69 Animal Models of Multiple Sclerosis

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ABSTRACT

To determine whether an immunological or pharmaceutical product has potential for therapy in treating multiple sclerosis (MS), detailed animal models are required. To date many animal models for human MS have been described in mice, rats, rabbits, guinea pigs, marmosets, and rhesus monkeys. The most comprehensive studies have involved murine experimental allergic (or autoimmune) encephalomyelitis (EAE), Semliki Forest virus (SFV), mouse hepatitis virus (MHV), and Theiler's murine encephalomyelitis virus (TMEV). Here, we describe in detail multispecies animal models of human MS, namely EAE, SFV, MHV, and TMEV, in addition to chemically induced demyelination. The validity and applicability of each of these models are critically evaluated.

Key Words: Multiple sclerosis, Experimental autoimmune encephalomyelitis, Semliki Forest virus, Mouse hepatitis virus, Theiler's murine encephalomyelitis virus.

INTRODUCTION

Multiple sclerosis (MS) affects about 350,000 people in the United States and is a major cause of nervous system disability in adults between the ages of 15 and 45 years. The symptoms are diverse, ranging from tremor, nystagmus, paralysis, and disturbances in speech and vision. Extensive demyelination is seen in the neuronal lesions. The clinical heterogeneity of MS, as well as the finding of different pathological patterns, suggests that MS may be a spectrum of diseases that may represent different pathological processes.[1] This has led to the development of many different animal models, including rodents and nonhuman primates, that reflect the pathological processes and could allow for the development of therapeutic approaches. At the present time, the exact etiological mechanism in humans is not clear; however, several animal models are available providing insight into disease processes. The relative inaccessibility and sensitivity of the central nervous system (CNS) in humans preclude studies on disease pathogenesis, and so much of our understanding of infections and immune responses has been derived from experimental animal models. The experimental systems include Theiler's virus, mouse hepatitis virus, and Semliki Forest virus infections of laboratory rodents. Additional information has been obtained from studies of experimental infections of other animals that result in demyelination, notably maedi-visna virus in sheep and canine distemper virus in dogs. In humans and animals, most natural cases of demyelinating disease are rare complications of viral infections. One possible reason for the low incidence of demyelination following viral infections could be the low efficiency of neuroinvasion. However, a correlation between CNS infection and clinical disease is difficult to determine.

The role of genetics and environmental factors in MS is complex. Factors such as geographical location, ethnic background, and clustering in temperate climates all contribute to susceptibility. Individuals with a North European heritage are statistically more susceptible to MS than those from a more tropical environment and it is more common in women.[2] Epidemiological data indicate that MS is not a single-gene disorder and that additionally environmental factors contribute to the disease.[3] Data from genetic studies indicate that although MHC genes clearly contribute to disease susceptibility and/or resistance, it is probable that a combination of environmental factors may additionally contribute to disease development in genetically predisposed individuals.

To understand the initiating factors and progression of MS, researchers have turned to experimental model systems. Since this disease cannot be recreated in a tissue culture system, much effort has been directed to the use of laboratory animals. Those animal models should mirror the clinical and pathological findings observed in human MS. Ideally, the animal model should be in a species that is easy to handle, inexpensive, can be kept in large numbers, and is easily bred in laboratory conditions. The most frequently used animals are laboratory rodents, including mice, rats, guinea pigs, and hamsters. One of the most useful aspects of laboratory rodents as animal models of disease is the vast array of inbred strains of the species available, most notably in experimental mice. Additionally, very valuable information has been obtained from studies using larger animals including sheep, dogs, cats, and nonhuman primates.

Models of MS fall into two main groups: viral and nonviral. Viral models are immensely relevant since epidemiological studies suggest an environmental factor, and almost all naturally occurring CNS demyelinating diseases of humans and animals of known etiology are caused by a virus. These include in humans, subacute sclerosing panencephalitis (SSPE)—caused by measles or rubella viruses, progressive multifocal leukoencephalopathy (PML)—caused by JC virus, and human T lymphotrophic virus-1

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(HTLV-1)-associated myelopathy (HAM)—caused by HTLV-1; in animals, these include visna virus in sheep and canine distemper in dogs. However, no one virus has consistently been associated with human MS, although it is likely that more than one virus could trigger the disease.

Of the nonviral models of MS, experimental allergic encephalomyelitis (EAE) is the most widely studied. EAE is characterized by inflammatory infiltrates in the CNS that can be associated with demyelinating lesions. In EAE, the disease is initiated by the extraneural injection of CNS material, or purified myelin components, emulsified in an adjuvant, the most commonly employed one being complete Freund's adjuvant containing *Mycobacterium tuberculosis* H37Ra. However, no naturally occurring autoimmune correlate of this experimental disease is known, although it is extensively researched as a model of MS, with the reasoning that MS may be such a disease.

The most widely studied models of MS are the experimental infections of rodents resulting in an inflammatory demyelinating disease in the CNS, such as Theiler's virus, mouse hepatitis virus, and Semliki Forest virus.[4] Each of these infections gives rise to lesions of mononuclear cell inflammatory demyelination throughout the brain and spinal cord but not in the peripheral nervous system. As such, this histopathology correlates with human MS, although it does not preclude the fact that the viruses could gain access to the CNS via the peripheral nervous system. These viral models demonstrate how a virus can easily reproduce CNS disease, which is comparatively rare in humans, and how this can be influenced by many factors including both genetic and immunological.

Experimental studies in induced animal models have the advantage over studies in spontaneous models in that the onset and progression of the disease can be controlled. Although it has been proposed that some autoimmune diseases may have a viral etiology, virus-induced autoimmunity is a controversial subject. Epidemiological studies of MS provide strong evidence for the involvement of a viral etiology in the onset of disease. Theiler's virus-induced demyelination, a model for human MS, bears several similarities to the human disease: an immune-mediated demyelination, involvement of $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells, delayed type hypersensitivity responses to viral antigens and autoantigens, and pathology. Indeed this mouse model may provide a scenario that closely resembles chronic progressive MS.

THEILER'S VIRUS-INDUCED DEMYELINATION

Theiler's murine encephalomyelitis virus (TMEV) is a picornavirus that causes an asymptomatic gastrointestinal infection, followed by occasional paralysis. There are two main strains of TMEV, the virulent strains and persistent Theiler's original (TO) strains. The virulent GDVII strains of Theiler's virus are highly neurovirulent and when injected intracranially, cause death by encephalitis within 48 h. GDVII strains also cause differing forms of paralysis depending on the route of inoculation (see Table 69–1). From these studies it appears that the GDVII virus may gain access to the CNS by retrograde axonal transport rather than by a hematogeneous route.[5]

Infection of susceptible strains of mice with the persistent TO strains BeAn, DA, WW, or Yale results in a primary demyelinating disease that closely resembles human MS.[6] Infection of resistant strains of mice with BeAn does not result in demyelinating disease, since these mice are able to clear virus from the CNS. Susceptible mice fail to clear virus from the CNS, possibly resulting from poor natural killer (NK) cell and cytotoxic T lymphocyte (CTL) responses. Persistent viral infection of the CNS is required for demyelination. Following the intracranial injection of susceptible mice with BeAn, virus replicates both in the brain and spinal cord.[7] One month postinfection, viral titers decrease, and high levels of neutralizing antibodies are detected (Figure 69–1). At this point in the disease, neurons may become infected with virus and mice may develop a nonprogressive flaccid paralysis of the forelimbs and/or hindlimbs.[8] This is sometimes referred to as a polio-like disease, but this is confusing since flaccid paralysis in mice infected with poliovirus is progressive and normally results in death. In the late phase of the disease, astrocytes, oligodendrocytes, and macrophage/microglial cells become infected with virus. Also in the demyelinating disease there is both B and T cell autoimmunity, directed against myelin and its antigenic components.

GENETICS OF PERSISTENT INFECTION AND DEMYELINATING DISEASE All inbred mouse strains inoculated intracerebrally with TMEV show early encephalomyelitis, but not all strains remain persistently infected. Resistant strains normally

Table 69–1
Specificity of paralysis due to routes of injection of Theiler's murine encephalomyelitis virus (TMEV)

Route of injection[a]	*Volume of virus*[b]	*pfu injected*[c]	*Paralysis, encephalitis, or mortality*[d]	*Days of paralysis*[e]
Intramuscular	100 µl	10^6 pfu	URLP/BRLP paralysis	2–4
Intragastric	100 µl	10^6 pfu	0%	0
Intravenous	100 µl	10^6 pfu	Fore/hindlimb paralysis 80%	6
Intraperitoneal	100 µl	10^6 pfu	Fore/hindlimb paralysis 100%	6
Intratongue	100 µl	10^6 pfu	Tongue paralysis 100%	4
Intracranial	10–20 µl	10^5 pfu	No paralysis, 100% mortality, 100% clinical encephalitis	0
Footpad	100 µl	10^6 pfu	Fore/hindlimb paralysis 100%	2–4

Source: Adapted from Villarreal *et al.*[5]
[a]Route of injection of TMEV in CBA mice.
[b]Amount of virus injected.
[c]pfu injected.
[d]Incidence of paralysis or encephalitis or mortality observed in mice.
[e]Onset of paralysis in CBA mice.

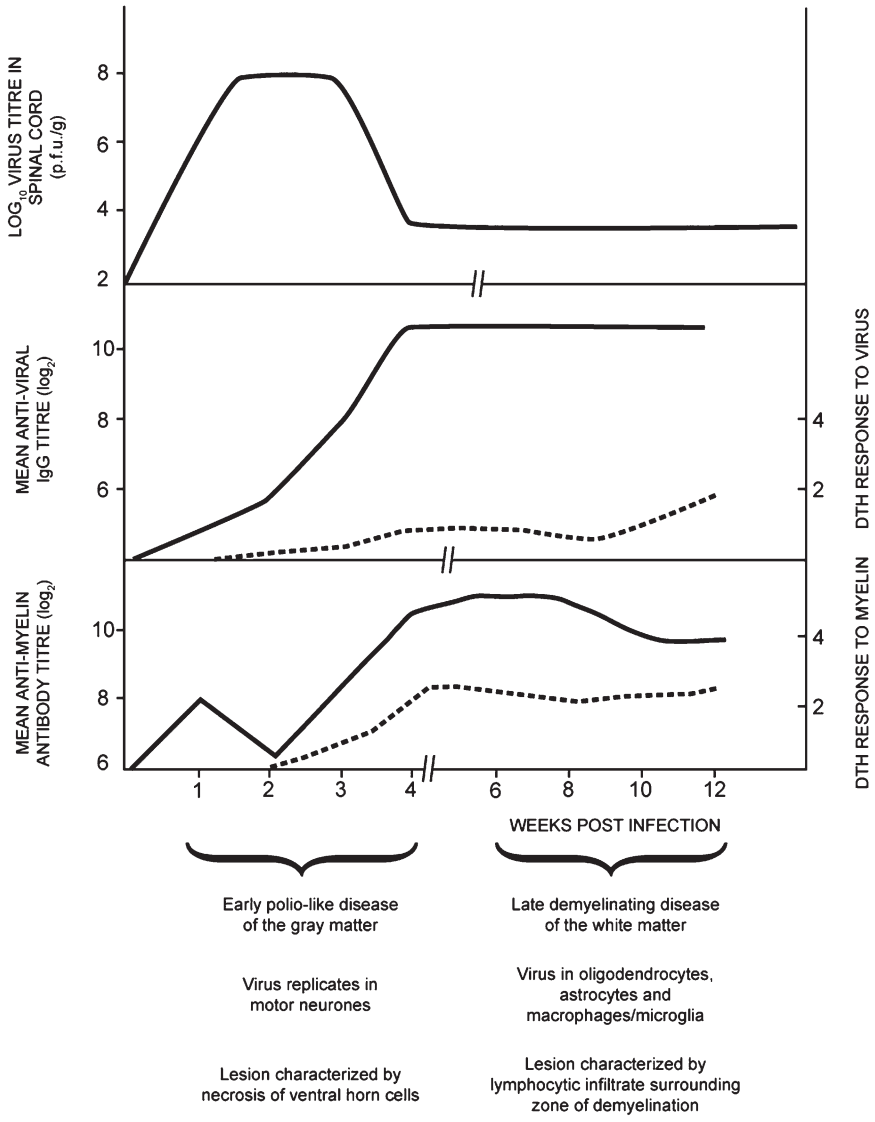

Figure 69-1. Disease course of Theiler's virus-induced demyelination in CBA mice. Intracerebral infection of CBA mice with 5×10^4 pfu of the BeAn strain of Theiler's virus results in high levels of virus in the spinal cord during the first month of infection (top panel). The CNS viral titers decrease at 4 weeks pi when neutralizing antiviral antibodies and viral T cell responses are detected (middle panel). During late disease, the virus is detected in astrocytes, oligodendrocytes, and macrophage/microglial cells and autoreactive T and B cell responses to myelin are detected in TMEV-infected mice (lower panel). (Reprinted from Welsh *et al.*[8] Copyright 2006 with permission from Springer.)

clear the virus after 14 days, whereas susceptible strains remain persistently infected for life. Thus, susceptibility/resistance refers to the demyelinating late disease and not to the encephalomyelitis. CNS viral load during persistence varies among susceptible strains, making susceptibility a quantitative trait.[9] This trait is under multigenic control, with H-2 MHC class I genes being the most prominent. Additionally, several non-H-2 quantitative trait loci (QTL) have been identified within the same H-2 haplotypes that control persistence. There is generally a good correlation in inbred strains between susceptibility to three phenotypes (viral load, pathology, and symptoms), suggesting that variations in both demyelination and clinical disease may result from how each mouse strain can control the viral load during the persistent infection.[10] Using B10 congeneic and recombinant strains of mice, susceptibility to disease has been mapped to the H-2D region.[11] Furthermore, resistant haplotypes are dominant and the same locus controls viral load during persistence and demyelination. Currently, 11 non-H-2 susceptibility loci have been identified as having an effect on susceptibility to Theiler's virus-induced disease (TVID) (see Table 69–2).

The mechanism(s) of TVID may be different for different mouse strains, but most of the information has come from studies of SJL/J mice infected with the DA or BeAn strain of virus. The virus infects oligodendrocytes, and the resulting demyelinating disease could be due, in part, to the virus killing oligodendrocytes directly or by the virus-specific $CD8^+$ CTLs present in the lesions.[7]

Table 69–2
Non-H2 loci of susceptibility to Theiler's virus-induced disease

Locus	Chr	Location (cM)	Phenotype	Viral strain
Tmevd1	6	22	Clinical signs	BeAn
Tmevd2	3	46	Clinical signs	BeAn
Tmevd3	14	12.5	Demyelination	DA
Tmevd4	14	39.5	Demyelination	DA
Tmevd5	11	60	Clinical signs	DA
Tmevd6	1	19.5	Clinical signs	BeAn
Tmevd7	5	72	Clinical signs	BeAn
Tmevd8	15	4.7	Clinical signs	BeAn
Tmevd9	1	32.8	Clinical signs	BeAn
Tmevp2	10	51.5–62	Viral load	DA
Tmevp3	10	69–70	Viral load	DA

Source: Adapted from Brahic *et al.*[9]

A series of experiments has demonstrated that demyelination correlates with the presence of a $CD4^+$ T cell-mediated response against viral epitopes. These cells secrete cytokines such as interferon (IFN)-γ that activate both microglial cells and invading monocytes, which subsequently secrete factors such as tumor necrosis factor (TNF)-α and thus can cause "bystander" demyelination. Activated macrophages ingest and degrade damaged myelin. Autoantibodies[12,13] and myelin-specific $CD4^+$ T cells have been shown in SJL/J mice several months after intracranial inoculation.[14,15] Epitope spreading in these mice commences with recognition of a proteolipid protein (PLP) epitope, and then progresses to additional PLP epitopes and then to myeloid basic protein (MBP) epitopes.[14] A direct demonstration that disease can be maintained on a purely autoimmune footing, after infection has been eradicated, has not been shown.

IMMUNITY AND THEILER'S VIRUS The first response to viral infection is the production of type I interferons, which are critical for viral clearance. IFN-α/β receptor knockout mice injected with TMEV die of encephalomyelitis within 10 days of infection. NK cells are activated early in infection with certain viruses. In TMEV infection susceptible SJL mice have a 50% lower NK cell activity in comparison to the highly resistant C57BL/6 mice. This low NK activity in SJL mice is in part due to a defect in the thymus impairing the responsiveness of NK cells to stimulation by IFN-β. The pivotal role of NK cells in early TMEV clearance is demonstrated by the finding that resistant mice depleted of NK cells by monoclonal antibodies to NK 1.1 develop severe signs of gray matter disease.[16]

In the early disease, both $CD4^+$ and $CD8^+$ T cells have been shown to be important in viral clearance. In early disease $CD4^+$ T cells are required for B cells to produce antibodies for viral clearance.[12] These $CD4^+$ T cells secrete IFN-γ, which *in vitro* inhibits TMEV replication and has a protective role *in vivo*. $CD8^+$ T cells are also important in viral clearance, as demonstrated by the finding that $CD8^+$ T cell-depleted mice fail to clear virus and develop a more severe demyelinating disease.[17] $CD8^+$ T cells also provide protection against TVID when adoptively transferred to a TVID-susceptible strain, BALB/c.AnNCr. Thus, $CD8^+$ T cells are implicated in viral clearance and resistance to demyelination. Higher CTL activity has been demonstrated in TVID-resistant C57BL/6 mice as compared to resistant SJL/J mice.[18] These CTLs may play an important role in viral disease since they may recognize viral determinants and/or they may inhibit delayed type hypersensitivity responses.

The relative roles of Th1/Th2 cells in TVID are very complex, and a simple picture of a Th1 or Th2 polarization during infection may not be apparent. A pathogenic role for Th1 cells during late demyelinating disease is demonstrated by the finding that both TVID correlates with delayed type hypersensitivity responses to TMEV and that the depletion of $CD4^+$ T cells during late disease results in the amelioration of clinical signs. High levels of the proinflammatory Th1 cytokines IFN-γ and TNF-α in late disease correlate well with maximal disease activity. Evidence demonstrating the protective role of Th2 in TVID has been shown in experiments in which skewing the immune response toward Th2 immunity in TMEV infection diminishes the later demyelinating disease.[19] However, other studies have shown that the Th1/Th2 balance did not explain the difference in susceptibility to TVID. Th1 cytokines are generally pathogenic during late demyelinating disease, whereas Th2 cytokines are protective.

USE OF THEILER'S MURINE ENCEPHALOMYELITIS VIRUS MODEL TO STUDY REMYELINATION Remyelination in MS lesions was first documented in 1906[20] and is characterized by abnormally thin myelin sheaths in relation to axon diameter. To date, however, there are few reliable data on the frequency of remyelination in MS patients. Stimulation of remyelination is a potential treatment for MS. The TMEV model of MS can be used to study remyelination using remyelination-promoting antibodies. In this remyelination model, SJL/J mice, aged 4–8 weeks, are injected with a 10 µl volume containing 200,000 pfu of Daniel strain intracerebrally. All animals develop mild encephalitis, which resolves within 14 days after the injection. The infected mice then develop the chronic demyelinating disease that gradually progresses over several months. To study remyelination, mice that had been infected with TMEV for 6 months receive a single intraperitoneal injection of 0.5 mg (~0.025 g/kg body weight) of a recombinant remyelinating antibody (rHIgM22) in phosphate buffered saline (PBS).[21] In one study, 82.8% of lesions in animals treated with rHIgM22 showed retraction of varying degrees, presumably the effect of remyelination in these lesions. The direct binding of rHIgM22 to demyelinated lesions is consistent with the hypothesis that these antibodies work directly in the lesions, probably by binding to the CNS glia to induce remyelination. Thus, this murine TMEV model can also be used as a model with which to examine different modes of remyelination.

MOUSE HEPATITIS VIRUS

Mouse hepatitis virus (MHV) is a member of the Coronaviridae, a group of large positive sense enveloped RNA viruses. Depending on the strain of virus used, MHV causes a variety of diseases including enteritis, hepatitis, and demyelinating encephalomyelitis.[22] Infection of mice with the neutrotropic JHM strain of MHV causes encephalitis, followed by chronic demyelination. Virus is not cleared from the CNS, resulting in a persistent infection.

After intracerebral or intranasal infection with MHV, virus enters the brain and causes encephalitis.[23] Intranasal infection with MHV-JHM or -A59 leads to viral spread through the olfactory bulb and along the olfactory tracts, as well as along the trigeminal nerve to the mesencephalic nucleus.[23] Up to 4 days postinfection (pi) early viral spread is via specific neural pathways and neural connections. Viral titers peak at about day 5 pi in the brain and later in the spinal cord and virus is cleared by days 8–20 pi.[24] However, viral antigen is still detectable up to day 30 pi. Additionally, viral RNA is detectable in the brain as late at 10–12 months postinfection, although the amount of RNA decreases with time. Liver infection can occur after any route of infection (in, ic, ig, or ip), with viral titers peaking at day 5 pi and hepatitis developing during the first 1–2 weeks.[25]

CNS demyelination develops as active MHV infection resolves. The lesions observed are histologically very similar to those observed in MS patients. These MHV lesions are characterized by primary demyelination accompanied by naked axons,[23] and are found scattered throughout the spinal cord.[26] The peripheral nervous system is not affected. Chronic lesions are associated with lipid-laden macrophages, scattered lymphocytes, and perivascular cuffing. These chronic lesions can persist as late as day 90 pi, and demyelinating axons can be seen as late as 16 months postinfection.

Chronic diseases in MHV-infected mice are associated with ataxia, hindlimb paresis, and paralysis, followed by a recovery. This animal recovery is mediated by CNS remyelination, beginning anywhere from 14 to 70 days pi.

C57BL/6 mice (H-2^b) are susceptible to MHV infection. In this murine model adult mice (of weight 20–22 g) are anesthetized by inhalant anesthesia and receive an intracerebral injection of approximately 500 pfu of a neurotropic MHV strain in a volume of approximately 20 µl of PBS. This intracerebral injection of MHV results in a biphasic disease: an acute encephalomyelitis with myelin loss, followed 10–12 days later by an immune-mediated demyelinating encephalomyelitis with progressive destruction of the CNS.[27] There is an 80–90% survival rate of mice injected with this MHV, with animals usually succumbing during the first 2 weeks of acute infection. Animals surviving this acute stage show a 95% chance of survival.[28] Control animals injected intracerebrally with sterile PBS show no clinical signs or histological defects.

Electron micrographs of demyelinating lesions show that macrophage processes slip between layers in the myelin sheath, implying that macrophages could indeed be mediating demyelination.[29] The appearance of macrophages within the CNS also correlates with the development of lesions. Additionally, they do not appear in large numbers in the absence of lymphocytes, so it is possible that myelin damage is caused by a nonmacrophage-dependent mechanism and that macrophages may only clear up the damaged myelin. In contrast to other mouse models of demyelination, there does not appear to be a clear role for any single lymphocytic or monocytic subset mediating the demyelination. Rather, it appears that a balance of immune components may be necessary for viral clearance and that various pathways, both immune and nonimmune, may cause the ensuing demyelinating events.

Recently, progress has been made in further identifying the immune cells required for demyelination. Experimental infection of severe combined immunodeficiency (SCID) mice, lacking T cells, results in fulminate encephalitis without demyelination.[30] Adoptive transfer of splenocytes from syngeneic immunocompetent mice into infected SCID mice results in demyelination within 7–9 days posttransfer. Additional experiments indicated that either $CD4^+$ or $CD8^+$ T cell subsets are capable of initiating this process. However, mice that receive splenocytes depleted of $CD4^+$ T cells survive longer and develop more demyelination than mice receiving splenocytes depleted of $CD8^+$ T cells. Thus, experimental SCID mice demonstrate that the roles of each T cell subset in demyelinating diseases are not equal.[31]

IFN-γ is a critical mediator of homeostasis and inflammation in MS and many of its rodent models. Bone marrow chimera mice have been used to address the role of IFN-γ in bystander demyelination mediated by $CD8^+$ T cells. These chimeras as rodent models for JHM have addressed a hypothesis that IFN-γ produced by $CD8^+$ T cells, and not from other sources, was the critical component in mediating bystander demyelination. This chimeric approach did not compromise IFN-γ production by cells such as NK cells and dendritic cells, thus preserving the innate immune response to the virus.[32] The results demonstrated that IFN-γ produced by these innate cells was unable to initiate the demyelinating disease, even in the context of activated $CD8^+$ T cells lacking only the ability of produce IFN-γ. These findings highlight the role that $CD8^+$ T cells have in demyelination in JMH-infected mice.[33] It has been demonstrated that IFN-γ is critical in other animal models of demyelination and in MS.

SEMLIKI FOREST VIRUS

Semliki Forest virus (SFV) is an alphavirus of the Togaviridae. The virus has been isolated from mosquitoes, but the natural host is unknown. SFV is a single-stranded positive strand RNA virus that has been cloned and sequenced. The most commonly studied strains used in adult mice are the virulent L10 strain and the avirulent A7(74) strain. Both of these strains are avirulent in neonatal and suckling mice by all routes of infection. Experimental infection of mice with SFV is widely used as a model to study the mechanism of virus-induced CNS disease. SFV has the advantage of being neuroinvasive as well as neurotropic, thus allowing studies of viral entry into the CNS and the integrity of the blood–brain barrier (BBB). Following intraperitoneal injection with 5000 pfu SFV in 0.1 ml PBS containing 0.75% bovine serum albumin,[34] all strains replicate in muscles and other tissues, resulting in a plasma viremia. Virus then crosses the cerebral vascular endothelial cells, resulting in infection of neurons and oligodendrocytes.[35] In neonatal or adult mice, infection with virulent strains results in widespread infection that is lethal within a few days. In contrast, infection of mice with the A7(74) strain results in a CNS infection, and infectious virus is cleared from the brain by day 10. Infiltrating mononuclear cells are observed 3 days pi and peak at about day 7. Focal lesions of demyelination throughout the CNS are observed 10 days pi and peak between 14 and 21 days pi.[36]

SFV-induced demyelinating diseases have been widely studied following intraperitoneal injection of adult mice with the A7(74) strain of the virus. Following intraperitoneal injection, virus is detected in the brain by 24 h. Viral titers then rise, but rapidly decline following initiation of the immune response. Interestingly, although infectious virus can be detected only up to day 8 pi, real-time polymerase chain reaction (rt-PCR) studies detect viral RNA up to day 90 pi.[37] Thus, it is possible that there is persistence of viral antigen(s). Disturbance of the BBB occurs between 4 and 10 days pi, which corresponds to the increase in inflammatory cell infiltration and reduction in viral titer and which may be related to the influx of cells or cytokine-mediated effects. The presence of macrophages, activated microglia, and the proinflammatory cytokines TNF-α, IFN-γ, interleukin (IL)-1α, IL-2, IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF) during SFV-induced demyelination, in addition to enhancing the inflammatory response, may also play a role in controlling viral infection since IL-6, IFN-γ, and TNF have direct antiviral activity.[38] Additionally, IFN-γ and TNF production peripherally coincides with SFV-induced encephalitis in SJL and B6 mice. Interestingly, these same cytokines predominate in MS lesions.[39] An intense inflammatory response characterized by perivascular cuffing is apparent histologically from 3 days. Demyelination, as demonstrated using luxol fast blue staining of sections, is apparent by 14 days. However, small focal lesions of demyelination can be observed using electron microscopy by day 10. A striking feature of SFV infection appears in the optic nerve, where there are demyelinating lesions and changes in visually evoked responses and axonal transport.[40] This optic neuritis also occurs in human MS.

It appears that SFV-induced demyelination in this mouse model is accompanied by neurophysiologically demonstrable

visual deficits very similar to those found in MS patients. Thus, this may provide a very useful animal model for research into MS. The advantages of this model are that genetic and environmental factors can be readily controlled, while the low cost and fast reproductive rate make experimental design considerably easier.

No demyelination is observed following SFV infection of SCID mice or athymic mice. In the absence of specific immune responses, SCID mice infected with SFV A7(74) have a persistent viremia, a persistent and restricted CNS infection, and no lesions of demyelination. Comparison of the infection to that in nu/nu and BALB/c mice and studies on the transfer of immune sera show that immunoglobulin M (IgM) antibodies clear the viremia but not the brain virus and that infections of brain virus can be reduced by IgG antibodies. These IgG antibodies can abolish infectivity titers in the brain but cannot remove all viral RNA.[41] Adoptive cell transfer studies and administration of anti-CD8 antibodies demonstrate that demyelination following SFV infection is dependent on $CD8^+$ T cells.[4] This is consistent with the finding that the CNS inflammatory infiltrate is dominated by $CD8^+$ T cells. This finding is analogous to that in MS and is in contrast to that in EAE, where $CD4^+$ cells predominate.[42]

In the EAE autoimmune model of MS, studies suggest that a Th1 cytokine profile predominates. Another point of difference between the EAE model and the SFV model is shown in the Th1/Th2 profiles. Following infection with SFV, Th1 and Th2 cytokines were detected in the CNS and both were present throughout the time course studied, indicating that there was no bias of Th response in the CNS, nor were changes apparent with time.[43]

EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

The experimental disease EAE has been investigated in many strains of animals including mice, rats, guinea pigs, rabbits, marmosets, and rhesus monkeys. EAE is an autoimmune inflammatory disease of the CNS and is characterized by perivascular and subpial inflammatory infiltrates and demyelinating lesions. The disease is usually initiated by injection of autoantigens emulsified in an adjuvant. The progression and pathology of lesions observed depend on the type of antigen used in the injection, the method of injection, and the strain of animal used. Because of its very nature, EAE as a model of MS does not address certain pertinent questions relating to MS, such as age-related onset of disease or epidemiology. A major difference between EAE and viral models of MS is that in EAE the inflammatory response is directed to autoantigens. A feature of the EAE model is that the course of the disease can be relapsing and remitting.

Studies of EAE have been used to identify antigenic determinants on components of myelin. Using bioinformatic technology these determinants have been used to search available databases of viral and bacterial proteins. Results indicate numerous viral and bacterial protein segments with probable sequence similarity to myelin basic protein determinants.[44]

EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN RABBITS EAE has been induced in rabbits by footpad inoculation with rabbit spinal cord homogenate, resulting in hindlimb paresis or paralysis.[45] Rabbits with 5-day paraplegia showed increased spinal cord incorporation of radioactive drugs administered in the epidural space. Thus, this demyelinating disease process may expose the spinal cord to larger amounts of substances administered neuraxially. It is therefore possible that this rabbit model could be used to investigate the incorporation of radioactive therapeutic drugs in the epidural space.

EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN GUINEA PIGS Guinea pigs have also been investigated to determine whether they may serve as useful EAE models of MS. The interest in guinea pigs stems from the fact that group 1 CD1 glycoprotein homologues, which in humans present foreign and self lipid and glycolipid antigens to T cells, are not found in mice and rats but are present in guinea pigs. In this guinea pig model, animals have been sensitized for EAE, and CD1 and MHC class II expression has been measured in the CNS. In normal guinea pigs low level MHC class II occurred on meningeal macrophages and microglial cells, whereas immunoreactivity for CD1 was absent. In the EAE CNS, however, the majority of infiltrating cells were MHC II^+ and microglia showed increased expression, whereas CD1 immunoreactivity was detected on astrocytes, B cells, and macrophages. Minimal CD1 and MHC II coexpression was detected on inflammatory cells or glia. Thus, in this guinea pig EAE model group 1 CD1 molecules are upregulated in the CNS on subsets of cells distinct from the majority of MHC II-bearing cells.[46] This expression of CD1 proteins in such EAE lesions broadens the potential repertoire of antigens recognized at these sites and highlights the value of this guinea pig model of human MS.

EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN RATS Rats were injected with spinal cord homogenate or the encephalitogen; myelin basic protein induced EAE in genetically susceptible Dark Agouti (DA) rats but not in Albino Oxford (AO) rats. Here 8- to 12-week-old rats were immunized in either or both hind footpads with 0.1 ml antigenic emulsion containing 100 μg rat spinal cord tissue in complete Freund's adjuvant (CFA).[47] Rats are monitored from day 5 after inoculation and the severity of disease was assessed by grading tail, hindlimb, and forelimb weakness, each on a sale of 0 (no disease), 1 (loss of tail tonicity), 2 (hindlimb weakness), 3 (hindlimb paralysis), to 4 (moribund or dead). Clinical disease in susceptible strains of rats is apparent in all animals, and the onset of disease occurs at day 11 postinjection. At the peak of the clinical manifestation of EAE there is a marked increase in the level of infiltration of cells accompanied by a lack of activation in susceptible DA rats, whereas it remains elevated in resistant AO rats. At the peak of clinical disease DA rat spinal cords contain high levels of $CD4^+$ T cells. DA rats also contained 10 times as many live $CD4^+$ T cells as AO rats. Astrocytosis, as an indication of CNS reaction to the presence of inflammatory cells, was clearly observed in both rat species. Microglial activation persists in resistant AO rats, whereas activation is downregulated in DA rats. In this model it is speculated that at the peak of disease, infiltrating monocytes and macrophages are the main antigen-presenting and effector cells.

Rat EAE may also be induced by the injection of xenogeneic myelin. For example, 8- to 12-week-old Lewis rats injected in both hind footpads with an emulsion containing 100 μg of guinea pig myelin basic protein and CFA develop acute EAE. Also chronic relapsing EAE (CR-EAE) may also be induced in this rat model using a regimen of intraperitoneal injections of 4 mg/kg of cyclosporin A.[48] Pathology studies indicate that in acute and CR-EAE, MCP-1 and its receptor CCR2 are significantly upregulated throughout the course of CR-EAE and that a large number of macrophages infiltrated the CR-EAE lesion. This suggests that

macrophages recruited by MCP-1 and CCR2-expressing CNS cells are responsible for the development and relapse of EAE. Thus, in this rat model, in addition to T cells, macrophages are another target for immunotherapy studies for neurological autoimmune diseases.

A more recent development of a rat EAE model involves using human MBP as antigen. Here EAE was induced by the immunization of female Wistar rats with human MBP. It was found that most of the rats developed tail tone loss and hindlimb paralysis together with demyelination, infiltrative lymphocyte foci, and "neurophagia" in the cortex of cerebra and in the white matter of the spinal cord.[49] This study further demonstrated that this rat model of EAE induced by human MBP resembles many features of human MS and may promise to be a better animal model for the study of MS.[49]

The use of CFA is not a prerequisite for the development of rat EAE. For example, EAE can be induced in 10- to 16-week-old DA rats by a single hind footpad injection of an encephalitogenic emulsion consisting of rat or guinea pig spinal cord homogenate (SCH) in PBS.[50] The reason for not wanting to use CFA is that in itself it induces a strong inflammatory response and exerts numerous immunomodulatory properties. Additionally, CFA induces a strong anti-purified protein derivative (PPD) response and may induce adjuvant arthritis, another autoimmune disease. The susceptibility of DA rats to EAE induction with SCH depends upon the origin of the CNS tissue, the homologous tissue being the more efficient encephalitogen. DA rats that recovered from EAE that had been induced with homologous SCH without adjuvant and then immunized with the encephalitogenic emulsion containing CFA developed clinical signs of the disease. Neurological signs in rechallenged rats were milder, but first signs appeared earlier. The earlier onset of EAE observed in DA rats after rechallenge has been attributed to the reactivation of memory cells. Taken together, these experiments demonstrate that EAE can be efficiently and reproducibly induced in DA rats without the use of CFA. This experimental model for understanding the basic mechanisms involved in autoimmunity within the CNS, without the limitations and inherent problems imposed by the application of adjuvants, may represent one of the most reliable rodent models of MS.

The rat as an experimental model could be used to evaluate new immunotherapies of EAE. These include antigen-induced mucosal tolerance, treatment with cytokines, and dendritic cell-based immunotherapy.

The ideal treatment of diseases with an autoimmune background such as MS should specifically eliminate autoreactive T cells without affecting the integrity of the immune system. One way to achieve this would be to induce immunological tolerance to autoantigens by the oral or nasal administration of autoantigen. Several studies have shown that nasal administration of soluble antigens results in peripheral tolerance by immune deviation or the induction of other regulatory mechanisms. In the rat model this tolerance has been investigated using synthetic peptides of MBP, MBP68–86, 87–99, and 110–128. Nasal administration of the encephalitogenic MBP68–86 or 87–99 suppresses EAE. MBP68–86 and 87–99 given together had synergistic effects in suppressing EAE and reversed ongoing EAE. A problem, however, of antigen-specific therapy by the nasal route is that one antigen, or peptide, may be effective in inducing tolerance in one strain of animal but not in another. One way of treating ongoing EAE may be the use of an altered peptide ligand with high tolerogenic efficacy when administered nasally.[51]

Cytokines have been widely used in disease prevention and treatment. Cytokine immunotherapy in MS could employ one or two basic strategies: first, to administer immune response downregulatory cytokines, or second, to administer inhibitors of proinflammatory cytokines. The nasal route of administering these cytokines has been studied in the rat EAE model. Nasal administration of low doses of antiinflammatory or regulatory cytokines such as IL-4, IL-10, or tumor growth factor (TGF)-β1 inhibits development of rat EAE when given before or on the day of immunization, but by differing mechanisms. Nasally administered IL-10 reduced both peripheral immune responses and microglia activation in the CNS, whereas nasal administration of IL-4 or TGF-β1 triggered the activation of dendritic cells (DCs).

However, nasal administration of cytokines alone fails to treat ongoing Lewis rat EAE. Interestingly, nasal administration of MBP68–86 + IL-4 or MBP68–86 + IL-10 suppresses ongoing EAE in Lewis rats. The suppression of EAE by MBP68–86 + IL-10 is associated with the induction of a broad lymphocyte hyporesponsiveness. Although this combined administration of autoantigen plus cytokine may be effective in treating rat EAE, the applicability of this to human MS is severely limited by the lack of knowledge of the pathologically relevant autoantigen(s) in MS.

DCs not only activate lymphocytes, but also induce T cell tolerance to antigens.[52] Use of tolerogenic DCs is thus a possible immunotherapeutic strategy for treatment of EAE, and indeed this has been studied in some detail. However, MBP68–86-pulsed DCs only prevented the development of EAE and failed to treat ongoing EAE in Lewis rats.[53] In an attempt to treat ongoing EAE, splenic DCs have been isolated from healthy Lewis rats and modified *in vitro* with cytokines IFN-β, IL-2, IL-10, or TGF-β1. Upon subcutaneous injection into Lewis rats on day 5 pi with MBP68–86 + FCA, IFN-β or TGF-β1-modified DCs promoted immune protection from EAE.

EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN NONHUMAN PRIMATES

The common marmoset *Callithrix jacchus* is an outbred species characterized by a naturally occurring bone marrow chimerism. The marmoset is a primate phylogenetically close to humans, and has been studied as an animal model for MS.[54] EAE can be induced in the common marmoset by the injection of human brain white matter, dispersed in demineralized water to a concentration of 30 mg/ml and emulsified with CFA containing 0.5 mg/ml of *Mycobacterium butyricum* H37A. Monkeys are injected intracutaneously with 600 µl of emulsion into the dorsal skin at several locations. Clinical disease in this model is scored daily on a scale from 0 to 5: 0 = no clinical signs; 0.5 = apathy, loss of appetite, and an altered walking pattern without ataxia; 1 = lethargy and/or anorexia; 2 = ataxia; 2.5 = paraparesis or monoparesis and/or sensory loss and/or brainstem syndrome; 3 = paraplegia or hemiplegia; 4 = quadriplegia; and 5 = spontaneous death attributable to EAE.[55] Here the onset of disease, as measured by clinical scores, is variable among animals between 7 and 13 weeks postinoculation. Additionally, the maximal clinical scores are variable among animals and range between 2 and 4. On histopathological examination, large plaques of demyelination are observed in the white matter of cerebral hemispheres, mainly localized around the

wall of lateral ventricles, in the hemispheric white matter, corpus callosum, optic nerves, and optic tracts. The demyelinated areas show a moderate or severe degree of inflammation characterized by perivascular cuffs of mononuclear cells. In the spinal cord, widespread demyelination is also observed. Areas of demyelination involve the ventral, lateral, and dorsal columns of the spinal cord, especially in the outer part of the spinal tracts. Thus, pathology in the marmoset model is characterized by inflammation, demyelination, and astrogliosis. Interestingly, this model demonstrates the presence of axonal damage in demyelinating plaques. Indeed, axonal damage and loss are well-known events in MS. In MS, axonal damage appears to be an early event, related to an acute inflammation. In the marmoset EAE, axonal damage also occurs in areas of acute and early inflammation and demyelination. This EAE in *C. jacchus* is of special interest because of the resemblance of this model to the human disease, and the similarity between the immune systems of marmosets and humans.

The type of clinical signs of EAE in marmosets depends largely on the antigens used for disease induction. Sensitization of marmosets to human myelin induces a relapsing-remitting, secondary-progressive disease course.[56] Lesions in this model represent all stages present in chronic MS. Marmosets inoculated with MBP develop only mild inflammatory disease unless *Bordetella pertussis* is used with the encephalitogen. CNS demyelination critically depends on the presence of antibodies to myelin oligodendrocyte glycoprotein (MOG), a minor CNS component. Marmosets sensitized to a chimeric protein of MBP and proteolipid protein (of myelin) develop clinical EAE only after the autoimmune reaction has spread to MOG. Marmosets immunized with recombinant human MOG 1–125 do not develop relapsing-remitting disease but only chronic-progressive disease.[57] During the asymptomatic phase of this primary progressive-like disease, which can last from 2 to 20 weeks, brain lesions are detectable using magnetic resonance imaging (MRI), but are not expressed clinically.

The induction of EAE with MBP or white matter tissue homogenate (WMH) has been well established in rhesus monkeys (*Macaca mulatta*). The rhesus monkey was the first animal species in which EAE was deliberately induced.[58] That autoimmunity to brain antigens could induce paralytic disease was confirmed by studies in rhesus monkeys given repeated inoculations of brain homogenates.[58] MOG-induced EAE has also been produced in this nonhuman primate species[59] that is a highly relevant model for the human disease. The close similarity of the human and rhesus monkey immune system is illustrated by the high degree of similarity between the polymorphic MHC and T cell receptor genes between these two primates. To produce this MOG-induced EAE, monkeys are injected, under anesthesia, with a total of 1 ml of 1:1 emulsion composed of 320 μg MOG in PBS and CFA at 10 sites into the dorsal skin. Overt clinical signs are scored daily according to the following criteria: (0) no clinical signs; (0.5) loss of appetite, apathy, and altered walking; (1) lethargy, anorexia, substantial reduction of the general condition, and loss of tail tonus; (2) ataxia, tail biting, sensory loss, and/or blindness; (2.5) incomplete paralysis of one (hemiparesis) or two sides (paraparesis); (3) complete paralysis of one (hemiplegia) or two sides (paraplegia); (4) complete paralysis (quadriplegia); and (5) death. The onset of clinical disease varies between animals, and occurs at days 15–23 after encephalitogenic challenge. All monkeys, however, develop clinical disease and all achieved a score of 4 on clinical severity.

The current available panel of nonhuman primate EAE models may reflect the spectrum of inflammatory demyelinating diseases in the human population. These EAE models can therefore be used to investigate pathogenic mechanisms and to develop more effective therapies.

EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN MICE

The most widely studied animal model of EAE is that of the mouse. In common with other animal models of EAE, disease induction varies depending on both the sex of the animals, the mouse strain used, as well as the origin of the spinal cord encephalitogen. In this model mice, aged 6–8 weeks, are immunized subcutaneously in four sites over the back with 200–400 μg of guinea pig MBP emulsified in equal volumes of CFA containing 200–400 μg heat-killed *Mycobacterium tuberculosis*.[60] Mice also receive 200 μg of pertussis toxin in 0.2 ml PBS intraperitoneally at the time of immunization and 48 h later. Mice are then scored daily for clinical signs of EAE for at least 35 days as follows: 0, no clinical signs; +1, limp tail or waddling gait with tail tonicity; +2, ataxia or waddling gait with tail limpness; +3, partial hindlimb paralysis; +4, total hindlimb paralysis; and 5, moribund/death. For each strain of mice there is variation in day of onset of disease, varying from day 7 to day 22 postinfection; incidence of disease, varying from 30% to 100% of animals; incidence of mortality, varying from 0% to 40% of animals; and mean clinical scores, varying from 0 to 1.6. Many mouse strains have been employed in the study of EAE, and while the SJL strain has been most frequently used to model gender differences in both disease onset and severity, the SJL model has some limitations due to its diminished $CD4^+$ T cell repertoire. Certain susceptible strains of mice, such as FVB mice, show a relapsing-remitting course of disease that bears some resemblance to MS. FVB mice therefore may serve as a mouse strain into which various transgenes may be introduced for the purpose of studying their influence on EAE and for exploring new therapeutic approaches.[61]

Since EAE is a well-studied disease in mice, mimicking many clinical and pathological features of MS, including CNS inflammation and demyelination, it is of significance that it can also be used as an appropriate model to study MS-related pain. It has been clearly demonstrated in SJL that in both "active" and "passive" EAE, there is an initial increase in tail withdrawal latency (hypoalgesia) that peaked several days prior to the peak in motor deficits during the acute disease phase. During the chronic disease phase, tail withdrawal latencies decreased and were significantly faster than control latencies for up to 38 days postimmunization. Thus, it is possible to use both murine active and passive EAE as models for MS-related pain.[62]

While specific immunotherapeutic strategies are effective in experimental model systems, translation to the human disease has genetically been poorly tolerated or has proved to be ineffective. This conflict may in part be due to the model systems used as well as the poor correlation of *in vitro* findings compared to those observed *in vivo*. In Biozzi ABH mice, which express the novel MHC class II A, EAE occurs following immunization with myelin proteins and peptide epitopes of these proteins; however, only PLP peptide 56–70, MOG peptide 8–21, or spinal cord homogenate reproducibly induces chronic relapsing EAE (CREAE) with inflammation and demyelination.[63] CREAE provides a well-characterized reproducible system to develop therapeutic strate-

gies during established relapsing autoimmune neurological disease and is pertinent to MS. In CREAE in ABH mice, relapse and progression of disease are associated with emergence and broadening of the immune repertoire due to release of myelin antigens following myelin damage.[64] Thus, this CREAE model in Biozzi ABH mice is very well suited as a model with which to examine the effect of therapeutic strategies in a dynamic system.

Disease susceptibility in human MS is associated with three MHC class II alleles in the HLA-DR2 haplotype, DRB1*1501, DRB5*0101, and DQB1*0602.[65] An autoimmune pathogenesis has been hypothesized in which one or more of these MHC class II molecules presents CNS-derived self-antigens to autoaggressive $CD4^+$ T cells, which infiltrate the CNS initiating an inflammatory response. However, the target autoantigens in MS are unknown. Immunization of mice with myelin or other brain-associated proteins induces EAE, a disease resembling MS both clinically and pathologically. The proteins, MBP, PLP, and MOG, components of the myelin sheath, are candidate antigens.[66] Indeed, T cells that are reactive to these antigens have been demonstrated in MS patients.[67] Mice expressing the human HLA-DR2 (DRB1*1501) molecule are capable of presenting peptides from all these three MS candidate autoantigens. It is possible in MS that while T cells responding to one of these antigens may initiate the disease, epitope spreading and the recruitment of T cells with additional specificities, as the disease progresses, could lead to inflammatory responses to several proteins resulting in an escalation of the autoimmune response.[68]

Transgenic mouse models of multiple sclerosis are now well established. The following are two examples of such transgenic models. First, MS is associated with HLA class II molecules HLA-DR2, -DR3, and -DR4. In humans it is difficult to analyze the individual roles of HLA molecules in disease pathogenesis due to the heterogeneity of MHC genes, linkage disequilibrium, the influence of non-MHC genes, and the contribution of environmental factors. However, the specific roles of each of these class II molecules can be addressed using transgenic models expressing these HLA genes. This model could prove useful in deciphering the role of HLA molecules and autoantigens in MS.[69] Second, while EAE has been a valuable model for the immunopathogenesis of MS, it has sometimes been difficult to reconcile the findings and therapies in the rodent models and the cellular and molecular interactions that can be studied in human disease. Humanized transgenic mice offer a means of achieving this, through the expression of disease-implicated HLA class II molecules, coexpressed with a cognate HLA-class II-restricted, myelin-specific T cell receptor derived from a human T cell clone implicated in disease. Such transgenic mice could provide an excellent model for studying epitope spreading in a humanized immunogenetic environment and for testing of immunotherapies.[70]

APPLICABILITY OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS MODELS OF MULTIPLE SCLEROSIS

The majority of the current therapies being planned for phase II and III trials in MS were first examined in EAE. Thus a particularly pertinent question is whether EAE is a suitable relevant research tool for MS? Some researchers believe that while EAE is a useful model of acute human CNS demyelination, its contribution to the understanding of MS is limited.[71] EAE is an acute monophasic illness, as compared to MS, which is a chronic relapsing disease, and may be more suited as a model of acute disseminated encephalomyelitis (ADEM). Drawbacks of the EAE model include the following: (1) the nature of the inflammatory response in EAE as compared to MS; (2) Th-1-mediated disease in EAE as compared to MS; (3) differences in the pathology between EAE and MS; and (4) pitfalls in extending immunotherapies from EAE to MS (see Table 69–3).

Consequently it may be concluded that the clinical picture of EAE presented depends not only on the animal species used, but also on the route of administration of the encephalitogen and the nature of the encephalitogen, MBP, PLP, or MOG. It is thus possible that these EAE models are somewhat imprecise methods to study the pathogenesis of MS or to develop therapeutic strategies.

The nonhuman primate EAE models are of primary importance for the safety and efficacy of testing new therapeutics for

Table 69–3
A comparison of some of the characteristics of EAE and MS[a]

Characteristics	*EAE*	*MS*
Inflammatory response	$CD4^+$ MBP-reactive T cells in perivascular lesions	Predominantly $CD8^+$ T cells in lesions, whereas $CD4^+$ T cells are infrequent
Th-1 or Th-2-mediated disease	Adoptive transfer of EAE by Th-1 cells	IFN-γ (Th-1 cytokine) seen in MS lesions
	Not true in all EAE models since MBP-reactive Th-2 cell clones can also cause EAE	No clear cytokine preponderance
Fundamental differences in pathology		
Location of pathology	Perivenous sleeves of myelin loss in spinal cord and brain	Demyelination not restricted to perivenous regions of white matter. Extensive demyelination of cerebral cortex
Location of lesions	Dependent on encephalitogen used; MBP-and PLP-induced EAE shows inflammation in lumbar spinal cord, MOG-induced EAE shows inflammation in the brainstem	Periventricular, brainstem, optic nerves, upper cervical cord
CSF immunology	Antibodies to myelin antigens in CSF	Antibodies to myelin antigens in CSF infrequent

continued

Table 69–3 (continued)

Characteristics	*EAE*	*MS*
Effect of immunotherapies		
IFN-γ	Variable and depends on route of administration	Worsens the inflammatory lesions?
IFN-β	Variable and depends on the route of administration	Decreases relapse rate
Anti-TNF Ab	Reverses EAE	Worsens MS
Anti-CD4	Cures EAE	No evidence of clinical efficacy on relapse or progression

Source: Adapted from Siriam and Steiner.[70]

[a]EAE, experimental allergic encephalomyelitis; MS, multiple sclerosis; MBP, myeloid basic protein; IFN, interferon; TNF, tumor necrosis factor; PLP, proteolipid protein; MOG, myeloid oligodendrocyte glycoprotein; CSF, colony-stimulating factor.

MS that may not work sufficiently well in species distant from humans, such as rodents. Questions concerning the immunogenicity of biological therapeutics have also been addressed in nonhuman primates. Many biological therapeutics, such as anti-CD4 antibodies[72] and altered peptide ligands,[73] have been investigated in rodents. Although some of these therapeutics have been effective in treating EAE in rodents, they have proven to be partially effective, or in some cases detrimental, in MS patients.[74] This ultimately raises the question of whether rodent models are the appropriate animal models for testing new therapeutic strategies for use in human MS.

CHEMICALLY INDUCED DEMYELINATION

There are several examples, in humans and animals, of demyelinating diseases not associated with viral infections, such as demyelination associated with vitamin deficiency or toxins. Many different animal models of EAE have been studied using various MRI techniques.[75] The clinical features of such models depend greatly upon the route of inoculation of the encephalitogen as well as the species and strain of animal used. Inoculation routes such as subcutaneous, footpad, or intraperitoneal are not helpful in determining the onset or location of the lesion in the brain or spinal cord. Thus, to create demyelinating lesions of precisely known locations and time courses, stereotaxic techniques are used to inoculate animals with chemicals that induce demyelinating lesions in the brain. Several chemicals, such as ethidium bromide, cuprizone, and lysophosphatidylcholine (LPC), when injected directly into nerves or into the CNS, produce lesions of demyelination. For demyelination studies with LPC, male Wistar rats are anesthetized with sodium pentathal and fixed in a rat head restraining stereotaxic surgical table, head shaved, a burr hole created, and 0.2 µl of a 1% LPC solution in isotonic saline injected using an injector cannula. Then LPC is infused at the rate of 0.05 µl/min for the next 4–5 min. The cannula is then removed and the burr hole closed using bone wax. Rats are then observed daily and histological studies are carried out from day 3 to day 15 after LPC injection to cover the entire process of disease evolution.[76]

Using this LPC-induced demyelination it is possible to observe the complete pathological process of demyelination and remyelination in this animal model of MS. Demyelination can be observed with the maximum value occurring on day 10. After day 10, remyelination starts with a reduction in edema. This model could be particularly useful for studying remyelination. One prominent feature of all chemically induced lesions is that the demyelinating lesions, and subsequent remyelination, can be studied without the interference of immune mechanisms. This has a tremendous advantage over virally induced models of MS: since no virus was inoculated none can remain to affect the remyelination.

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