

Chapter 3

Animal Models for the Study of Neuroimmunological Disease

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Abstract The development and use of numerous animal models of human autoimmune diseases have provided important advances in our understanding of pathogenic mechanisms of disease and provided robust and reliable models to test novel therapeutic strategies. However, few preclinical studies of therapeutic treatments have demonstrated efficacy in the clinic, possibly because of the biological differences between humans and other animals. Although animal models of human disease are imperfect, it is important to understand the differences between the human disease and its animal models and to design experimental studies using animal models appropriately for the questions being asked. This review provides an overview of the currently used animal models of three human neuroimmunological diseases, multiple sclerosis, Guillain-Barré syndrome, and myasthenia gravis, as well as the advantages and disadvantages of each model and how they correlate or differ from their human counterpart.

Keywords Multiple sclerosis • Neuritis • Myasthenia gravis • Animal models • Neuroimmunology • Virus models • Myelin

3.1 Introduction

Following decades of research, scientists have developed a large number of drugs and therapeutic agents that can be used to reduce the symptoms and severity of a number of neuroimmunological diseases such as multiple sclerosis (MS), neuritis, and myasthenia gravis. However, none of these treatment methodologies is curative, and many have a limited life span, such as interferon (IFN)- β that induces neutralizing antibodies in some MS patients [1], or cause serious side effects (progressive multifocal leucoencephalopathy) such as monoclonal anti-very late antigen (VLA)-4 antibodies (natalizumab, Tysabri®) [2] and thus cause patients to drop out of clinical trials or stop taking the treatment. In addition, although many

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studies have dissected the intricate pathways involved in the aetiology, development, and pathogenesis of diseases such as MS, we still do not have a definitive understanding of how these diseases manifest and, indeed in the case of MS, whether it is a single disease or rather a spectrum of disorders with similar characteristics. Therefore, researchers have used animal models to aid our understanding of disease pathways, immune cell functions, and pathology. An example of the importance of animal models was the demonstration that CD4 T cells specific for a myelin epitope injected into naïve animals were sufficient to induce a central nervous system (CNS) demyelinating disease with CNS lesions similar to those observed in MS [3, 4]. In addition, these models are a useful preclinical aid to developing novel therapeutic agents. Many different animal models of neuro-immunological disorders have been developed over the last few decades with some success. However, it is important to understand the advantages and limitations of each model to ensure that the correct model is being used for the purpose of the study.

In this review, we provide an overview of the currently available neuro-immunological disease animal models, describing their advantages and disadvantages as well as their relationship and correlation to the human disease they are attempting to model. Although no one animal model is completely identical with human disease, they are nevertheless very useful and important tools with which to increase our understanding of neuroimmunological disease when used correctly.

3.2 Animal Models of Multiple Sclerosis

3.2.1 *Active Experimental Autoimmune Encephalomyelitis*

MS is a human, inflammatory, demyelinating disease of the CNS, with characteristic demyelinating lesions containing immune cell infiltrate and activated CNS-resident cells located in the white matter of the brain and spinal cord; therefore, studies to investigate its pathology are difficult and often rely on autopsy tissues. In these cases, patients might have had the disease for decades and therefore the tissues are representative of disease at the end stage. Furthermore, epidemiological studies have suggested that the initiating factors for MS might be infections that occur during childhood [5]. Therefore, animal models of MS are critical for the study of susceptibility, disease initiation, and pathology during the early stages of disease. Experimental autoimmune encephalomyelitis (EAE) is an autoimmune T cell-mediated disease of the CNS mediated primarily by CD4+ T cells and a commonly used animal model for the study of MS. Disease is characterized by perivascular lesions containing inflammatory infiltrates and nerve conduction block that causes reversible hind-limb paralysis. In addition, late-stage EAE animals also develop axonal demyelination and loss, a pathological hallmark of MS, which leads

to severe permanent disability. Characterization of the immune cell infiltrate (T cells, B cells, activated macrophages, and microglia) and the pathology of perivascular demyelinating lesions have shown similarities with human MS lesions [6, 7] although in EAE the spinal cord is the target organ, whereas in MS the brain is more often targeted, especially the white matter. However, EAE is a useful model to study the inflammatory stage of MS and shares some characteristics of MS such as optic neuritis, increased susceptibility of females, perivascular lesions, axonal demyelination, and partial remyelination, as well as eventual axonal loss and flaccid-limb paralysis.

There are two commonly used models of EAE. Active EAE is induced by subcutaneously immunizing genetically susceptible animals, usually rodents, with myelin antigen in Freund's complete adjuvant-containing mineral oil and *Mycobacterium tuberculosis* strain H37RA. In some EAE low-responder mouse strains, such as C57BL/6, additional injections of pertussis toxin are required. During the induction phase, myelin-specific T cells are activated by antigen-presenting cells (APC) presenting myelin peptide fragments in the draining lymph nodes to produce T helper 1 (Th1) type cytokines, such as interferon (IFN)- γ and tumour necrosis factor (TNF)- α , which allows them to escape the lymph nodes and traffic to the CNS. The effector phase of disease involves the extravasation of activated myelin-specific T cells through the blood-brain barrier and into perivascular spaces in the spinal cord. Here, the encephalitogenic T cells encounter CNS-resident cells mediating further stimulation of pro-inflammatory cytokines and chemokines that mediate a secondary influx of other peripheral inflammatory cells including B cells and mononuclear phagocytes. A study in EAE and a viral model of MS demonstrated that APCs in the CNS restimulated myelin-specific T cells to initiate epitope spreading to other myelin antigens and perpetuate disease [8]. The consequence of this pro-inflammatory cytokine milieu is the demyelination of CNS axons, thought to be mediated in part by numerous mechanisms including phagocytosis by activated mononuclear cells, destructive effects of anti-myelin antibodies, the production of free radicals, and the direct cytotoxic effects of pro-inflammatory cytokines secreted by activated CD4+ T cells and monocytes.

Initially guinea pigs and rats were the animals of choice for EAE studies. However, with the advent of transgenic and gene knockout technology, mice have become the more commonly used animal for EAE studies. Numerous mouse models of EAE have been developed, and these are differentiated by the strain of mouse used and the immunodominant myelin peptide for that particular strain. Commonly used EAE-susceptible mouse strains include SJL, B10.PL, C57BL/6, C3H, SWR, and Biozzi ABH. Depending upon the immunizing myelin antigen and mouse strain used, different forms of EAE can be induced. The most commonly used models are SJL mice immunized with proteolipid protein (PLP) 139–151 that induces a relapsing-remitting form of disease, the most common form of MS, and C57BL/6 mice immunized with myelin oligodendrocyte protein (MOG) 35–55 that initiates a chronic-progressive type of EAE. Other strains, such as Biozzi ABH mice, can be immunized with a suspension of whole Biozzi ABH spinal cord to develop a very reproducible relapsing-remitting disease [9]. The clinical symptoms

of EAE usually develop at 7–15 days post-immunization and include weight loss, loss of tail tone and gait, and eventually paralysis in either or both hind limbs. Weight loss precedes the onset of disease symptoms and thus can be used as a marker for disease onset. Daily observation of EAE symptoms and body weight is noninvasive and is therefore a great advantage to researchers, allowing the disease process to be followed, especially when studying the effects of drug treatments.

Of note, some mouse strains are resistant to EAE (A/J, C3H/HeJ, AKR, NZW, and DBA/2). In addition to the importance of environmental factors, it is thought that multiple predisposing genetic elements might be involved in susceptibility to MS (reviewed by Ebers 1994) [10]. Therefore, the backcrossing of EAE-resistant mice with EAE-susceptible strains has been useful for genetic susceptibility studies.

3.2.2 Passive Experimental Autoimmune Encephalomyelitis

The second model of EAE is passive and involves the *in vitro* restimulation of encephalitogenic T cells with the myelin peptide used to immunize the original T-cell donor animals [11]. The successful culture of these cells usually requires the addition of Th1 cytokines such as IL-12 [12]. Once cells have been sufficiently activated, they are intravenously administered to naïve animals, where they traffic to the CNS and induce disease. The CNS pathology and disease course are similar to that for active EAE. The ability of MBP-specific T cells to induce EAE in rats and mice was some of the earliest evidence to suggest that MS has an autoimmune aetiology [3, 4]. Another evidence for an autoimmune inflammatory pathogenesis includes the presence of MHC class II-restricted CD4+ T cells that recognize myelin antigens such as myelin basic protein (MBP), PLP, and MOG in MS patients as well as healthy individuals and that MHC class II genes are associated with MS susceptibility [13–15]. Because T cells are already activated when administered into naïve animals, passive EAE is a suitable model for investigating the effector phase of disease. It has some advantages over active EAE as an immunization step is not required; thus, there is no antigen reserve that continually activates naïve T cells, which likely boosts the immune system nonspecifically. Furthermore, the direct injection of effector T cells into naïve mice allows the definitive starting point of disease induction to be known, which might be useful for treatment studies, and finally encephalitogenic T cells can be directly tracked *in vivo* to study methods of extravasation into the CNS and for isolation of antigen-specific T cells.

Early studies demonstrated that activated myelin-specific Th1 CD4+ T cells secreting IFN- γ , TNF- α , and IL-2 were encephalitogenic and sufficient to induce EAE when transferred into naïve mice of a susceptible genetic background [16–18]. However, the knockout of genes encoding IFN- γ or TNF- α [19, 20] exacerbated EAE and therefore the role of Th1-induced EAE was not straightforward. IL-17 production by CNS-infiltrating T cells is important for blood-brain barrier dysfunction and lesion formation in MS patients [21, 22], and the genetic inhibition of IL-17A in mice was sufficient to partly ameliorate EAE in mice [23]. This indicated

that Th17 cells, an alternative effector T-cell subset to Th1, might be the critical effector cells in EAE. Indeed, Th17 cells cultured in the presence of IL-23 and other cytokines become highly pathogenic and can induce EAE when transferred into naïve mice [24]. Th17 cells show an enhanced efficiency at inducing EAE compared with Th1 cells [24] and therefore might require less cell manipulation in vitro for adoptive transfer studies.

3.2.3 Other Experimental Autoimmune Encephalomyelitis Models

3.2.3.1 CD8 Experimental Autoimmune Encephalomyelitis Models

Although there is a predominance of EAE papers investigating the role of myelin-specific CD4+ T cells in EAE, MS lesions have been reported to contain greater numbers of CD8+ T cells compared with CD4+ T cells [6, 25, 26]. However, the function of CD8 T cells in MS lesions is unclear, and therefore, the study of CD8+ T cells in EAE is important. A number of EAE models induced by MHC class I-specific CD8+ T cells have been reported. MBP79-87-specific CD8+ T-cell clones isolated from MBP-immunized C3H wild-type mice induced EAE with numerous neurological deficits (ataxia, spastic reflexes, spinning) as well as hind-limb paralysis when intravenously injected into C3H wild-type mice [27]. This form of EAE was very severe and all mice were moribund by day 14. Interestingly this model showed different clinical symptoms to the CD4+ T cell-mediated type of EAE, but importantly had some similarities to MS, in that perivascular lesions were predominant in the brain, compared with CD4+ EAE where perivascular lesions are located in the spinal cord.

Another study reported that MOG35-55-specific CD8+ T cell lines could also induce a severe, chronic form of EAE when adoptively transferred to naïve C57BL/6 mice [28]. In contrast to the MBP-induced CD8 T-cell model of EAE, lesions were present in both the spinal cord and brain. Differences in genetic background, availability of myelin antigens in the CNS, or induction procedure might explain the differences observed between the two models. In contrast to these early CD8 studies, more recent investigations have indicated inhibitory roles for CD8 T cells in EAE: neuroantigen-specific autoregulatory CD8+ T cells inhibited autoimmune demyelination by modulating dendritic cell functions [29] and IL-15-dependent CD8+ CD122+ T cells ameliorated EAE by reducing IL-17 production by CD4+ T cells [30].

Humanized mouse models have also been developed to study the effect of MS-related myelin epitopes presented to CD8 T cells by human HLA molecules and showed that MOG181-189 presented by HLA-A*201 to MOG-specific CD8 T cells exacerbated CD4 T cell-induced EAE [31].

Overall, these models of CD8 T cell-induced EAE are important for determining the function of CD8 T cells during CNS autoimmune disease, i.e. pathological

versus inhibitory/regulatory effects, and have an advantage over CD4 T cell-induced EAE in that the target organ is predominantly the brain, similar to that in MS.

3.2.4 Transgenic Models of Autoimmune Encephalomyelitis Models

A number of spontaneous mouse models of EAE have been developed by the transgenic expression of T-cell receptors (TCR) specific for myelin antigens (PLP, MOG, or MOG) in T cells that overcome the negative selection of autoreactive T cells in the thymus (reviewed by Croxford 2011) [32]. Therefore, these models represent a more “natural” type of disease and are useful for examining the role of autoimmune T-cell activation by environmental stimuli. However, depending on the type of study involved, for example, drug efficacy testing, the use of spontaneous EAE models is not recommended because of the wide variance in disease onset and severity.

3.2.4.1 Advantages of Autoimmune Encephalomyelitis Models

The advantages of EAE are its robust and reproducible disease course that is useful for studying the early stages of disease initiation; the activation of immune cells, especially antigen-specific T cells; as well as mechanisms in the effector phase such as the role of resident CNS cells, regulatory T cells, and other regulatory mechanisms. In addition, pathological studies at all stages of disease can provide important information regarding sites and mechanisms of demyelination, remyelination, nerve conduction block, and axonal loss. Furthermore, depending upon the models used, the different mechanisms involved in relapsing vs chronic forms of disease can be studied. The reader is encouraged to read review articles describing the specific induction protocols for the numerous EAE models that are available [11, 12].

3.2.5 Disadvantages of Autoimmune Encephalomyelitis Models

The most significant disadvantage of EAE is the location of pathology in spinal cord, unlike MS, where many lesions occur in the brain. Furthermore, although EAE is clearly an autoimmune disease, MS does not have all the hallmarks of an autoimmune disease, and evidence for a specific immunodominant myelin antigen is lacking. Although CD4+ T cells are critical for disease induction in most EAE

models, their role in MS is less clear, where many other types of immune cells such as CD8+ T cells, B cells, and monocytes probably also have important roles. For example, B cells have little or no role in many EAE models, at least at the early time points usually studied; however, their importance in MS is suggested by the beneficial effects of anti-CD20 therapy in some MS patients [33].

EAE is a very useful tool to test potential new immunomodulatory therapies. Although four approved MS treatments were studied in EAE (natalizumab, mitoxantrone, glatiramer acetate, and fingolimod), the large majority of new treatments fail when they enter MS clinical trials. An explanation for this might be that treatment studies using the EAE model do not mimic the patient disease course in the clinic. For example, the administration of drugs before the initiation of EAE disease is only useful for indicating an effect on the activation of T cells and has no real clinical significance for MS treatment, where patients have often experienced symptoms for some time before treatments are started. In addition, treatments administered before the onset of symptoms that prevent paralysis are often said to prevent demyelination. However, if these treatments are immunomodulatory, then it is difficult to differentiate between their anti-demyelination and immunosuppressive effects. Therefore, potential treatments should be administered after the onset of EAE symptoms for clinically meaningful results. In summary, although EAE is an imperfect model of MS, its correct usage by investigators is critical when studying novel therapeutic compounds.

3.2.6 Virus Models of Multiple Sclerosis

A number of etiological studies have indicated the potential role of viruses in the susceptibility, onset, and exacerbation of MS. Therefore, in addition to immunization models that are useful to investigate the early immunological pathways involved in pathogenesis, a number of virus-induced demyelinating models have been developed that allow the study of the potential viral aetiology of MS. Using these models a number of hypotheses of the mechanism of disease onset have been developed including molecular mimicry, epitope spreading, direct bystander activation, and release of cryptic epitopes.

3.2.7 Theiler's Murine Encephalomyelitis

Theiler's murine encephalomyelitis (TMEV) is a single-strand RNA virus that belongs to the cardiovirus group of the Picornaviridae and is a natural mouse pathogen. TMEV-induced demyelinating disease (TMEV-IDD) develops in susceptible strains of mice (SJL/J) upon intracranial injection of the BeAn 8386 strain of TMEV, which persistently infect microglial cell populations in the CNS [34, 35]. The persistent infection of TMEV is thought to cause demyelination

mediated by macrophage bystander destruction activated by TMEV-specific CD4+ T-cell responses that target the persistent viral infection, leading to the release of myelin antigens that can activate autoreactive PLP156-171-specific T cells that have escaped negative selection [36]. Epitope spreading to other myelin epitopes propagates the disease, which shows similar inflammatory and demyelinating pathological hallmarks as those seen in MS patients [37, 38]. The onset of a chronic progressive demyelinating disease with no recovery or remission periods occurs around day 30–35 post-infection and continues for over 100 days. TMEV-IDD is characterized by the development of a spastic hind-limb paralysis and perivascular and parenchymal lesions in the spinal cord with demyelination of white matter tracts containing mononuclear cell infiltrates.

Although epitope spreading is thought to be involved in TMEV-IDD, molecular mimicry, the mistaken recognition of a pathogenic epitope for a “self” epitope due to shared amino acid sequences, is another potential mechanism for the induction of autoimmunity. Early studies demonstrated that the immunization of viral peptides could stimulate myelin peptide-specific T-cell responses in vivo [39, 40]. However, the need for epitope processing and the role of a “live” viral infection to stimulate the host innate immune system are not addressed by short-length peptide immunization. Therefore, recombinant TMEV strains engineered to incorporate 30-mer myelin or bacterial/viral myelin mimic epitopes were used to study the potential viral induction of autoimmunity by molecular mimicry [41, 42]. Infection of SJL mice with TMEV engineered to express a PLP mimic peptide derived from *Haemophilus influenzae*, a natural mouse pathogen, with 6/13 homologous amino acids to PLP139-151, including primary TCR and MHC class II contact residues, induced a mild but rapid onset CNS disease [42]. Interestingly, the immunization of mice with the *Haemophilus influenzae* peptide in complete Freund’s adjuvant failed to induce overt disease, indicating the importance of pathogen-delivered innate immune signals for the induction of disease by molecular mimicry. Of note, viral peptide sequences in TMEV do not share any sequence homology with PLP, the immunodominant myelin peptide in TMEV-IDD in SJL mice, therefore indicating the role of the engineered *Haemophilus influenzae* peptide sequence in disease induction. These studies were expanded to include TMEV expressing 30-mer from murine hepatitis virus (MHV), which only shares 3/13 amino acids with PLP139-151, and demonstrated the importance of a proline residue at the secondary MHC class II contact point [43].

3.2.7.1 Semliki Forest Virus

Semliki Forest virus (SFV) strain A774 is a neurotropic, single-stranded RNA alphavirus of the *Togaviridae* family that induces a demyelinating disease upon intraperitoneal injection in SJL/J mice. Disease is characterized by virus-induced demyelination of the CNS. Despite the clearance of the virus by the immune system, maximal demyelinating lesions with the expression of IFN- γ and TNF- α are observed at 14 days post-infection up to 1 month in BALB/c mice, whereas both

demyelination and pro-inflammatory cytokine expression can be detected in SJL/J mice up to 1 year [44]. SFV infection induces an MBP-specific T-cell response [45] and demyelination is mediated by CD8 T cells [46, 47].

3.2.7.2 Mouse Hepatitis Virus

MHV, a group II positive-strand RNA coronavirus, is a natural pathogen of mice that upon intracranial injection causes an acute encephalomyelitis that develops into a chronic CNS immune-mediated demyelinating disease with some clinical and pathological similarities with MS [48]. MHV can induce either acute or chronic forms of disease. The acute form is characterized by the production of pro-inflammatory cytokines and chemokines that eventually reduce MHV viral load in the CNS. However, persistent infection of spinal cord white matter tracts propagates and promotes antiviral responses that cause demyelination leading to symptoms of limp tails and partial to complete hind-limb paralysis, similar to that in EAE. In contrast to EAE and other viral models of CNS demyelinating disease, myelin-specific T cells and epitope spreading are not thought to be required for demyelination; rather it is the persistent effect of antiviral immune responses including macrophages and CD4+ and CD8+ T cells that cause chronic demyelination (reviewed by Lane 2010) [49].

3.2.7.3 Uses of Viral Models of Multiple Sclerosis

In summary, TMEV-IDD is a demyelinating model that is associated with persistent infection of the CNS, whereas SFV infection of SJL mice might represent a model of MS where immune-mediated demyelination is triggered by a virus infection of the CNS that is cleared efficiently by the host immune system. Although some studies have indicated MS might have a viral aetiology, the mechanisms involved are unknown but might involve an infection in early life that primes autoreactive T cells by molecular mimicry or a persistent infection of the CNS that causes demyelination via epitope spreading and/or molecular mimicry [50]. Thus, both mouse models allow the study of how viruses might induce CNS demyelinating autoimmune disease. In contrast, MHV provides a different scope for study, the underlying mechanisms that mediate host defence against an acute viral infection that later becomes chronic and which is associated with CNS demyelination and neurological symptoms in the absence of myelin-specific T-cell responses.

3.3 Animal Models of Guillain-Barré Syndrome

Guillain-Barré syndrome (GBS) is a rapid autoimmune-mediated acute disease of the peripheral nervous system, which is often caused by infection with *Campylobacter jejuni*. Clinical disease characteristics usually occur rapidly following onset, usually within 4 weeks, and include neuromuscular paralysis, progressive limb weakness, and deficits of the sensory and autonomic systems as well as cranial nerve involvement. The generation of antibodies to cell-surface gangliosides highly expressed on peripheral nerves by cross-reactivity to *Campylobacter jejuni* outer membrane components (lipo-oligosaccharides) mediates disease [51] and is thought to be caused by postinfectious molecular mimicry (reviewed by Shahrizaila 2011) [52].

Two forms of GBS have been characterized: (i) a primary demyelinating form (acute inflammatory demyelinating polyradiculoneuropathy [AIDP]) and (ii) those with axonal involvement (acute motor axonal neuropathy [AMAN], acute motor-sensory axonal neuropathy [AMSAN]), with either the presence or absence of anti-ganglioside antibodies [53, 54]. The pathogenic mechanisms of the AIDP form of GBS include the demyelination of the peripheral nerve myelin sheath by inflammation including upregulated adhesion molecules, pro-inflammatory cytokines, and the infiltration of CD4+ T cells and plasma cells secreting anti-ganglioside antibodies and macrophages [53–56], whereas the axonal forms of GBS (AMAN, AMSAN) usually do not include lymphocyte or monocyte involvement, rather Wallerian degeneration as well as complement and anti-ganglioside antibodies that directly mediate myelin destruction [57].

Disease is often acute and the recovery of motor function is common in approximately 60% of GBS patients. However, in other cases, residual sensory deficits remain, and in extreme cases, severe alterations to the autonomic nervous system can cause death by respiratory failure, embolism, or cardiac arrest. Current treatment of GBS is generally nonspecific and typically includes the administration of intravenous immunoglobulins or plasmapheresis, which reduce the duration to recovery. However, neither of these treatments is curative, and therefore, the use of animal models of GBS to identify pathogenic mechanisms and to test novel treatments is important.

3.3.1 *Experimental Autoimmune Neuritis*

Experimental autoimmune neuritis (EAN) is a commonly used, robust, and highly predictable animal model of GBS. It was originally developed in rabbits [58], but has since been induced in a wide variety of animals including rats, mice, rabbits, guinea pigs, and monkeys. Disease is induced by the active immunization of susceptible animals with purified whole peripheral nerve myelin or specific myelin protein components (myelin protein 2 [P2], myelin protein zero [p0]) in complete

Freund's adjuvant [58–60]. EAN in rats and mice is a monophasic acute demyelinating inflammatory disease of the peripheral nervous system [58] with many similarities to GBS including clinical, immunological, and morphological characteristics. In rats, onset of disease is observed at 12–13 post-immunization with a peak of disease severity at day 16. Clinical disease is characterized by tail and limb weakness, and histopathological analyses have shown the presence of nerve oedema and demyelinated peripheral nerves accompanied by the infiltration of inflammatory cells, features which are present in GBS. Symptoms are likely to be caused by a combination of nerve conduction block and the effects of demyelination. During the effector phase of EAN disease, typical pro-inflammatory components including chemokines (MIP-1 α and MIP-1 β , MCP-1, RANTES, and IP-10) (reviewed by Fujioka 1999) [61, 62], cytokines (reviewed by Zhu 1998) [63], and adhesion molecules (VCAM-1) [64] have been shown to be upregulated. Th1-type cytokines might mediate the demyelination of peripheral nerves as evidence shows that the addition of IFN- γ can enhance EAN, whereas blockade of IFN- γ with neutralizing antibodies can ameliorate EAN symptoms and disease course [65].

EAN mouse models with severe clinical symptoms and pathological features similar to rat EAN have also been reported. An early study reported the induction of EAN SJL/J mice, which showed subclinical damage to peripheral nerve myelin but without clinical symptoms, in contrast to EAN Lewis rats, that developed typical hind-limb weakness and histopathology of the peripheral nervous system [66]. A subsequent study demonstrated that the addition of pertussis toxin to SJL/J mice immunized with bovine peripheral nerve myelin in complete Freund's adjuvant enhanced the mild disease seen in the absence of pertussis toxin [67]. When immunized mice with pertussis toxin were treated with recombinant mouse IL-12, the disease course duration was prolonged and recovery was delayed. Histological analysis demonstrated severe demyelination of the caudae equinae and sciatic nerves during the recovery stage as well as mononuclear cell infiltration. Of note, although C57BL/6 mice were initially thought to be resistant to EAN induction, immunization of male C57BL/6 mice with a synthetic P0_{180–199} peptide induced the clinical and pathological characteristics of acute monophasic EAN [68]. As previously shown, the addition of intravenously administered pertussis toxin increased the incidence of EAN and enhanced inflammation and demyelination of peripheral nerves.

3.3.2 Adoptive Transfer Models of Experimental Autoimmune Neuritis

Similar to that seen for EAE, in addition to immunization models of EAN, adoptive transfer models of EAN have been reported. P2- and P0-specific CD4⁺ T cell lines have been shown to transfer histopathologically similar EAN to naïve syngeneic Lewis rat recipients when injected intravenously [69–71]. However, the onset of

disease was earlier (day 7) in a P2-adoptive transfer EAN model compared with active immunization EAN models (day 12–13).

3.3.3 *Correlation of Experimental Autoimmune Neuritis with Guillain-Barré Syndrome*

Although EAN is a robust, reproducible model of neuritis, sharing many pathological and clinical features with GBS, one critical disadvantage is that *Campylobacter jejuni* infection, which is thought to be involved in a high percentage of human cases of GBS, does not induce disease in rats. Furthermore, immunization of rats with various gangliosides, the main immunological target of the immune system in *Campylobacter jejuni*-induced GBS, also does not induce disease [72]. The addition of gangliosides to immunization protocols failed to have an enhancing effect of EAN severity or disease onset. Although other species of animals have shown some promise in terms of developing conduction block (rabbits immunized with GM1 demonstrated sciatic nerve conduction block [73], 33/100 chickens administered *Campylobacter jejuni* isolated from a Chinese patient showed sciatic nerve Wallerian degeneration with minor demyelination) [74], these models have not been studied extensively. Therefore, the existing EAN model is useful for studies related to the effector phase immune-mediated mechanisms of peripheral nerve demyelination; it is less useful for studies to determine the pathogenic mechanisms involved following *Campylobacter jejuni* infection.

3.4 Animal Models of Myasthenia Gravis

Myasthenia gravis (MG) is a rare neuromuscular disease that causes excessive fatigue and generalized muscle weakness that can fluctuate and is characterized by clinical symptoms including ptosis and diplopia. Muscle weakness often worsens upon use but usually improves with rest. As the disease course of MG progresses, bulbar and respiratory muscle weakness worsens, which can become life-threatening, often requiring the use of mechanical ventilation by intubation.

MG is thought to be a T cell-dependent antibody-mediated autoimmune disease because approximately 80% of MG patients have autoantibodies against acetylcholine receptors (AChR) [75, 76], possibly as a consequence of the loss of “self”-tolerance in the thymus [77]. AChR antibodies bind to AChR expressed at neuromuscular junctions (NMJ) and block neuromuscular transmission. Interestingly, the first experimental evidence to suggest AChR antibodies might be pathogenic was demonstrated using an experimental rabbit model where immunization with purified acetylcholine receptor in complete Freund’s adjuvant induced the

production of antibodies to acetylcholine receptor, which mediated neuromuscular blockade that caused flaccid paralysis and MG-like symptoms [78].

Despite the early identification of AChR antibodies as potential mediators of disease, the mechanisms involved that precede the production of AChR antibodies are less clear, although involvement of the thymus has been indicated. The symptomatic treatment of MG using acetylcholinesterase inhibitors and/or immunosuppressive drugs can improve muscle function although these treatments are limited by either a reduction in efficacy over time or severe side effects. Furthermore, plasma exchange and surgery in patients that develop thymoma are invasive procedures. Currently, no treatments directly target the autoimmune component of disease, and therefore, there is still a need for the further elucidation of MG pathogenesis and the development of novel drugs, which highlights the importance of using animal models of MG.

3.4.1 Active Experimental Autoimmune Myasthenia Gravis

Experimental autoimmune myasthenia gravis (EAMG) was originally induced in rabbits by immunization of highly purified AChR isolated from the electric organ of *Electrophorus electricus* emulsified in complete Freund's adjuvant [78]. This induced the production of antibodies that specifically recognize AChR and bind to these receptors at NMJs, subsequently blocking neurotransmission, causing muscle fatigue and weakness, which mimic the symptoms observed in human MG. Since the first description of EAMG, it has been induced in a wide variety of animals including rabbits [78, 79] rats [80], mice [81, 82], guinea pigs [80], goats [83], monkeys [84], and frogs [85].

Currently, rat and mouse models are the animal models of choice, as gene knockout and transgenic technology has allowed a greater in-depth investigation into the specific molecules involved in disease pathogenesis. Susceptible rat strains include Lewis, Fischer, and Wistar-Munich rats, and nonresponder strains include Wistar Furth and Copenhagen rats. It is important to note that some mouse strains have different susceptibilities to EAMG; H-2^{b, s} haplotype strains (C57BL/6 and SJL/J) are high responders, whereas H-2^{k, p} haplotypes are nonresponders; therefore, it is important to determine the strain used before initiating studies [81, 86]. Interestingly, rats appear to be more susceptible to EAMG than mice, as usually only one immunization with AChR in complete Freund's adjuvant is required to induce autoantibodies to AChR. In contrast, susceptible mouse strains usually require two or three immunizations with AChR in complete Freund's adjuvant. Furthermore, disease severity in mouse EAMG is reduced compared with the rat model, and therefore, this is an important consideration if the efficacy of novel therapeutic agents is to be tested. Of note, susceptibility to both MG and EAMG is linked to the HLA/MHC region [87]. Thus, EAMG has many clinical and pathological similarities to human MG, especially the presence of autoantibodies that recognize and bind to AChR.

Following immunization, T cells mediate the production of AChR-specific antibodies in murine EAMG as well as human MG [88–90], and treatment with anti-CD4 or anti-Ia antibodies can block the induction or induce remission of EAMG [89, 91]. Further evidence for a role of T cells in EAMG pathogenesis was shown by the use of lymphocyte immunosuppressive agents and oral tolerance to AChR that inhibited disease onset [92–94]. Similar to that observed in EAE, the role of pro-inflammatory cytokines including IL-1, IL-12, IFN- γ , and TNF- α is also important in the onset of EAMG, with functions related to T-cell development, proliferation, and differentiation. Studies in EAMG demonstrated that treatment of EAMG rats or mice with anti-TNF- α treatments reduced EAMG development [95] and significantly improved established disease [96]. Confirmation of a role for TNF in MG was demonstrated by a trial investigating the use of a TNF inhibitor (etanercept) in MG patients that reduced muscle weakness [97].

Once the autoreactive T cells become activated, they stimulate B cells to produce and secrete anti-AChR antibodies that induce the observed clinical symptoms. EAMG studies have indicated that following the binding of AChR antibodies to NMJs, complement activation including C3 and C9 deposition and membrane attack complex might mediate the destruction of NMJ plasma membranes [98–100]. Confirmation of a role for complement in EAMG was shown in studies reporting that the blockade of the complement system by complement inhibitors protected rats against the induction of EAMG [101–103]. Importantly, the role of complement was indicated in human MG [99, 100, 104], indicating the validity of EAMG.

Clinical signs of active EAMG (tremor, hunched posture, muscle weakness, and fatigue) usually occur 3–10 days after the second immunization, and mice are observed for signs of muscle weakness by the paw-grip test at least once a week. In addition, a number of other tests have been developed to assess the extent of disease including the quantitative measurement of muscle weakness by electromyography, the evaluation of EAMG induction by the quantification of muscle AChR loss, and serum anti-AChR antibody levels by radioimmunoassay or ELISA.

Therefore, active EAMG is a useful model for myasthenia gravis and the extent of disease can be measured using fairly noninvasive techniques.

3.4.2 Passive Experimental Autoimmune Myasthenia Gravis

Another method for the induction of EAMG is the passive transfer model, where autoantibodies specific for AChR from donor AChR-immunized animals are injected daily into naïve animals, and this was first shown in a rat model [83, 105]. Another method for the passive induction of EAMG is the injection of AChR-specific antibodies isolated from the serum of MG patients [106].

Interestingly, the transfer of EAMG by autoreactive lymphocytes is less robust than that by autoantibodies [107] and indicates that autoreactive antibodies are probably the major mechanism involved in the onset of NMJ destruction.

In summary, active EAMG is useful for investigating the induction phase of disease (T- and B-cell activation and autoantibody production) including loss of self-tolerance, mechanisms of antigen-specific immune response induction, and their modulation by therapeutic agents (to induce tolerance or immunosuppression), whereas passive EAMG is useful for the study of the effector phase of disease including IgG deposition in NMJs, complement molecules, and investigating treatments using regulatory proteins to prevent the degradation of NMJs. Clinical disease in the passive model of EAMG is similar to that observed in active EAMG and therefore serves as a useful model of MG when full activation of the immune system is not required, as is seen following active immunization protocols.

3.4.3 Experimental Autoimmune Myasthenia Gravis Induced by Musk Antibodies

Although most MG patients develop autoreactive antibodies to AChR, approximately 20% of MG patients are AChR antibody negative; however, 30–40% of these MG patients have antibodies that recognize muscle-specific kinase (MuSK) [108]. MuSK is a tyrosine kinase receptor that is involved in the development of postsynaptic membranes in the NMJ. However, whether MuSK antibodies are involved in the pathogenicity of MG is unclear and it is unknown whether they contribute to muscle weakness. Therefore, the use of animal models is useful to help dissect the potential pathogenic function of MuSK antibodies. A recent study demonstrated that immunization of rabbits or mice with a MuSK ectodomain induced muscle weakness as measured by electromyographic analysis and flaccid paralysis, which was similar to that in human MG [109, 110]. However, the appearance of disease-related symptoms is longer than that for AChR-active and AChR-passive models of EAMG, and the passive transfer of MuSK antibodies is less effective than the equivalent AChR-passive model. Therefore, MuSK antibody-related MG might represent a subtype of MG or reflect differences in environmental or genetic susceptibility factors.

3.4.4 Relationship of Experimental Autoimmune Myasthenia Gravis with Human Myasthenia Gravis

EAMG is a very useful animal model for the study of the pathways involved in disease pathogenesis as well as investigating novel therapeutic strategies. Importantly, EAMG shares similar symptoms with MG, especially muscle weakness and fatigue. Furthermore, they share such immunopathological features as AChR-specific antibodies in the serum, muscle AChR loss, presence of complement factors such as C3 and C9 at NMJs, and a supportive role for T and B cells as

regards autoantibody production. One of the major differences between EAMG and MG is the involvement of the thymus (loss of self-tolerance) in human MG, which is absent in EAMG, where tolerance must be “broken” by immunization of the autoantigen in complete Freund’s adjuvant. Although many therapeutic strategies have been demonstrated to be beneficial in EAMG, few have translated to the clinic for human MG. This might be due to the human form of disease having a more complex aetiology than EAMG. However, EAMG still has an important role to play in preclinical studies, whether to investigate pathogenic mechanisms or potential therapeutic strategies.

3.5 Conclusions

Despite the numerous beneficial advances of researchers in each of these human neuroimmune diseases, the underlying mechanisms of many of these disease processes are still unclear and this has hindered the search for curative therapies. Although the use of human tissues and samples is critical for these types of studies, often they are invasive and provide an indication of a single time point within a disease process that might have been ongoing for years or decades. Therefore, it is vital to use animal models to fill in the “gaps” that cannot be analysed by research using human tissues. However, whilst animal models of disease can provide useful information, it is important to note that no single model is a perfect model of its human counterpart and each has their advantages and disadvantages. Therefore, it is important to use the correct model for the study involved, i.e. immune cell activation, mechanisms of demyelination, immune cell trafficking into the target organ, induction vs effector phases of disease or routine drug testing. In addition, it is important to remember that each mouse strain used is genetically representative of a single human individual; therefore, multiple strains should ideally be used when developing novel treatments or investigating pathogenic mechanisms, to reduce the possibility of strain irregularities confounding the results.

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