Chapter 21

Animal Models of Demyelination of the Central Nervous System

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Experimental Autoimmune Encephalomyelitis

Experimental autoimmune encephalomyelitis (EAE) is possibly the best animal model for studying autoimmune diseases and in particular demyelinating diseases of the central nervous system (CNS) such as multiple sclerosis (MS). Since the classic studies of Rivers et al. [1] in monkeys immunized with CNS homogenate, EAE has been an invaluable tool for dissecting mechanisms of the immune response against self-antigens within the CNS, as well for testing new therapies for the treatment of autoimmune diseases. An autoimmune response leading to EAE in susceptible species can be obtained by active immunization with CNS proteins or by passive transfer of T lymphocytes reactive against myelin antigens to syngeneic recipients. The role of T lymphocytes in EAE was first demonstrated by Paterson who succeeded in transferring disease by means of T cells from immunized animals [2]. Since then, many researchers have attempted to characterize the role of T cells in EAE. Over the years it became clear that activated $CD4^+$ T cells mediate EAE upon recognition of the target antigen bound to class II molecules of the major histocompatibility complex (MHC) [3]. Encephalitogenic T cells can be retrieved from the blood of immunized as well as naive animals, supporting the concept that autoaggressive lymphocytes are part of the natural immune repertoire [4, 5].

Virtually all mammalian species can be susceptible to EAE as long as they are properly immunized; several species and strains have been utilized including mice, rats and guinea pigs (Table 1). The clinical, pathological and immunological picture of autoimmune models of demyelination depends upon the mode of sensitization, the nature of the immunogen, and the genetic background of each species and strain.

Modes of sensitization include the route of immunization, primarily subcutaneously, and the use of immunogens emulsified with an equal volume of complete Freund's adjuvant containing *Mycobacterium tuberculosis* to create an antigen depot. Boosts with *Bordetella pertussis* are often used to help open the blood-brain barrier (BBB).

Whole myelin homogenate as well as distinct myelin proteins, including myelin

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Table 1. Models of experimental autoimmune encephalomyelitis

Species	MHC	Immunogen	Encephalitogenic epitope (amino acid sequence)	TCR	Course	Reference
Mice	**Inbred**					
Biozzi/ABH	dq1	MH	–	–	RR	Baker et al. [27]
		MOG	1-22/43-47/134-148	–	CR	Amor et al. [28]
SJL	H-2^S	MH	–	–	AM	Brown [15]
		MBP	89-101	Vβ17	AM	Zamvil [3]
		PLP	139-151	–	RR	Tuohy [95]
		PLP	40-70/100-119/178-209	–	RR	Greer [30]
		MOG	92-106	–	CR	Amor [28]
PL/J	H-2^U	MH	–	–	AM	Zamvil [3]
		MBP	Acl-9	Vβ8.2/Vα2	CR	Zamvil [3]
		MBP	35-47	–	CR	Zamvil [3]
		MOG	35-55	–	CR	Kerlero de Rosbo [97]
C57BL/6	H-2^B	MOG	35-55	Vβ8	CP	Mendel [96]
C3H.SW	H-2^K	MOG	35-55	Vβ8	CP	Mendel [96]
B.10.PL	H-2^U	MH	Acl-9	Vβ8.2/Vα2	AM	Zamvil [3]
		MBP	1-37	–	CR	Zamvil [3]
Rats	**Inbred**					
Lewis	RTI.B^1	MH	–	–	AM	Hoffman et al. [98]
		MBP	68-88	Vβ8.2/Vα2	AM	Burns et al. [18]
		MOG	35-55	–	RR	Linington et al. [8]
DA	av1	MH	–	–	CR	Lorentzen et al. [29]
Guinea Pig	**Inbred**					
13	–	MH	–	–	AM	Freud [99]
Primates	**Outbred**					
Macaque	–	MH	Heterogeneous	–	AM	Rivers [1]
Marmoset	–	MH	Heterogeneous	Diverse	CR	Massacesi et al. [78]

MHC, major histocompatibility complex; *MH*, myelin homogenate; *MOG*, myelin oligodendrocyte glycoprotein; *MBP*, myelin basic protein; *PLP*, proteolipid protein; *RR*, relapsing-remitting; *CR*, chronic relapsing; *AM*, acute monophasic; *CP*, chronic progressing; –, Not determined.

basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), and proteolipid protein (PLP), have been used to induce EAE in different species. The role of MBP, an abundant hydrophilic myelin protein, was first characterized by Ben-Nun and colleagues who successfully induced EAE by transferring MBP-specific T cell lines to naive Lewis rats [6]. Passive transfer studies also elucidated the role of other myelin antigens, including PLP [7] and MOG [8]. MOG, a minor glycoprotein exposed on the surface of the myelin sheath, is the target of both humoral and cellular immune responses. The importance of the humoral response to MOG was first demonstrated by Linington et al. who showed the presence of sharp demyelination following the injection of anti-MOG auto-antibodies into animals with T-cell-mediated EAE [9]. Other encephalitogenic proteins include a lipid-bound form of MBP [10] as well as non-myelin auto-antigens such as the astrocyte-derived calcium binding protein S100β [11].

Depending upon the species, the antigen and the mode of sensitization, the course of EAE can be monophasic acute, chronic relapsing, or even primarily progressive, mimicking human MS. The classic picture of acute EAE is characterized by perivascular inflammation mainly represented by $CD4^+$ and $CD8^+$ T lymphocytes and macrophages within the cerebral white matter. Nevertheless, manipulation of the above-mentioned factors can lead to a wide spectrum of neuropathological patterns including demyelination, remyelination, gliosis, loss of axons and, in certain species, also necrosis [12].

EAE in Inbred Species

Olitsky and Yager were the first to establish EAE in mice [13]. Since then, thousands of scientists have used inbred rodents as the most suitable species for EAE studies. EAE has been successfully induced in guinea pigs [14] as well as in several strains of rats and mice. Some mice strains such as SJL/J develop a relapsing-remitting disease following active immunization with spinal cord homogenate [15]. Pathological investigations show the presence of mononuclear cell infiltrates within the CNS and demyelination. A similar disease can also be obtained by the adoptive transfer of MBP-specific T lymphocytes [16]. In this $H\text{-}2^S$ strain, a few epitopes of MBP concentrated within the sequence of amino acids 89-101 are recognized by encephalitogenic T cells which preferentially use the T cell receptor (TCR) $V\beta17$ segment [17]. A more restricted response to encephalitogenic determinants of MBP has been reported for EAE-susceptible $H\text{-}2^U$ mice such as PL/J and B.10.PL. Both encephalitogenic and non-encephalitogenic T cells recognize the N-terminal peptide Ac1-9 utilizing the same TCR $V\beta8.2/V\alpha2$ (or $V\alpha4$) gene combination [3]. Interestingly, the same TCR gene segments are characteristic of the T cell response to MBP in Lewis rats [18]. In Lewis rats, EAE induced either by active immunization or by passive transfer of T cells is an acute monophasic disease mostly characterized by inflammation [19]. In this species, EAE is mediated by $CD4^+$ encephalitogenic T lymphocytes that are specific for the peptide MBP 68-88 in the context of the class II MHC molecule $RT1.B^l$ [20] and that rapidly home to and persist at the site of inflammation [21]. The dominant use of a restricted TCR repertoire has been successfully exploited in designing immunospecific therapies by means of anti-$V\beta$ monoclonal antibodies [22] and active vaccination with epitopes of the encephalitogenic TCR molecule [23]. Nevertheless, the presence of an epitope dominance, as well as limited TCR usage within the T cell response to MBP, seems to be confined to the early phases of EAE and remains controversial in humans. Later stages of EAE are characterized by a more diverse recognition of previously cryptic determinants inside the MBP molecule (intra-molecular spreading) and within other CNS antigens (inter-molecular spreading) [24]. The appearance of a diverse T cell repertoire is further confirmed by the presence, in the spinal cord of rats with EAE, of a heterogeneous $V\beta$ population during the recovery phase of disease [25]. This may result from apoptosis of encephalitogenic T cell clones [26] followed by secondary recruitment of activated cells from the recirculating T cell pool specific for minor antigens.

Recently, a chronic relapsing form of EAE has been successfully induced in Biozzi (AB/H) mice by immunization with spinal cord homogenate [27] or MOG [28]. The full spectrum of pathological lesions seen in MS, including sharp demyelination and remyelination, is seen in this species. Another useful model, recently described in DA rats, is characterized by a chronic relapsing course, inflammation and demyelination [29].

In contrast to the rather restricted response to MBP, a diverse recognition of determinants within other myelin antigens such as PLP [30] and MOG [28] seems to occur in most rodents. However as a general rule for all inbred species, strains of different haplotype appear to react with different epitopes of myelin antigens.

The presence of encephalitogenic T cells reacting against self-antigens in naive animals is of remarkable conceptual importance and supports the fact that autoaggressive T cells escaping thymic deletion are maintained within the normal circulating T lymphocyte pool [4, 5]. A possible explanation for self-reactive T cells escaping negative selection has been recently clarified in a MBP-/- transgenic model in which endogenous MBP inactivated high avidity clones reactive against the immunodominant epitope and made that determinant appear cryptic [31].

Thus, other factors are also necessary to cause autoimmune disease. The genetic background is a major factor conferring susceptibility to EAE; a number of murine loci have already been identified [32]. Both MHC and non-MHC genes have been reported to control the development and severity of EAE [33]. The role of environmental factors has been elegantly elucidated by Goverman and colleagues [34] who created transgenic mice expressing an MBP-specific TCR; these mice spontaneously developed EAE only when challenged with microbial stimuli. In a similar model, the complete cohort of anti-MBP TCR transgenic mice, deficient for mature T and B cells, developed spontaneous EAE, suggesting that other cells may have a protective role counteracting encephalitogenic cells [35]. A protective or regulatory role has been claimed for almost all cells involved in the immune response including $CD4^+$ [36], $CD8^+$ [37], and $CD4^-CD8^-$ [38] T cells, macrophages [39], B cells [40], $\gamma\delta$ T cells [41] and NK cells [42].

The environment exerts a major effect on EAE, leading to the activation of potentially autoaggressive T cells which consequently home to the brain and induce disease [43]. Activation state is the necessary prerequisite for T cells to migrate through the BBB irrespective of their antigen specificity. Activation of myelin-specific T lymphocytes in the peripheral compartment can occur through a mechanism of molecular mimicry [44] and by stimulation with microbial superantigens [45]. Other theoretical possibilities such as activation of T cells carrying two sets of receptors, one specific for a foreign protein and another for a self-antigen, have never been demonstrated to play a role in autoimmunity [46]. Migration through the BBB involves adhesion molecules on both T cells (LFA-1 and VLA-4) and endothelial cells (ICAM-1 and VCAM-1) [47]. Following migration through the BBB, neuroantigen-specific $CD4^+$ T cells are reactivated in situ by fragments of myelin antigens presented in the framework of class II MHC molecules on the surface of local antigen-presenting cells including macrophages, microglia and, although less efficient, astrocytes [48]. These events are associated

with the release of proinflammatory cytokines leading to the upregulation of MHC molecules on a variety of resident, antigen-presenting cells [49]. The kinetics of cytokines along the EAE clinical course suggests the role of T helper 1 (Th1) cytokines such as tumor necrosis factor (TNF)-α, TNF-β, interleukin (IL)-12, and interferon (IFN)-γ before and at the peak of disease. The recovery phase correlates with Th2 cytokines such as transforming growth factor (TGF)-β, IL-10 and possibly IL-4 [50]. The onset of overt inflammation also maintains endothelial activation and leads to a second wave of inflammatory recruitment, including T cells and macrophages that damage tissue by means of TNF-α [51], oxygen and nitrogen intermediates, perforin and complement [52] and demyelinating antibodies [9].

Thus far, no consistent evidence differentiates between activated MBP-specific T lymphocytes with no encephalitogenic capabilities and their pathogenic counterparts, in spite of identical growing and stimulation conditions, sharing of epitope specificity, and MHC restriction. Some studies suggest that differences in encephalitogenicity correlate with a predominant Th1 cytokine profile [53], their brain homing capacity [54] and the ability to mediate a delayed-type hypersensitivity (DTH) response [55]. Moreover, encephalitogenic T cells, despite their $CD4^+$ phenotype, were cytotoxic for cells (e.g. astrocytes) presenting myelin antigens in an MHC-restricted manner [56]. On the other hand, non-encephalitogenic T cells may initiate autoimmune regulatory mechanisms through the production of IL-3 [57]. At least in Lewis rats, encephalitogenicity of MBP-specific T cells may correlate with the cytokine profile which depends on the MHC haplotype of the strain [58].

A rather simplistic picture of the EAE cytokine network suggests that TNF-α, TNF-β, IFN-γ and IL-12 (proinflammatory cytokines) have a disease-promoting role while TGF-β, IL-10 and possibly IL-4 (anti-inflammatory cytokines) protect from disease. Although a detailed analysis of the current literature on this topic is beyond the scope of this chapter, an enormous amount of data states that the real picture is much more complicated. Several factors influence the cytokine profile of effector and regulatory cells in EAE and, therefore, the final outcome of the immune response within the target organ. These include age of the animal [59], nature of antigen-presenting cells [60, 61], local cytokine micro-environment [62], selective engagement with costimulatory molecules [63] interaction with altered forms of the immunizing antigen [64], and the route of immunization [65].

Based on the hypothesis that Th1 cytokines play a promoting effect on autoimmunity while Th2 cytokines may have a protective role, immune deviation toward a Th2 profile has been exploited for successfully treating EAE by administration of anti-inflammatory cytokines [66], altered peptide ligands [64], monoclonal antibodies (MAb) affecting B7/CD28 interactions [63] or anti-inflammatory cytokines [67, 68], and by induction of oral tolerance [65]. Despite the success of most experimental treatments targeting Th1 cytokines, the Th1 versus Th2 dichotomy underscores the complexity of interactions that lead to reciprocal cross-regulation of Th1/Th2 responses. This has been dramatically elucidated by

the severe aggravation of EAE in primates due to an enhanced Th2 response occurring after discontinuation of treatment for immune deviation [69]. Moreover, conflicting results arise from the utilization of genetically manipulated mice either lacking or over-expressing cytokines, as is the case in a number of TNF-α studies which demonstrated a different clinical phenotype depending on the experimental conditions. For example, over-expression of TNF-α in transgenic mice leads to spontaneous inflammation and demyelination within the CNS [70], while TNF-α deficient knock-out mice can still develop EAE, thus challenging the role of this cytokine in EAE pathogenesis [71]. Surprisingly, in a recent study, MOG-immunized, TNF-deficient mice developed severe EAE but were remarkably ameliorated by the administration of TNF-α, possibly supporting a protective role for this cytokine [72]. Targeting cytokine genes has helped to elucidate the role of other cytokines such as INF-γ [73], IL-4 and IL-10 [74], nitric oxide [75], and also of Fas/Fas-ligand and perforin pathways [76]. Nevertheless, it must be kept in mind that genetic manipulation results in experimental conditions that only partially represent the in vivo situation and that likely underscore the redundancy of the cytokine system.

Overall, the deep knowledge of the immunogenetics of inbred species and the possibility of successfully manipulating these animals, together with their accessibility and moderate costs, make these species the first choice for studies on autoimmune diseases of the CNS.

EAE in Outbred Species

EAE in non-human primates represented the first experimental model for demyelinating diseases of the CNS [1]. The recent advances in primate housing and handling techniques, knowledge of primate anatomy, immunology and genetics, and the compatibility with most human reagents and diagnostic techniques have sparked wide interest in EAE in these species. A unique advantage of monkeys arises from their outbred condition that closely resembles the human status. Moreover, the transfer of immunocompetent cells in outbred primates is allowed by the possibility of crossing the trans-species barrier among closely related species [77] and by the natural bone marrow chimerism in some others [5]. Therefore, in primates it is possible to elucidate the role of pathogenic cells by means of passive transfer experiments in a polymorphic setting. Recently, EAE has been induced in the common marmoset *Callithrix jacchus*, a unique primate species whose offspring develop in utero as genetically distinct twins or triplets sharing bone marrow-derived elements through a common placental circulation [78]. It has been recently demonstrated that *C. jacchus* TCR genes are extensively conserved [79] and that class II MHC region genes, despite a relatively low polymorphism, encode the evolutionary equivalents of the HLA-DR and -DQ molecules [80]. A fully demyelinating form of EAE has been induced by active immunization with whole myelin [78], MOG or MBP followed by administration of MOG-specific antibodies [81]. Passive transfer experiments have demonstrated that encephalitogenic MBP-specific T cells are part of the normal marmoset

repertoire [5]. As in humans, MBP-reactive T cells recognize different determinants by means of a diverse TCR repertoire (A. Uccelli, unpublished results). EAE induced with whole myelin or MOG is characterized pathologically by perivascular inflammation with conspicuous primary demyelination whose topography correlates with magnetic resonance imaging (MRI) abnormalities [82]. On the contrary, immunization with MBP or passive transfer of MBP-reactive T cells leads to mild inflammation and no demyelination ([5] and G.L. Mancardi and A. Uccelli, unpublished results). A complex role for cytokines, possibly released by activated T lymphocytes and macrophages during the immune response, is suggested by the high expression of CD40 and CD40-ligands in marmoset active lesions [83]. The role of Th1 cytokines has been demonstrated by the prevention of disease following treatment with the cAMP-specific type IV phosphodiesterase inhibitor Rolipram [84]. On the other hand, the ambiguous role of Th2 cytokines was highlighted by the enhancement of EAE occurring after discontinuation of a MOG-based tolerization treatment due to an enhanced proliferative and antibody response to the antigen. Hence, it is likely that Th2-like T cells play a different role, protecting or favoring autoimmunity, under different conditions [69].

EAE has also been induced in macaques by immunization with myelin or MBP emulsified in complete adjuvant [85]. The EAE course is primarily hyperacute or acute, often with a lethal outcome, and is characterized by intense inflammation associated with hemorrhages and necrosis resembling acute disseminated encephalomyelitis [86]. The association of EAE-susceptibility with a class II MHC allele [87], the presence of myelin-reactive T cells correlating with the course of EAE [86], the beneficial effect of anti-CD4 antibodies on EAE outcome [88], and the possibility of inducing a mild form of EAE by adoptive transfer of MBP-specific T cells from unprimed animals [89] all provide strong evidence that T cells play a central role in this model.

At the moment, the major advantage of a non-human primate EAE model for human MS resides in the molecular and functional organization of the primate immune system, leading to the possibility of evaluating the safety and efficacy of biological molecules as therapy for MS.

Virus-Induced Demyelinating Diseases

CNS demyelination spontaneously occurs following infection with neurotropic viruses such as Theiler's virus [90]. Theiler's murine encephalomyelitis virus (TMEV), a natural mouse pathogen, is a picornavirus that induces a chronic demyelinating disease with a clinical course and histopathology similar to that of chronic-progressive MS. Viral persistence within the CNS is required for the immune system to mount a cellular and humoral response leading to demyelination [91]. The host response may trigger both protective and pathogenic immune responses which are the result of a balance between persistent viral infection and immune injury mediated by $CD4^+$ or $CD8^+$ T cells and antibodies [92]. In susceptible animals the lack of virus-specific cytotoxicity has been postulated to lead to demyelination. On the other hand, resistant strains clear the infection following

acute encephalomyelitis, possibly due to the ability to generate an effective class I-restricted T-cell response [93]. As in EAE, epitope spreading to endogenous myelin determinants has been shown to play a key role in the chronic-progressive course of disease. Demyelination in TMEV-infected mice is initiated by a mononuclear inflammatory response mediated by virus-specific $CD4^+$ T cells targeting viruses, which chronically persists in the CNS. Following myelin destruction, activation of $CD4^+$ T cells specific for multiple myelin epitopes occurs, leading to disease progression [94]. Other models of virus-mediated demyelinating disease of the CNS are obtained by infections with the mouse hepatitis virus strain JHM (MHV-JHM) and corona virus.

Although almost the complete spectrum of MS-like lesions can be observed in virus models of demyelination, the mechanisms underlying the pathogenesis of immune response within the CNS are extremely complex and depend on the mutual interaction between virus and host, thus making it difficult to dissect the role of each factor in the pathogenesis of demyelinating diseases of the CNS.

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