et al., 1991; Miller et al., 1992; Chen et al., 1994), intraperitoneally (Gaur et al., 1992), or intravenously administered (Critchfield et al., 1994; Racke et al., 1996), has been shown to be effective in preventing or reversing established EAE. In some mouse strains the administration of high-dose soluble peptide intravenously leads to anaphylaxis (Smith et al., 2005). Additionally, oral tolerance mediated by soluble peptide administration has been less successful when treatment is initiated after established EAE (Meyer et al., 1996; Bai et al., 1997; Kennedy et al., 1997; Benson et al., 1999). However, IL-10 administration along with mucosally administered peptide can ameliorate ongoing established EAE (Slavin et al., 2001). Given these potential caveats of oral tolerance induction in EAE, the failure of oral bovine myelin administration in a large multicenter trial of MS patients in preventing disease relapse (Weiner, 2004) indicates that this approach may not be ideal for antigen-specific tolerance therapy.

The use of intravenous administration of myelin antigen-coupled cells has yielded more promising results in EAE. For example, intravenous administration of syngeneic leukocytes that have been coupled to myelin antigen via ethylene carbodiimide (ECDI) can completely inhibit the development of EAE as well as ameliorate ongoing EAE (Miller et al., 1979; Sriram et al., 1983). This method does not carry the same risk of anaphylaxis as with soluble peptide administration (Turley and Miller, 2007), and early results in clinical trials involving the administration of antigen-coupled peripheral blood mononuclear cells (PBMCs) indicate that it is safe and well tolerated in human subjects (Lutterotti et al., 2013). More recently, intravenous administration of ECDI antigen-coupled polystyrene nanoparticles has been used to target antigen to natural apoptotic debris uptake pathways with comparable clinical efficacy in preventing or treating EAE to ECDI-coupled syngeneic leukocyte administration (Getts et al., 2012).

Glatiramer acetate

GA (Copaxone, Teva Pharmaceuticals) is an FDA-approved immunomodulatory therapy for the treatment of MS. GA is a random copolymer of four amino acids (alanine, lysine,

glutamate, and tyrosine) in a specific ratio of 4.2:3.4:1.4:1 respectively, with an average molecular weight of 7.5 kDa, within a range of 40–90 amino acids in length (Dhib-Jalbut, 2003). It was originally designed as a synthetic MBP mimic for use in the induction of EAE (Teitelbaum et al., 1974). However GA (known as copolymer-1 at the time of publication) failed to induce EAE, and when administered in the immunizing MBP/CFA emulsion, GA suppressed the incidence of EAE (Teitelbaum et al., 1971). GA has shown efficacy in the treatment of RRMS and limits disease relapse by 30% (Johnson et al., 1995). GA is thought to modulate the immune system in many ways, such as induction of APC promotion of Th2 responses, cross-reactivity with MBP-associated peptides as an altered peptide ligand, and deviation of immune responses, also known as “bystander suppression” (Dhib-Jalbut, 2003). Interestingly, GA is also effective in ameliorating MOG peptide (Ben-Nun et al., 1996) and PLP peptide (Teitelbaum et al., 1996)-induced EAE when administered in the immunizing emulsion, suggesting that its immunomodulatory effect may stem from a mechanism separate from the driving of MBP-specific cross-reactive Th1 cells to a Th2 phenotype, perhaps by interfering with the binding of encephalitogenic peptide to MHC (Teitelbaum et al., 1996). In fact, GA was shown to be neuroprotective in EAE through the modulation of neurotropic factors produced directly by CNS-resident cells and infiltrating T cells (Aharoni et al., 2005).

It has also been reported that GA therapy during the chronic phase of EAE leads to increased proliferation, differentiation, and survival of oligodendrocyte precursor cells (Aharoni et al., 2008). Adoptive transfer of GA-specific T cells alone does not confer full protection afforded by administration of GA, suggesting that the effect of GA is multifaceted and may affect multiple cell types, including APCs such as B cells, macrophages, and dendritic cells. The successful development of GA therapy from studies in EAE demonstrates the power of this model in evaluating potential therapies.

Modulating cytokines and T-effector cell fates

Studies in the EAE model have yielded an extensive understanding of the role of T-effector cell differentiation, but have also led to an overreliance on paradigms that were later shown not to be entirely accurate, such as the Th1/Th2 paradigm. For example, TNF-α is a prototypic Th1-associated proinflammatory molecule that is highly elevated in the CNS of mice undergoing EAE (Merrill et al., 1992). Treatment of mice with anti-TNF-α antibodies ameliorated clinical symptoms of EAE (Ruddle et al., 1990; Selmaj et al., 1991). Yet mice genetically deficient in TNF-α remained susceptible to EAE induction, albeit with a delayed onset of disease (Frei et al., 1997; Riminton et al., 1998). Additionally, exogenous treatment of EAE with recombinant TNF-α in genetically deficient animals actually decreased the severity of the disease (Liu et al., 1998; Riminton et al., 1998). It is not surprising then, given these discordant results, that anti-TNF-α therapy using a recombinant TNF-receptor fusion protein (lenercept) failed to ameliorate MS, and in some patients may have even exacerbated symptoms of MS patients by increasing the frequency of relapses (Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group, 1999). In fact, a single nucleotide polymorphism in the TNFR1 gene that was found to be associated with MS in a genome-wide study results in the increased expression of a mutant soluble TNFR1 that antagonizes the action of TNF (Gregory et al., 2012), intriguingly bolstering the evidence that TNF antagonism exacerbates MS.

Similarly, in a large placebo-controlled phase II clinical trial, targeting the polarization of Th1/Th17 responses with monoclonal antibody to IL-12p40 did not substantially affect disease progression (Segal et al., 2008). This is in contrast to what was observed in EAE, where administration of anti-IL-12p40 or anti-IL-23p19 monoclonal antibodies had a substantial effect in preventing and ameliorating ongoing EAE (Chen et al., 2006). Perhaps the failure of this type of therapy is due to the fact that much of the autoimmune T-cell

response that persists in MS patients is likely unaffected by therapies that interfere with early T-cell polarization events.

Although targeting single cytokines in the Th1/17 pathway has been largely clinically unsuccessful in the treatment of MS thus far, administration of the type I interferon, IFN-β, has long been used clinically in the treatment of MS (Ebers et al., 1998). Like all currently approved therapies for the treatment of MS, IFN-β can successfully ameliorate the symptoms, progression, and development of EAE (Yu et al., 1996). Recent evidence in the EAE model suggests that IFN-β therapy may have an effect in limiting both IFN-γ and IL-17 responses *in vivo* but is only effective in ameliorating EAE disease initiated by transfer of Th1 cells, but not Th17 cells (Axtell et al., 2010). Furthermore, in MS, higher concentrations of serum IL-17 correlate with non-responsiveness to IFN-β therapy (Axtell et al., 2010).

Lymphocyte-depleting therapies

Alemtuzumab (trade name: Campath, Genzyme Corp.) is a monoclonal antibody against CD52, an antigen present on the majority of circulating lymphocytes. Treatment with alemtuzumab causes a profound lymphopenia in the blood that is long-lasting. Early reports from phase II clinical trials indicate that CD52 is effective in reducing relapse and lesion burden by magnetic resonance imaging (MRI) in RRMS patients (Coles et al., 2008). However, in earlier open-label studies, it was observed that this treatment immediately but transiently increased MS symptoms, which correlated with a significant increase in circulating proinflammatory cytokines (Moreau et al., 1996; Coles et al., 1999). Although anti-CD52 monoclonal antibody therapy has not been examined in EAE because the expression pattern of CD52 differs between mouse and human, lymphocyte-depleting therapies have been examined previously such as anti-CD4 monoclonal antibody-depleting therapies, which were shown to be effective in ameliorating EAE (Waldor et al., 1985). Some have put forth the argument that the failure of anti-CD4 monoclonal antibody therapy in ameliorating MS (van Oosten et al., 1997) highlights, among other things, the significant difference between murine EAE and human MS, and the validity of assessing therapeutic efficacy in EAE as a precursor to studies in human MS (Sriram and Steiner, 2005). However the failure of anti-CD4 monoclonal antibody therapy is likely due to the fact that it ineffectively depleted IFN-γ-secreting Th1 effector CD4+ T cells (Rep et al., 1997).

Immunoablative whole-body irradiation followed by syngeneic bone marrow transplantation resulted in the prevention of EAE symptoms and tolerance to repeated immunization of inducing antigen (Karussis et al., 1992). Similarly, since 1995 over 400MS patients have undergone autologous bone marrow transplantation following immunoablative therapy in several small noncontrolled studies, reviewed by Mancardi and Saccardi (2008), with significant efficacy in preventing relapse and disease progression. This therapy is also effective in ameliorating progression of relapsing EAE in SJL/J mice (Burt et al., 1998). The success of lymphocyte-depleting therapies in ameliorating MS significantly bolsters the idea that specifically targeting the autoimmune reaction for therapy development, as is often done in EAE, is a particularly important therapeutic avenue that needs to continue to be explored.

B-cell depletion therapy

Although EAE is clearly a CD4+ T-cell-mediated disease, EAE can be exacerbated by demyelinating antibodies which augment the disease when induced with MOG. Rituximab is a chimeric anti-CD20 antibody that was originally developed for the treatment of non-Hodgkin's B-cell lymphoma. Rituximab effectively depletes B cells in most stages of development, excluding early development (pro-B cells) and late terminally differentiated plasma cells, as both of these cell populations lack expression of surface CD20. Although early studies in EAE had suggested that B cells were dispensable for EAE initiation with

peptide in CFA adjuvant (Wolf et al., 1996; Dittel et al., 2000), it was clear that, in at least some models, induction of EAE with properly glycosylated recombinant whole protein was dependent on B cells, suggesting that B cells may have a role in antigen processing and presentation during the initiation of disease (Lyons et al., 1999). Given the relative dearth of studies that would indicate that B cells were important in the pathogenesis of EAE, B-cell depletion therapy in MS was not given high priority. However, there is clearly a B-cell response associated with MS, as over 90% of RRMS patients have oligoclonal banding in the CSF.

Rituximab treatment showed efficacy in reducing inflammatory brain lesions and clinical relapses in RRMS compared to placebo (Hauser et al., 2008) and also showed some efficacy in patients with progressive MS who had active gadolinium-enhancing lesions (Hawker et al., 2009). More recently, ocrelizumab (Roche & Biogen Idec), a fully humanized anti-CD20 monoclonal antibody, was also demonstrated to have similar efficacy in reducing Gad +lesions by MRI and limiting relapses in RRMS (Kappos et al., 2011). Although this class of B-cell-depleting drugs was not developed in an animal model of MS prior to assessment in MS patients, recent evidence suggests that this type of therapy is effective in ameliorating the symptoms of EAE when initiated during disease onset (Matsushita et al., 2008; Weber et al., 2010). Because of the rapid kinetics of disease amelioration and the lack of a detectable reduction in circulating immunoglobulin in MS patients treated with rituximab (Hauser et al., 2008), it seems unlikely that the efficacy of B-cell depletion therapy is due to effects on autoantibody production. More likely the function of B cells in MS is through antigen presentation, proinflammatory cytokine production, or lymphoneogenesis. Recent evidence suggests that B-cell depletion in EAE at disease onset is effective because B cells are a major source of the proinflammatory cytokine IL-6, which can help to drive encephalitogenic Th17 responses (Barr et al., 2012). Indeed, Th17 antigen-specific responses following B-cell depletion therapy are typically reduced in EAE (Matsushita et al., 2008; Weber et al., 2010; Monson et al., 2011; Barr et al., 2012). Continuing studies in EAE will allow further understanding of the role of B cells in MS and may allow for the development of more specifically targeted therapies.

Quinoline carboxamines

Laquinimod is an orally active quinoline carboxamine, a derivative of linomide (Roquinimex) that is currently being assessed in phase III clinical trials for the treatment of RRMS. Linomide originally was shown to be efficacious in inhibiting EAE when administered during the priming phase and in ameliorating disease symptoms when administered during the acute phase (Karussis et al., 1993a, b). These early studies prompted the assessment of linomide in the treatment of MS; however the drug was found to have an unacceptable level of cardiopulmonary toxicity during clinical trials (Noseworthy et al., 2000). These unforeseen side-effects of linomide administration in human patients highlight the limitations of the use of EAE in assessing potential toxicity in humans. Clearly additional safety and toxicity studies must be undertaken after a disease-modifying therapy has been shown to be efficacious in the EAE model, but large-scale controlled trials are currently the best was to assess these potential effects directly. These results do not however undermine the utility of the EAE model in identifying disease-modifying reagents, as, despite linomide's toxicity, it was shown to be efficacious in the attenuation of disease relapses.

Given the clinical efficacy of linomide in ameliorating MS, the second-generation quinoline carboximide, laquinimod, was assessed in EAE, with similar but more potent efficacy in relapsing EAE (Brunmark et al., 2002). Similarly, it was shown to be effective in ameliorating EAE in the Lewis rat model of EAE (Yang et al., 2004). Additional toxicity studies were carried out in canines and laquinimod was shown to have less toxicity than

linomide (Jonsson et al., 2004). Although the mechanism by which laquinimod is effective in ameliorating EAE and MS was unclear until recently, several different pathways seem to be affected. For example, laquinimod's effect may be due to interference between the proinflammatory molecule S100 and its receptor RAGE (Bjork et al., 2009) and it has also been shown to down-regulate leukocyte VLA-4 adhesiveness and IL-17 secretion (Wegner et al., 2010). Several recent reports suggest that laquinimod may induce type II anti-inflammatory monocytes that are protective in EAE (Schulze-Topphoff et al., 2012; Thone et al., 2012) or at least prevent the entrance of proinflammatory monocytes into the CNS (Mishra et al., 2012).

Dimethyl fumarate

Dimethyl fumarate (DMF: BG-12, Biogen Idec) is the second orally available disease-modifying therapy that is expected to be approved by the US FDA, based on the recent completion of the phase III DEFINE clinical trial in RRMS, with demonstrated efficacy in reducing relapse rate and Gad+lesions by MRI (Gold et al., 2012). This therapy was originally approved for the treatment of psoriasis. While the mechanism of action is unclear, DMF can modulate Th1 cells to reduce the secretion of IFN-γ and increase IL-10 production (Ockenfels et al., 1998). More recently, DMF was found to potently suppress allo-specific T-cell responses in human PBMC mixed leukocyte reactions, as well as inhibiting TNF-α, IL-12, and IFN-γ from activated human PBMCs (Lehmann et al., 2007). Although the use of DMF in RRMS patients did not grow out originally from its efficacy in EAE, the compound was concurrently tested and found to be effective in attenuating EAE disease severity as well as macrophage infiltration into the CNS (Schilling et al., 2006).

Daclizumab—Daclizumab is a humanized monoclonal antibody that targets human IL-2Rα chain (CD25) and is currently being assessed in clinical trials for its efficacy in the treatment of patients with RRMS. In mouse models of EAE anti-CD25 monoclonal antibody is typically used to functionally inactivate or deplete Treg cells (Kohm et al., 2006). In general EAE progression and severity are either unaffected or exacerbated with anti-CD25 treatment depending on optimal or suboptimal immunization (Kohm et al., 2006). In contrast, daclizumab was shown to improve clinical outcomes in RRMS patients who failed IFN-β therapy (Bielekova et al., 2004, 2009; Rose et al., 2007). It has been demonstrated that daclizumab expands a population of immunoregulatory $CD56^{bright}$ NK cells which act to kill activated T cells (Bielekova et al., 2006). As of yet, there are no reports examining this effect in EAE, most likely because an analogous murine population of immunoregulatory NK cells (CXCR3+CD27+) has only recently been identified (Marquardt et al., 2010). This therapy is one of the only examples in which the clinical efficacy of the therapy in humans is not observed in EAE.

CONCLUSIONS

EAE is an animal model of autoimmune-mediated inflammatory demyelination that resembles the pathology and symptoms of MS in many ways, but also differs significantly in many aspects of the disease. Much of our understanding regarding the autoimmune and inflammatory process in general comes directly from this model. The successful development and examination of therapies for MS clearly demonstrate the utility of EAE as a model for MS.

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