



# Viral mouse models used to study multiple sclerosis: past and present

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## Abstract

Multiple sclerosis (MS) is a common inflammatory demyelinating disease of the central nervous system. Although the etiology of MS is unknown, genetics and environmental factors, such as infections, play a role. Viral infections of mice have been used as model systems to study this demyelinating disease of humans. Three viruses that have long been studied in this capacity are Theiler's murine encephalomyelitis virus, mouse hepatitis virus, and Semliki Forest virus. This review describes the viruses themselves, the infection process, the disease caused by infection and its accompanying pathology, and the model systems and their usefulness in studying MS.

## Introduction

The most common central nervous system (CNS) demyelinating disease in humans is multiple sclerosis (MS). There are an estimated 2.3 million people worldwide, and one million people in the United States alone, who are living with MS [1]. MS is 2–3 times more common in women than in men and is usually diagnosed between the ages of 20 and 50 [1]. Demyelination occurs when the protective myelin sheaths that surround axons are destroyed. Myelin is produced and maintained within the CNS by oligodendrocytes, one of the cell types that comprise glia. Primary demyelination is due to direct damage to the myelin and/or death of oligodendrocytes. In contrast, secondary demyelination occurs following neuronal damage and axon loss [2–6]. Axons are the long processes emanating from neurons along which impulses travel via saltatory conduction from the nerve cell body to other neurons and cells. Destruction of the myelin sheath results in disruption of saltatory conduction, slowing of the nerve impulses, axonal damage, and neuron death. The location of the lesions within the CNS determines the neurological symptoms, with the autonomic, visual, motor, and sensory functions being most commonly affected. MS patients can experience cognitive impairment, weakness,

spasticity and limb incoordination due to musculoskeletal dysfunction, impairment in speech and swallowing, bladder and bowel dysfunction, and optic neuritis, to name but a few of the symptoms of MS [7]. Ultimately, demyelination is debilitating.

Much of the research into demyelinating diseases has been focused on using animal models for MS, even though MS only occurs in humans. Demyelination can be caused by viral infections [8], and many of the animal models for MS are viral infection models. The most common animal used in these viral infection models is the mouse. In this review, we will take a closer look at three past and present murine viral infection models for MS/demyelination: Theiler's murine encephalomyelitis virus (TMEV), mouse hepatitis virus (MHV), and Semliki Forest virus (SFV). We will compare the viruses themselves and the disease pathology that they each induce in the mouse. Finally, we will discuss the usefulness of these viral infection models for the study of MS.

## The viruses

The three viruses – TMEV, MHV, and SFV – are members of three different virus families/genera: *Picornaviridae/Cardiovirus*, *Coronaviridae/Betacoronavirus* and *Togaviridae/Alphavirus*, respectively. As such, they are three very different viruses. TMEV and MHV are naturally occurring enteric pathogens of mice, although MHV can also naturally infect the respiratory system in mice [9, 10]. The natural route of infection for TMEV is thought to be fecal/oral [9, 11], while MHV can naturally infect through the fecal-urine/oral,

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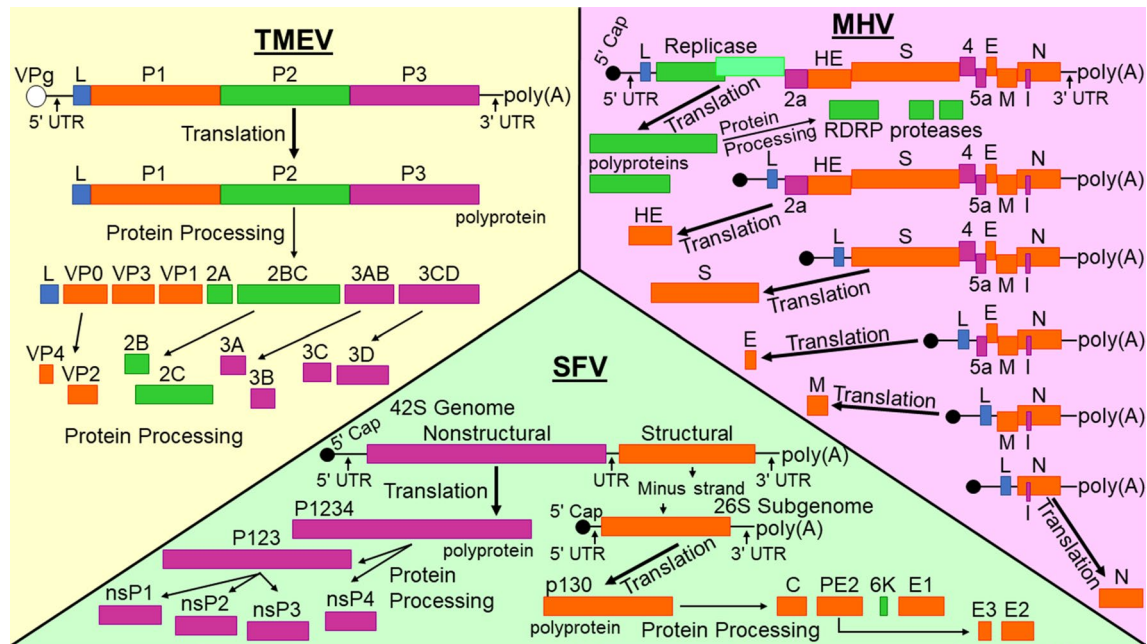
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intrauterine, and respiratory routes [10]. These two viruses do not naturally infect humans. SFV can be found in nature in humans, mosquitoes, wild birds, rodents, domestic animals, and non-human primates [12], although the natural host reservoir is unknown [13]. Transmission of SFV to humans occurs through mosquito bites [12, 13]. SFV infection of humans, although usually asymptomatic, was shown to cause an outbreak of febrile illness in central Africa, with patients, new to the area, presenting with the symptoms of fever, severe persistent headache (lasting up to 15 days, accompanied by a general weakness), myalgia (muscle ache), and arthralgia (joint pain) [14]. Additionally, SFV was found to be the etiological agent in a laboratory-acquired fatal case of meningoencephalomyelitis without demyelination [15].

All three viruses were isolated in the first half of the 20<sup>th</sup> century. TMEV and MHV were isolated – five strains of TMEV between 1932 and 1934 [11, 16], the Daniels (DA) strain of TMEV in 1948 [17], and MHV in 1947 [18] – from the CNS of mice with spontaneous hind limb flaccid paralysis. SFV was originally isolated from wild-caught *Aedes abnormalis* mosquitoes in 1942 in the Semliki Forest of western Uganda [19]. When injected intracerebrally into adult white mice, the filtrate from a ground up mosquito suspension caused hind leg paralysis, followed by death [19]. An avirulent strain of SFV (SFV A7) was subsequently isolated from *Aedes argenteopunctatus* mosquitoes in 1959 in Namancurra, Mozambique [20, 21]. A mosquito suspension proved fatal following intracerebral injection of infant mice [21]. However, intracerebral injection of a virus that had been passaged in infant mouse brains into adult mice resulted in 23% of them showing signs of illness, with most of the infected mice surviving with no signs of illness. This virus was determined to be immunologically identical to the SFV isolate from 1942 [21]. Thus, TMEV and MHV were isolated from mice and SFV was isolated based on its pathogenicity in mice. Additionally, all three viruses were serially passaged in the CNS of mice following the original isolation [11, 16–19, 21]. This passaging initially resulted in an increase in virulence (TMEV, MHV, and SFV) and a shortening of the incubation time (MHV and SFV) needed for the development of clinical signs of disease: encephalitic signs (ruffled fur, hunching, stunted growth, general lassitude, or excitability), paralysis, and death. For example, the original isolate of SFV was highly virulent following CNS passaging [19], causing death within two days in 3-day-old mice, and was invariably fatal in older mice (21 and 50 days of age) regardless of the route of infection (intracerebral, intraperitoneal, footpad) [22]. Passaging in tissue culture followed: TMEV in BHK-21 (baby hamster kidney) cells (cytopathic effect – rounding and detachment of cells, cell lysis) [23, 24], SFV in BHK-21 cells or chick embryo cells (cytopathic effect – rounding and detachment of cells, cell

lysis) [25–28], and MHV in DBT (murine-delayed brain tumor) cells (cytopathic effect – syncytium formation) [29]. Passaging and adaptation ultimately resulted in the production of tissue-culture-adapted viruses that consistently caused CNS demyelinating disease in mice (A7(74) strain of SFV; variants of the JHM [named for Prof. John Howard Mueller] strain of MHV; DA strain of TMEV) [17, 18, 30–32], which are the viral strains that will be explored in depth below. Of course, there are other strains/variants of all three of these viruses, such as the BeAn strain of TMEV, variants of JHM that do not cause demyelination, and further derivatives of SFV A7(74), but discussion/inclusion of all of the different versions of these viruses is beyond the scope of this review. For a direct comparison of the DA and BeAn stains of TMEV, see Zoecklein *et al.* [33].

All three of these viruses – TMEV, MHV, and SFV – are positive-sense single-stranded RNA viruses containing a nucleocapsid; however, MHV and SFV are enveloped viruses [28, 34], while TMEV is a nonenveloped virus [35]. Previously, plus-strand RNA viruses were grouped into three supergroups based on sequence relationships, and the picornaviruses (TMEV) and coronaviruses (MHV) group together in supergroup 1 while the alphaviruses (SFV) fall into supergroup 3 [36]. Furthermore, the sizes of the genomes of these viruses are different. TMEV is the smallest, at ~8,100 nucleotides, SFV is slightly larger, at ~13,000 nucleotides, and MHV is much larger, at ~31,000 nucleotides. The production of a new positive-strand RNA viral genome for all three viruses involves negative-strand RNA intermediates and the activity of virally encoded RNA-dependent RNA polymerases. The RNA genomes of all three viruses are polyadenylated at the 3' end, and either bound to a small virally encoded protein – VPg for TMEV [35] – or capped at the 5' end in the case of MHV and SFV (Fig. 1) [34, 36]. Being of positive sense (i.e., mRNA) and capped or bound at the 5' end and polyadenylated at the 3' end, the RNA genomes of all three of these viruses are infectious when introduced by transfection into permissive host cells [37]. The TMEV genome encodes four capsid proteins (VP1, VP2, VP3, and VP4) and seven nonstructural proteins, including the polymerase and a protease, which are all proteolytically cleaved from a single precursor polyprotein (Fig. 1) [35]. The SFV genome encodes one capsid (C) protein, three envelope proteins (E1, E2, and E3) that are embedded within a host-cell-derived lipid membrane, and four nonstructural proteins, which are involved in viral RNA synthesis, plus a small protein (6 kDa), encoded in the structural region of the genome, that is not incorporated into virions (Fig. 1) [28]. In the case of SFV, the genomic 42S RNA encodes the nonstructural proteins and a subgenomic RNA species, the 26S RNA, encodes the structural proteins. The structural and nonstructural proteins are translated as separate precursor polyproteins, which are proteolytically cleaved (Fig. 1) [28].



**Fig. 1** Genome organization of TMEV, SFV, and MHV and the process by which their viral proteins are produced. The diagrams show the complete viral RNA genomes and subgenomes (SFV and MHV) and the steps performed (translation and protein processing) to produce the various virus-encoded structural (orange) and nonstructural (green and purple) proteins. One MHV subgenomic RNA, encoding the four nonstructural protein, has been omitted for space and clarity. 2A, 2B, 2C, 3A, 3B, 3C, 3D, 2a, 4, and 5a, encoded nonstruc-

tural proteins; 6K, 6-kDa protein; C, capsid protein; E, small envelope protein; E1-3, envelope proteins 1-3; HE, hemagglutinin-esterase protein; I, internal protein; L, leader; M, membrane protein; MHV, mouse hepatitis virus; N, nucleocapsid protein; nsP1-4, nonstructural proteins 1-4; RDRP, RNA-dependent RNA polymerase; S, spike protein; SFV, Semliki Forest virus; TMEV, Theiler's murine encephalomyelitis virus; UTR, untranslated region; VP1-4, viral capsid proteins 1-4; VPg, small virus-encoded protein

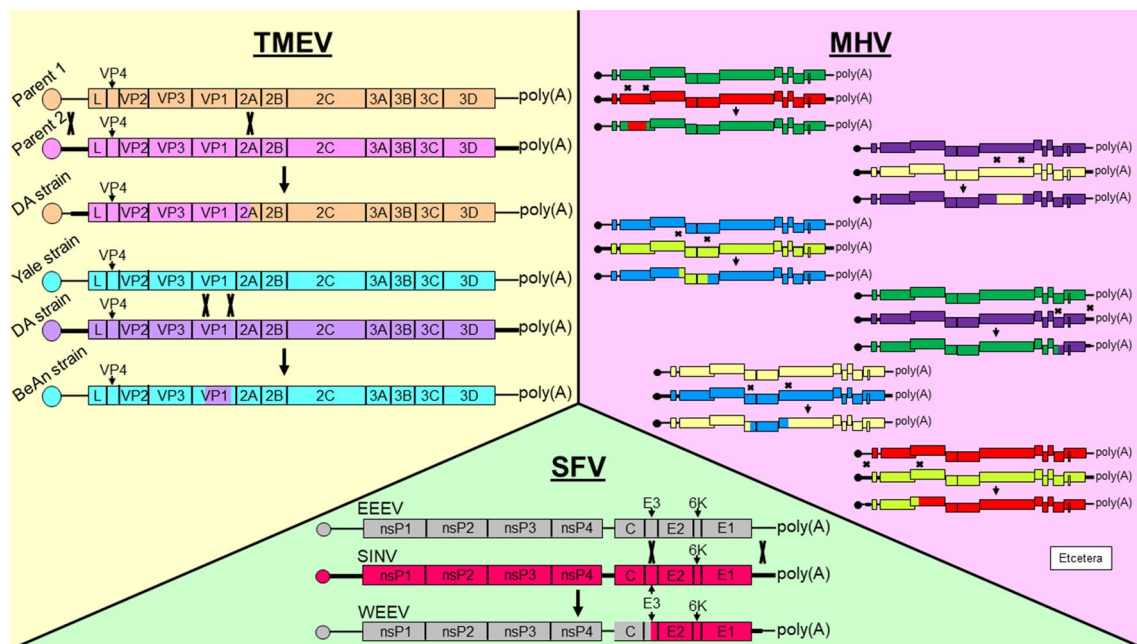
The MHV genome encodes one nucleocapsid (N) protein and three or four other structural proteins – the spike (S) protein, the membrane (M) protein, the small envelope (E) protein, and the hemagglutinin-esterase (HE) protein, which is encoded by some MHV strains (Fig. 1) – that get embedded into the host-cell-derived envelope [34]. In addition, up to 16 nonstructural proteins, including the polymerase and two proteases, are encoded within the replicase gene (Fig. 1) [34, 38, 39]. The replicase gene is the only MHV gene translated, utilizing a ribosomal frameshift mechanism, as two polyproteins (nonstructural proteins 1-11 and nonstructural proteins 1-10, 12-16) which are then proteolytically cleaved [37–39]. The rest of the MHV proteins are translated individually from a nested set of 3' co-terminal subgenomic mRNAs that are generated through a process of discontinuous transcription (Fig. 1) [34, 37, 38]. In addition to the structural and nonstructural proteins, TMEV and MHV also have a leader genomic region that is not present in SFV (Fig. 1). In the DA strain of TMEV, the leader (L) protein is processed from the N-terminus of the polyprotein, while the L\* protein is synthesized out of frame at the N-terminus of the polyprotein [40]. The L protein may play a role early in infection in the virus' escape from host immune defenses by inhibiting the expression of interferon  $\alpha/\beta$ , while L\* is important for virus

growth in macrophages, viral persistence, and demyelination [40]. On the other hand, MHV has an RNA leader sequence upstream of the protein coding region that functions to regulate and initiate subgenomic mRNA transcription [41]. All of the subgenomic mRNAs contain, at their 5' end, an identical 72-nucleotide leader sequence derived from the 5' end of the genomic RNA (Fig. 1) [41]. Although the three viruses have different-sized genomes, they all encode about the same number of proteins, eleven in TMEV, eight (plus up to 13 additional nonstructural proteins [from the replicase] and four additional accessory proteins [2a, 4, 5a, I]) in MHV, and nine in SFV. However, the number of proteins that make up the nucleocapsid differs among these viruses, four for TMEV and only one for both MHV and SFV. Both MHV and SFV encode three envelope proteins, although MHV sometimes encodes four. Additionally, the way in which the structural and nonstructural proteins are produced varies – from cleavage from a single polyprotein (TMEV) to cleavage from two separate polyproteins translated from the genomic and a subgenomic RNA (SFV) to both cleavage of a polyprotein and translation of individual proteins from a set of subgenomic mRNAs (MHV). Finally, the presence of leader proteins (L and L\* for TMEV), a leader RNA sequence (MHV), or no leader (SFV) is different for each virus.

Genetic recombination, which requires coinfection of a single cell with multiple viruses with compatible genomes, commonly occurs in positive-sense single-stranded RNA viruses [42]. Recombination contributes to the emergence of novel viruses, to changes in host tropism due to alterations in receptor usage, and to virus evolution, and it serves as a means to purge deleterious mutations generated through the action of the error-prone polymerase [42]. Early studies demonstrating recombination in RNA viruses used a picornavirus, poliovirus type I, and mixed infections with selectable mutants [43, 44]. It is thought that the primary mode of recombination involves switching of the RNA polymerase from one genome to another during synthesis of the negative-sense RNA, and there is evidence that the RNA-dependent RNA polymerase is necessary and sufficient to catalyze this switching of templates [35, 42]. A recent sequence comparison study using six strains of TMEV and analyzing for recombination events found two naturally occurring events (Fig. 2) [45]. One event demonstrated recombination between the DA and Yale strains of TMEV to produce the BeAn strain of TMEV. The second event demonstrated recombination between two unknown parental strains to produce the DA strain [45]. Recombination in togaviruses/alphaviruses is rare, other than a likely recombination event between eastern equine encephalitis virus (EEEV), contributing the nonstructural and C proteins, and an as

yet unknown Sindbis-like virus, contributing the envelope proteins, which produced western equine encephalitis virus (WEEV) (Fig. 2) [32, 42, 46–48]. A genome-scale phylogeny analysis of members of the genus *Alphavirus* ruled out additional recombination events in other genome regions and between other known alphaviruses [46]. In contrast, RNA recombination is an important characteristic of coronavirus RNA synthesis (Fig. 2) [34]. Mixed-infection experiments *in vitro* showed that the RNA genomes of different MHV strains could recombine at a very high frequency [49–51]. Recombinants of MHV could also be recovered at a high frequency from the brains of mice following mixed-infection experiments *in vivo* [52]. A study using only a small region of the MHV genome demonstrated that recombination was random over the entire region, but clustering of recombinant sites occurred as a result of selection for or against certain recombinants [53]. Additionally, “hotspots” for recombination have been shown to positively correlate with RNA secondary structure in coronaviruses, suggesting that both host selective pressure and viral RNA secondary structure contribute to the types of recombinants observed [54].

The physical structures of the viruses are alike in some ways and different in others. TMEV virions are 30-nm-diameter spheres made up of a protein shell, the capsid, surrounding the naked RNA genome, thus forming a nucleocapsid structure [35]. The TMEV capsid consists of the structural



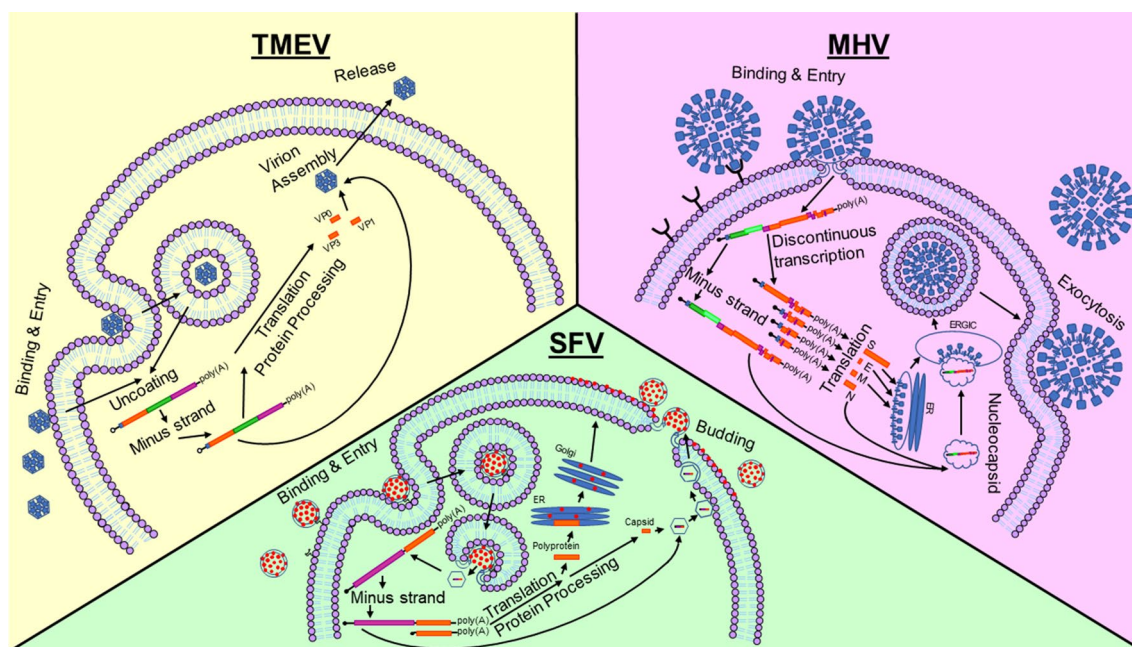
**Fig. 2** Genetic recombination of TMEV, SFV, and MHV. The diagrams show the specific known recombination events for TMEV (two events) and SFV (one event) and a representation of some of the many recombination events that are possible for MHV. 2A, 2B, 2C, 3A, 3B, 3C, and 3D, encoded nonstructural proteins; 6K, 6-kDa protein; C, capsid protein; E1–3, envelope proteins 1–3; EEEV, east-

ern equine encephalitis virus; L, leader; MHV, mouse hepatitis virus; nsP1–4, nonstructural proteins 1–4; SFV, Semliki Forest virus; SINV, Sindbis virus; TMEV, Theiler’s murine encephalomyelitis virus; VP1–4, viral capsid proteins 1–4; WEEV, western equine encephalitis virus

proteins VP1-3, which are external, and the VP4 protein, which is internal to the other three proteins [55]. The surface of the TMEV capsid contains 60 capsomers, each containing a single copy of the four structural proteins, arranged in an icosahedral lattice [35, 55]. VP1, VP2, and VP3 structural proteins are able to pack together into a sphere based on all of them having an eight-stranded antiparallel  $\beta$ -barrel topology. The surface topography of the TMEV capsid is dominated by a star-shaped plateau found at the fivefold axis of symmetry. There are also a series of pits in the surface of the TMEV capsid that have been proposed to function in receptor binding [35, 55]. Additionally, at the surface of the capsid near the rim of the pits, a gap created between exposed loops of both VP1 (loop II) and VP2 (puff B) is believed to participate in receptor/coreceptor binding [56–60]. At more than double the size, SFV virions are ~65-nm-diameter spheres [32, 61]. A protein capsid with icosahedral symmetry is formed by 240 copies of the single C protein, and this capsid encloses the genomic RNA, forming the nucleocapsid structure. The capsid is in turn enclosed within a lipid envelope derived from the plasma membrane of the host cell, and the envelope contains the viral E1 and E2 proteins. The 240 copies each, per virion, of the E1 and E2 proteins heterodimerize, and these heterodimers in turn form trimers, resulting in 80 knobs that protrude from the surface of the SFV envelope and participate in receptor binding (E2 protein, knobs) and membrane fusion (E1 protein,

skirt area under the knobs) [32, 61]. MHV virions are generally spherical and are larger than TMEV or SFV, with a diameter of ~85 nm [62], a size that is dictated by the much larger MHV genome. MHV virions do not contain a protein shell. Instead, the N protein complexes with the genomic RNA to form a compact, helical ribonucleoprotein structure that closely follows the lipid envelope, which, at twice the thickness of a typical biological membrane, is unusually thick. The S protein of MHV forms trimeric, club-like spikes that extend radially from the surface of the envelope, giving the virion a solar-like appearance (hence the name “coronavirus”), and which function in receptor binding and membrane fusion [62]. Thus, all three viruses are spherical, although their sizes differ. They also all have nucleocapsids, although their composition differs. Both TMEV and SFV have icosahedral protein shells, made up of four proteins and one protein, respectively, surrounding naked RNA, while MHV has a single protein associated with the genomic RNA without a protein shell. Additionally, the surface features of the virus that are involved in receptor binding differ – from pits and loops on the surface of the capsid of TMEV, to projections from the surface of the envelope: knobs for SFV and spikes for MHV.

Replication of the viruses (Fig. 3) also has common aspects among the three viruses, as they are all positive-sense RNA viruses, but glaring differences are evident as well. TMEV virions attach to an as yet unknown host cell



**Fig. 3** Replication of TMEV, SFV, and MHV. The diagrams show viral binding and entry, genome replication through minus-strand RNA, discontinuous transcription for MHV, virion/nucleocapsid assembly, and release. For details of translation and protein processing, see Fig. 1. E, small envelope protein; ER, endoplasmic

ERGIC, endoplasmic reticulum-Golgi intermediate compartment; M, membrane protein; MHV, mouse hepatitis virus; N, nucleocapsid protein; S, spike protein; SFV, Semliki Forest virus; TMEV, Theiler’s murine encephalomyelitis virus; VP0, 1, 3, viral capsid proteins 0, 1, 3

surface receptor, although sialic acid is thought to function as a coreceptor and the P0 protein may function as a receptor in the peripheral nervous system [57–60, 63, 64]. Following attachment, the virions must be uncoated, which involves capsid dissociation and release of the viral RNA into the cell – steps that have not been well characterized. Based on other picornaviruses, the virion has been suggested to enter the cell through the endocytic pathway [35]; however, there is evidence to suggest that capsid dissociation with RNA release occurs on the plasma membrane without involving endocytosis [65]. Also, unlike other picornaviruses, uncoating of TMEV occurs without the generation of stable intermediates [65]. The released RNA genome is then translated into proteins and used for the synthesis of negative- and positive-sense RNAs within the host cell cytoplasm [35]. Positive-stranded RNAs are incorporated into virions, which are then released from the host cell by lysis [35]. SFV virions may use the major histocompatibility complex class I molecule of mice or humans as a receptor, and the E2 protein of SFV plays an important role in receptor binding [32, 61]. Entry of the virion into the host cell is dependent on endocytosis and acid-induced fusion with the vesicle membrane facilitated by the E1 protein [32, 61]. Viral replication occurs in the host cell cytoplasm following release of the nucleocapsid core and core disassembly, which occurs via a mechanism yet to be determined [61]. The polyprotein that contains the envelope (E1 and E2) structural proteins is translocated across the endoplasmic reticulum membrane, where the proteins are processed and mature and are transported through the endoplasmic reticulum to the Golgi and on to the plasma membrane. Elsewhere in the cytoplasm, genomic RNA is packaged within the capsid, as certain amino acids in the capsid protein recognize a packaging sequence within the viral genome, and the resulting nucleocapsid core assembly associates with the envelope proteins at the plasma membrane, where budding occurs [61]. MHV virions attach through their surface spikes to their host cell surface receptor, mCEACAM1 (murine carcinoembryonic antigen-related cell adhesion molecule 1), mCEACAM2 [66–68], or an unrelated and as yet unidentified alternative receptor in the CNS [30], and fusion occurs between the viral envelope and the host cell plasma membrane [34, 37]. The ribonucleoprotein structure is delivered to the cytoplasm, where the genomic RNA is translated, using subgenomic mRNAs and discontinuous transcription, and viral replication takes place. The assembly of progeny virus involves the insertion of the M, S, E, and, sometimes, HE viral proteins into the endoplasmic reticulum membrane and the transit of these proteins to the endoplasmic reticulum-Golgi intermediate compartment. Here, the genomic viral RNA, containing packaging signals not present in the subgenomic mRNAs, complexed with the N protein associates with the viral envelope proteins to form new virions,

which bud into the endoplasmic reticulum-Golgi intermediate compartment. Progeny virions are then transported to the plasma membrane of the cell and released through exocytosis [34, 37]. Therefore, all three viruses replicate within the cytoplasm of the host cell, but the mechanisms of entry into the cytoplasm differ (Fig. 3). Endocytosis is employed by SFV and possibly TMEV, and membrane fusion is employed by both SFV and MHV, although the target membrane is different: the endocytic vesicle membrane for SFV and the plasma membrane for MHV. The mechanism of release of the viral progeny also differs: cell lysis for TMEV, budding from the plasma membrane for SFV, and, budding into the endoplasmic reticulum-Golgi intermediate compartment and exocytosis for MHV (Fig. 3).

Replication of positive-stranded RNA viruses can result in the production of defective interfering (DI) particles, which lack portions of their RNA genomes [42, 69]. DI particles are usually produced by high multiplicity passaging of viruses in cell culture or by persistent viral infections of cultured cells [28, 70]. DI particles have been found for MHV and SFV [27, 71, 72], and although DI particles have not been found for TMEV [24, 73, 74], other picornaviruses [75, 76] and other cardioviruses [76, 77] have been shown to produce DI particles. These aberrant/truncated genomes can replicate and be encapsidated, but they usually lack structural genes [42, 69]. As such, DI genomes can be encapsidated if they are in the presence of capsid proteins provided by other, full-length viral genomes. DI RNAs can compete effectively with full-length genomes for capsids, resulting in a reduction in the yield of viable progeny virus [42, 69, 70]. This interfering activity was identified *in vitro*, but some DI particles have been shown to be protective *in vivo* [72]. The sequence deletions present in DI RNAs may be the result of recombination events [42]. In alphaviruses, an increased error rate (reduced fidelity) of the polymerase has been linked to both an increase in recombination events and an increase in the accumulation of DI particles [78].

## The infection

All three viruses are neurotropic viruses that infect cells of the CNS [79]. SFV is neuroinvasive, meaning that the virus travels to and effectively enters the CNS following intraperitoneal infection. TMEV and MHV are much less efficient at neuroinvasion, e.g., neurologic disease develops in only about 1 in 2000 mice naturally infected with TMEV, and therefore these viruses are introduced intracerebrally into experimental mice. Following direct CNS infection, TMEV uses axonal transport within the CNS to spread from the gray matter of the brain and spinal cord to the white matter of the spinal cord [80]. MHV can also use axonal transport, as evidenced by entry of the virus into the CNS

via the trigeminal and olfactory nerves following intranasal inoculation [81, 82]. SFV is transported from the peripheral (intraperitoneal, subcutaneous) site of infection to the CNS via the blood (viremia) [79, 83]. This viremia is a plasma viremia with virus free in the plasma rather than being cell-associated [84]. The SFV A7 strain can be found in blood almost immediately following intraperitoneal infection. It reaches an equilibrium in the blood by 40 minutes postinfection, and virus can be detected in the brain by 24 hours postinfection [85]. SFV then spreads throughout the CNS via axonal transport [28, 86, 87]. SFV can also be inoculated intranasally, and in this case, transport to the CNS occurs through the olfactory nerves via both anterograde and retrograde axon transport without viremia; however, this axonal transport is reduced with neuronal maturation, which correlates with the age of the host [86, 87]. Upon CNS infection, TMEV [88–92] and MHV [93] can both be localized to neurons, oligodendrocytes, astrocytes, microglia, and macrophages. SFV can be localized to neurons [87, 94, 95], oligodendrocytes [31, 32], and vascular endothelial cells in the brain [94].

The three viruses vary in their ability to persist within the infected host. The DA strain of TMEV persists [11, 17] within glial cells (microglia, macrophages, oligodendrocytes) in the white matter of the spinal cord throughout the life of the susceptible mouse [80]. Both infectious virus and infectious RNA (30-fold more than virus) have been isolated from the spinal cords of DA-infected mice at six months postinfection [96]. The ability of the DA strain of TMEV to persist may be dependent on its ability to utilize sialic acid as a coreceptor [60]. Sialic acid facilitates the binding to and infection of macrophages and enables axonal transport of the virus from the gray matter to the white matter of the spinal cord. Finally, sialic acid sterically impedes the binding of antiviral antibodies, which otherwise could function to clear the virus, to virus attached to the surface of cells [60]. Indeed, TMEV is able to persist even in the presence of intrathecal virus-specific antibody production, which was found to correlate with the viral load within the CNS during chronic infection [97]. Chronic inflammation and demyelination result from this persistence of infectious TMEV [80]. In those strains of mice that are able to clear infectious MHV, virus is present in the CNS for one week postinfection, clearance is mainly through the actions of CD8<sup>+</sup> T cells [98], and the virus is undetectable in the CNS in the second week postinfection [79, 99]. MHV, however, also persists within survivors of acute infection that are unable to completely clear the virus from the CNS [79, 99]. In fact, low levels of viral RNA have been found to persist in the brain for over two years following intracerebral MHV infection [100]. MHV is thought to persist primarily in astrocytes within the brain and spinal cord [101], although a temperature-sensitive mutant of the JHM strain, which causes acute demyelination

in 90% of intracerebrally infected mice while having only a <5% mortality rate, was shown to persist mainly in oligodendrocytes, was infrequently found in astrocytes, and caused chronic recurrent demyelination [102, 103]. B cells play an important role in preventing reactivation of the persistent virus in the CNS following resolution of the acute disease [30, 104]. Antibody-secreting cells were retained within the CNS throughout the chronic MHV infection, suggestive of intrathecal virus-specific antibody production [105]. For MHV, chronic ongoing CNS demyelination occurs in the absence of detectable infectious virus [30, 99]. In contrast, infectious SFV A7 is cleared from the brain by the immune response of the host by day 12 postinfection [106]. Antibody-secreting cells were detectable in the CNS by day 9 postinfection, suggesting intrathecal antibody production, and these cells were later found associated with areas of demyelination [107]. As an exception to viral clearance, viral RNA from the avirulent M9 mutant strain (produced by chemical mutagenesis) of SFV has been detected for up to 90 days postinfection even though infectious virus was cleared by 7 days postinfection, depending on the mouse strain [108]. More recently, although infectious virus was undetectable in immunocompetent mice by standard infectivity assays by day 10 after infection with the SFV A7 avirulent strain, immunosuppression of antibody responses in previously infected aged mice (40 or 80 weeks postinfection) resulted in the renewed ability to detect infectious virus [109]. Therefore, full-length, functional, genomic viral RNA capable of producing infectious virus can persist within the brains of these mice.

Outside of the CNS, TMEV can be found early in the blood [17]. MHV can be found in liver, spleen, lungs, kidneys [18], intestine, and pancreas [10], and SFV can be found in the blood, lungs, heart, spleen, kidneys, liver [19, 22], and muscle (peritoneal wall, hind limb muscle) [110].

Infection with recombinant chimeric viruses, created through the exchange of genomic regions between closely related virulent strains that cause severe acute disease resulting in death and avirulent strains that cause mild acute disease that is survivable and may lead to chronic disease, or through selection of viral variants (such as neutralization escape mutants) with altered pathogenicity, followed by sequence comparisons and the subsequent generation of viruses with single point mutations, has allowed the viral determinants that influence virulence to be identified. For the DA strain of TMEV, in addition to its contribution to coreceptor binding, the VP1 protein is the main determinant of viral virulence (viral persistence and chronic demyelination within the CNS) [111–114]. Specifically, amino acid 101 of VP1, which is exposed at the tip of VP1 loop II, is a determinant of persistence and demyelination [115–117]. Another determinant of persistence and demyelination within the CNS for the DA strain of TMEV is amino acid

173 of VP2, which is located at the exposed tip of the VP2 puff B loop [118]. A third determinant of persistence and demyelination for DA is amino acid 141 of the VP2 protein, which is located at the exposed tip of the VP2 puff A loop, also at the rim of the pit, and which plays a role in the efficiency with which the virus is able to spread within the CNS and infect the white matter of the spinal cord [118–120]. Likewise, alterations in amino acid 81 of VP1 loop I, located at the exposed tip of loop I, and amino acid 91 of VP1, located in a hydrophobic patch on the wall of the pit, also affect the virulence of the DA strain of TMEV [121]. A threonine-to-aspartate (T81D) alteration at the tip of loop I resulted in much-reduced chronic demyelination and viral persistence, while a valine-to-threonine (V91T) alteration on the pit wall resulted in the absence of both chronic demyelination and viral persistence [121]. Finally, an additional VP1 determinant of demyelination (persistence was not examined) of the DA strain of TMEV is amino acid 268 at the carboxyl terminus of the protein, which is disordered and exposed on the surface of the capsid [118, 122]. For MHV, the S protein has been shown to be the predominant viral virulence factor [30, 123]. At least three domains within the S protein – the receptor-binding domain, the hypervariable region, and the heptad repeat domains – have an influence on neurovirulence (the ability to cause disease of the CNS). An additional viral virulence factor that influences neurovirulence is the N protein [30, 124]. For SFV, the determinants of viral virulence have not been examined in as much detail, but they are multiple, involving the E2 protein, the non-structural proteins nsP2 and nsP3, and the 5' untranslated region of the viral genome, possibly all acting in combination [13, 28, 125–129]. Additionally for SFV, it was found that the degree of virulence of chimeric viruses correlated well with the spread and distribution of the virus within the CNS [129]. In general, viral strains capable of inducing extensive damage to neurons were found to be more virulent, and viral virulence was at least in part dependent on proteins/regions involved in receptor/coreceptor binding, suggesting that alterations in receptor binding affinity affect pathogenicity.

Viral proteins/regions involved in receptor/coreceptor binding are also known targets of host-produced neutralizing antibodies. With TMEV infection, neutralizing antibodies appeared in the serum on day seven postinfection, rose until two months postinfection, and then remained steady until five months postinfection [130]. Additionally, neutralizing antibodies were found in the CNS of TMEV-infected mice during the chronic stage of infection [131]. Neutralizing antibodies with specificity for the VP1 capsid protein [132] and the VP2 capsid protein have been characterized [118]. In VP1, both amino acid 101, at the exposed tip of loop II, and amino acid 268, also exposed on the surface, have been found to be essential parts of neutralizing epitopes

recognized by specific neutralizing monoclonal antibodies [116, 133]. The neutralization site in VP2 for a well-characterized neutralizing monoclonal antibody consists of at least three amino acid residues: 141 and 143 at the exposed tip of VP2 puff A and 173 at the exposed tip of VP2 puff B [118]. In MHV infection, neutralizing antibodies were first detected as early as day six postinfection in surviving animals [134]. Neutralizing antibodies against MHV were sustained at constant levels following control of the viral infection in the CNS [105]. Neutralizing antibodies were induced by antigenic sites found on the glycoproteins on the surface of the MHV virion, with the S protein being the major target [10, 135–138]. More specifically, clustered neutralization epitopes were localized within 330 amino acids of the N-terminus of the S protein [137–140]. In SFV infection, antibodies were present in the blood at four days postinfection and in the brain at six days postinfection [85]. Neutralizing antibody in the serum reached a maximum by six days postinfection and was maintained thereafter [106]. Neutralizing antibodies were found directed against both the E1 and E2 proteins of SFV [141–143]. Neutralization can occur either against virus in suspension or against virus bound to the surface of cells, and, in addition to affecting receptor binding, neutralization can also affect later steps of the infection process, such as uncoating and RNA release in the case of TMEV [65], or internalization and membrane fusion in the case of SFV and MHV.

## The disease

The course of disease following TMEV infection depends on the passage history of the DA strain of TMEV used for inoculation. Intracerebral infection of susceptible mice (Swiss, SJL) with the DA strain of TMEV (brain passaged) causes a biphasic disease [130, 144]. One to two weeks after infection, a mild acute gray matter polioencephalomyelitis develops that lasts two weeks and from which the mice recover. Transient hind limb flaccid paralysis is observed during the acute phase. Then, 1–2 months after infection, a chronic, progressive, inflammatory demyelinating disease of the white matter develops. The demyelination mainly affects the spinal cord, resulting in a spastic gait disturbance [130, 144]. In contrast, infection with tissue-culture-passaged DA does not result in acute disease, but the late persistent demyelinating infection of the white matter with gait disturbance is still apparent [24]. Viral persistence is necessary for the induction of the demyelinating disease [145, 146].

Intracerebral infection of susceptible mice (C57BL/6, BALB/c, Swiss) with the original JHM strain of MHV results in a usually fatal, monophasic acute encephalitis with demyelination [10, 147]. However, one group found that the JHM strain of MHV that they received to study required



seven passages in suckling mouse brains (Swiss mice) before the pathologic sign of demyelination was apparent [134]. The inability to clear virus in rare survivors results in chronic ongoing CNS demyelination [99]. These mice display incoordination, and they develop hind limb paralysis and, in some animals, tetraplegia [10, 147]. Less virulent strains/isolates/variants/mutants of MHV result in milder acute encephalitis, with myelin loss, which the majority of the mice survive, followed by chronic ongoing demyelination [147]. The cellular tropism of the virus strain appears to determine the course of disease, with MHV strains with tropism predominantly for neurons causing fatal encephalitis and MHV strains with tropism predominantly for oligodendrocytes causing demyelination [148].

Intraperitoneal infection of Swiss/A<sub>2</sub>G mice with the avirulent A7 strain of SFV occasionally results in acute hind limb paralysis (5% of mice) and ruffled fur, from which the mice recover by day 14 postinfection [149, 150]. All adult mice survive. Intranasal SFV infection gives more consistent results, being a more direct route to the CNS [28, 125], although it requires a higher dose of virus [19]. Males have been found to be more susceptible than females to SFV infection [151].

Disability is due to both neuronal death and demyelination. Demyelination results from the death of the myelin-producing cells, the oligodendrocytes. The three viruses can induce neuronal and oligodendrocyte death through different mechanisms: necrosis, in which death is due solely to cellular injury or malfunction, or apoptosis, which is programmed cell death under the genetic and functional control of the cell and which requires energy [25, 152, 153]. In TMEV infection, the DA strain of TMEV induces apoptosis in neurons (many of which are infected by the virus) during the acute stage of infection when demyelination is absent, and apoptosis in oligodendrocytes (which are not infected by the virus) in the spinal cord white matter during the chronic demyelinating stage of the disease [154]. For those MHV strains with a tropism predominantly for neurons, cell death of neurons occurs via apoptosis [155]. Necrosis is induced in MHV-infected oligodendrocytes early in the development of the demyelinating disease, whereas later in the demyelinating disease, when only low levels of viral RNA can be detected, oligodendrocyte death occurs through apoptosis [156]. SFV infection of mature (adult) neurons is generally nondestructive, and this can result in viral persistence in immunocompromised hosts [13, 84]. However, SFV induces apoptosis in immature neurons and neurons that are continuously being replaced and undergoing differentiation, such as some found in the olfactory bulb, whereas the death of mature neurons occurs via necrosis [28, 157, 158]. SFV also induces apoptosis in oligodendrocytes in cell culture [25], but the mechanism of cell death of oligodendrocytes *in vivo* has not yet been determined. The nonstructural region of the

SFV genome has been determined to be necessary for the induction of apoptosis [28, 153].

The three viruses have been tested for their ability to confer protection against subsequent viral challenge. It was found that mice infected with TMEV by the intranasal or intracerebral route were protected against later challenge with TMEV administered by the intracerebral route [11, 16]. Likewise, mice, older than 19 days, infected with SFV A7 by the intraperitoneal, intracerebral, or intranasal route were protected against a later lethal challenge with the original isolate of SFV given intraperitoneally or intracerebrally [20]. In contrast, mice infected with MHV by the intramuscular, subcutaneous, or intravenous route were not protected from subsequent challenge with MHV administered intracerebrally [82].

The host range of TMEV by the intracerebral route is limited to mice and cotton rats [17, 159], and chronic demyelination and viral persistence only occur in mice. In addition to mice, MHV is able to cause disease in rats [160], hamsters [18], and non-human primates [161] following intracerebral infection. SFV is able to infect rats, following intranasal inoculation [158], and voles, following footpad and intracerebral inoculation [162], often without clinical signs of disease, as well as cause overt disease in hamsters following infection by inhalation [163] and guinea pigs, rabbits, and non-human primates following intracerebral inoculation [19].

## The pathology

TMEV infection of the CNS results in perivascular cuffing, necrosis of ganglion cells (only in suckling mice for DA) [11, 17], meningeal inflammation, demyelination, which presents as large demyelinating lesions of the white matter tracts of the spinal cord, and some axonal loss [17]. Of note, skeletal muscle lesions (myositis) occur following intracerebral injection of suckling mice and intramuscular injection of weaned mice [17]. Additionally, Tsunoda and colleagues have developed a mouse model of viral myocarditis, a virus-induced inflammatory disease of cardiac muscle, induced through intraperitoneal injection (also intracerebral injection with a slightly lower frequency of disease) of C3H mice with the DA strain of TMEV [164–166]. The acute phase of the biphasic CNS disease in weaned mice is characterized by virus-infected neurons and inflammation of the gray matter of both the brain and spinal cord [167], and this parenchymal involvement of the gray matter was seen by day 10 and reached a maximum at days 15–20 postinfection [130]. The chronic phase of the biphasic disease is characterized by virus-infected cells (glia, macrophages), inflammation, and demyelination of the white matter of the spinal cord [167], with maximal involvement of the spinal cord occurring at

2-3 months postinfection [130]. Axonal damage has been shown to precede demyelination in TMEV infection [3]. Demyelinating lesions occur in areas of inflammation [130], and the inflammatory cells found at the sites of white matter spinal cord lesions include lymphocytes (highly activated CD4<sup>+</sup> T cells), plasma cells, and monocytes/macrophages [93, 144]. Generalized immunosuppression has been shown to prevent both cellular infiltration and demyelination in TMEV-infected SJL mice [168]. However, athymic nude mice, which lack T cells, developed demyelination by day seven postinfection [169]. Also, specific genetic depletion of CD4<sup>+</sup> T cells increased the extent of demyelination and the incidence of clinical disease, while, in contrast, specific genetic depletion of CD8<sup>+</sup> T cells did not change the extent of demyelination but did reduce the incidence of clinical disease in TMEV-infected (DA strain) SJL mice [170]. However, antibody depletion of CD8<sup>+</sup> T cells did decrease the extent of inflammation and demyelination in the spinal cord during the chronic phase [171]. All evidence suggests that the early acute disease is likely to be caused by direct cell lysis by the virus, while the late disease, which may have some lytic component due to viral persistence, is most likely due to immunopathological mechanisms directed against infected glial cells [130, 168, 170]. Late in the chronic phase, autoimmune CD4<sup>+</sup> T cell responses against myelin are observed [172, 173].

Acute MHV infection of the CNS results in widespread demyelination, mostly in the spinal cord, meningeal inflammation, cellular infiltration of the brain parenchyma, and necrosis, all of which mainly affect the white matter of the CNS, plus liver lesions [82]. Perivascular cuffing is also present within the CNS [174–176]. Surviving, persistently infected animals appear to recover from the acute disease, starting in the third week following inoculation, and undergo remyelination (assessed via electron-microscopic autoradiography), apparently mediated by newly generated oligodendroglia. However, small areas of active demyelination persist or recur [177, 178]. Infection of susceptible strains of mice with neuroattenuated strains of MHV that do not cause fatal disease results in chronic demyelination characterized by destruction of the myelin sheaths [93, 134, 147]. Axonal damage has also been shown to precede demyelination in MHV infection [3], which can be seen as early as the first week postinfection, suggesting direct viral pathology [79]. Also, generalized immunosuppression reduces cellular infiltration, does not prevent demyelination, but allows a non-fatal viral infection to progress to a fatal viral infection [134]. Therefore, early demyelination seems to be due to direct cytolytic effects of the virus on oligodendrocytes, which are infected by the virus [179, 180]. In contrast to direct cell lysis by the virus, the role that the immune system plays in demyelination has also been studied for MHV infection, and an immunological basis for demyelination is

supported [181]. The inflammatory cells found at the sites of white matter spinal cord lesions include lymphocytes and macrophages [30, 93]. Although neither subset of conventional CD4<sup>+</sup> and CD8<sup>+</sup> T cells is required for demyelination, each is independently capable of producing MHV-induced demyelination in an adoptive transfer model [182]. Thus, demyelination appears to be due to both direct viral lysis and the persistent effect of antiviral immune responses. Auto-immune responses against myelin have not been found to contribute significantly to demyelination in MHV infection [183].

Peripheral infection with the original isolate of SFV results in perivascular cuffing, cellular infiltration, neuron loss, and demyelination [19, 22]. Peripheral infection with the A7 isolate of SFV results in demyelination in 25% of the mice, and inflammatory pathology (perivascular cuffing, meningeal inflammation, and microcystic foci) in 95% of the mice [149, 150]. Perivascular cuffs are apparent at three days postinfection [79], and the acute encephalitis affecting both the brain and spinal cord, with focal lesions and perivascular cuffing of lymphocytes, resolves by six weeks postinfection [184, 185]. Demyelination of the brain and spinal cord can be seen as early as day 10 postinfection, is maximal between 14 and 21 days postinfection, and is largely recovered (assessed via electron-microscopic autoradiography) in some mice (but not all) by 28–36 days postinfection [31, 186, 187]. Infection of Swiss/A<sub>2</sub>G mice with an avirulent SFV A7 variant of SFV was also shown to result in patchy demyelination, accompanied by cellular infiltration and microcystic changes, in the optic nerve between 11 and 21 days postinfection [188]. Additionally, intranasal infection of BALB/c or SJL mice with SFV A7 also resulted in demyelination of the olfactory pathways [189]. The demyelination induced by the A7 isolate is dependent on the immune response of the host, not on direct viral damage, as determined using irradiation [190] and chemical [186] immunosuppression experiments in which demyelination was reduced or eliminated. It is likely that antibodies are protective and that T cells contribute to the demyelination [186, 190]. This is suggested by the findings that activated lymphocytes are seen in close proximity to lesions via electron microscopy [31] and that demyelination is not observed after infection of athymic nude mice, which lack T cells, even though SFV persists in the CNS [106]. Also, demyelination can be prevented by *in vivo* depletion of CD8<sup>+</sup> T cells [191], and no demyelination is seen in SCID (severe combined immunodeficiency) mice, which lack T and B cells, even though SFV persists in the blood and CNS of SCID mice [79, 83]. Therefore, based on the nude and SCID mouse studies, it appears that serum antiviral antibodies can clear virus from the periphery, but not the CNS, while T cells are pathogenic [13, 83, 84, 106]. Based on the requirement for CD8<sup>+</sup> T cells for demyelination [191] and the infection of oligodendrocytes by SFV

A7 [31, 32], a likely mechanism for demyelination is CD8<sup>+</sup> T-cell-mediated destruction of infected oligodendrocytes [13]. Following viral clearance, autoimmune responses against myelin are observed [192, 193].

To recap, the timing, mechanism, and resolution of the demyelinating pathology varies for these three viruses. SFV demyelination is acute, reaching a maximum between 14 and 21 days postinfection, is immunopathological, and is fully reversible in most of the mice. MHV demyelination is both acute and chronic and caused by both immunopathology and direct cytolytic effects of the virus, and small areas of active demyelination persist or recur. TMEV demyelination is chronic at 2–3 months postinfection, is mostly immunopathological, and large demyelinating lesions persist for the life of the mouse.

## The models

Clinically, MS is characterized by acute relapses with remissions followed by a secondary progressive phase in 85% of patients; the other 15% of patients are progressive from onset [1]. The cause of MS is unknown and may be different in different patients; however, genetics and environmental factors, such as infectious agents, play a role [7, 194, 195]. Herpesviruses, such as Epstein-Barr virus and human herpesvirus 6, as well as human endogenous retroviruses, and torque teno virus have been suggested to trigger the disease process and have been implicated in disease exacerbations, as reviewed elsewhere [194, 195]. Potential trigger mechanisms for demyelination and disease induction include direct lysis of oligodendrocytes by viral infection, lysis of infected oligodendrocytes by virus-specific cells of the host immune response, lysis of uninfected oligodendrocytes by an autoimmune response triggered by infection, and lysis of infected and/or uninfected oligodendrocytes by an indirect nonspecific bystander immune response triggered by infection [145]. Over the many years that MS has been studied, several viruses, including coronaviruses, have been isolated from MS patients and thus have been circumstantially associated with the disease [145]. Coronavirus RNA and antigens have been found in active demyelinating plaques in the brains of MS patients [196, 197], and antibodies specific for coronavirus were more frequent and of higher titer in the cerebrospinal fluid of MS patients when compared to controls [198]. Although it is tempting to suggest that coronaviruses are pathogenic in MS based on this association, it is possible that patients with MS are just more susceptible to CNS infection [196]. Although the event or agent that triggers MS remains unknown, MS is considered to be the prototypical immune-mediated demyelinating disease [173]. MS manifests pathologically as acute focal inflammatory demyelinating lesions of the brain and spinal cord, with

varying degrees of axonal loss, developing with time into multifocal sclerotic plaques [7, 79]. The optic nerve, also a part of the CNS, is a major target in MS [199]. In addition to inflammation within the demyelinating lesions, inflammatory pathology in MS also includes perivascular cuffing and meningeal inflammation. Leptomeningeal inflammation has been found to be associated with cortical demyelination of the gray matter in early, acute MS [199–201] but has also been described for the late, chronic stage of the disease [202], as well as in all forms of the disease: clinically isolated syndrome, relapsing-remitting MS, and primary and secondary progressive MS [203]. The infiltrating cell population consists mainly of lymphocytes and macrophages [204, 205]. An additional characteristic of most MS patients is the presence of oligoclonal bands of unique antibodies in the cerebrospinal fluid, evidence of intrathecal antibody production [199, 206]. The B/plasma cell clones that produce the oligoclonal bands have been found to persist over time within a single MS patient but are highly heterogeneous among patients [207]. Extensive regions of apoptotic oligodendrocytes have been observed in association with the formation of new symptomatic MS lesions [208]. Also, in acute inflammatory demyelinating MS lesions, substantial numbers of axons are transected; however, initially, these damaged axons mostly survive and functionally recover [209]. Chronically demyelinated axons, which occur with the progression of disease, in contrast, ultimately succumb to necrosis [209]. Lastly, myelin antigen-directed autoimmune responses are found in MS patients and are thought to be pathogenic [205, 210, 211]. Studies have found that MS patients have an increased number of myelin-specific T cells and antibody-producing cells in the cerebrospinal fluid and the peripheral blood compared to controls, and the specificity broadly covers several myelin antigens and peptides thereof [205, 206, 211–219]. Other studies, in contrast, have found that myelin-specific T cells were found in comparable numbers in the cerebrospinal fluid and the peripheral blood when comparing MS patients and controls. However, the T cells from MS patients demonstrated the altered characteristics of an activated phenotype, proinflammatory cytokine production, and an increased frequency of high-avidity T cells, as reviewed elsewhere [220]. Although the myelin-specific antibody-producing cells found in MS patients have the potential to be pathogenic, the exact target antigens of these responses are unknown [221–223]. Identification of the targets of these myelin-specific T cells and antibody-producing cells could lead to an understanding of the heterogeneity of the disease presentation and would greatly advance the treatment of MS [222, 224]. Importantly, the autoimmune antibody response in MS is not limited to myelin but encompasses antigens from neurons, astrocytes, and immune cells, as well as other oligodendrocyte antigens and ubiquitous antigens [225]. In addition to producing antibodies, B cells

also produce cytokines, act as antigen-presenting cells, and participate in the formation of ectopic lymphoid tissues in the meninges in MS [207, 221, 223]. These proinflammatory functions of B cells may occur in an antigen-independent manner, thus resulting in bystander activation of T cells [223]. This deregulation of the B cell pool as a whole, not just autoreactive B cells, results in perpetuation of pathology beyond autoimmunity [221].

Experimentation and tissue culture adaptation of the three viruses discussed in this review have led to well-characterized models using defined viral strains and titers and defined routes of infection in susceptible mouse strains of defined gender and age, as the ability of various viruses to cause disease and demyelination in mice often depends on such variables. All three viral infections give rise to inflammatory demyelinating lesions of the CNS (brain and/or spinal cord), but not in the peripheral nervous system, reflective of what is seen with MS [146].

Other characteristics of TMEV infection that are reflective of MS include immune-mediated ( $CD4^+$  and  $CD8^+$  T cells) demyelination and pathology [146]. The presence of antibody, being both produced and deposited within the CNS, is also reflective of what is seen in MS [7, 97, 131, 226]. The TMEV mouse model most closely resembles chronic progressive MS [146]. For both TMEV and MS, the disease progresses through a significant portion of the animal's/patient's life span but then seems to plateau, as the progressive neurological disability does not tend to ultimately cause the death of the animal/person [226]. As there are currently effective therapies for the relapsing-remitting stage of MS, but few therapies that work during the progressive stages (secondary or primary) of MS, this makes the TMEV model a currently relevant model [226].

MHV lesions, characterized by focal areas of demyelination of the white matter of the spinal cord with myelin stripping accompanied by naked axons, are histologically very similar to MS lesions [82, 146, 147, 227]. Also for MHV, both the immune-mediated demyelination and the presence of antibody production within the CNS are reflective of what is seen in MS [7, 105].

SFV infection has been thought to be a more relevant model of MS because immune-mediated demyelination is triggered by infection but not linked to viral persistence, whereas in TMEV and MHV infection, demyelination is linked to viral persistence [28]. This, however, has recently been brought into question by the ability to detect infectious virus in aged previously SFV-infected mice following immunosuppression, demonstrating the persistence of functional viral RNA [109]. A second characteristic that makes SFV infection a good model for MS is the finding that the patchy demyelination seen in the optic nerve is accompanied by neurophysiologically demonstrable visual deficits with similarities to what is found in MS patients

[7, 228]. A third characteristic that makes SFV infection a good model for MS is the finding that the CNS inflammatory infiltrate is dominated by  $CD8^+$  T cells, much like what is found in MS lesions [7, 146].

For all of their similarities, there are also differences between the three viral infection models and MS. The locations of the demyelinating lesions vary for the three viral models. TMEV and MHV produce demyelination mainly within the spinal cord. SFV produces demyelination within both the brain and spinal cord, as is seen in MS [173]. The ability to efficiently remyelinate axons also varies. The TMEV model demonstrates an abortive remyelination (assessed via electron-microscopic autoradiography) [144, 173], while the MHV and SFV models both show remyelination. In MS patients, remyelination occurs in new lesions early in the course of disease, but only limited remyelination occurs in chronic lesions in MS patients who have had the disease for many years [173].

MS is not the only human inflammatory demyelinating disease of the CNS. Acute disseminated encephalomyelitis (ADEM) and neuromyelitis optica (NMO) are two additional diseases that are also inflammatory demyelinating diseases of the CNS. Comparison of MS to ADEM and NMO demonstrates heterogeneity in clinical presentation, severity, progression rate, prognosis, and immunopathological features, suggestive of distinct diverse effector mechanisms of disease [229]. Although TMEV, MHV, and SFV mouse models of demyelination, to our knowledge, have not previously been used to study ADEM or NMO, the possibility remains that the viral models may be useful for elucidating certain features of these diseases. ADEM typically occurs following an infection or immunization [229], so these viral mouse models may be appropriate. Also, SFV infection has optic nerve involvement [188] and as such may be used to study this aspect of NMO.

## Summary

For all their usefulness, no one model faithfully recapitulates all the clinical or pathological features of MS: relapses, remissions, progression, axonal damage, demyelination, remyelination, inflammation, disability, etc. (Table 1) [226]. In addition, it appears that the underlying mechanisms mediating demyelination differ from one model to another [173]. Nevertheless, these viral animal models allow for the examination of the interactions between viruses, the cells of the host CNS, and the host immune system response as a means of elucidating the link between inflammation, demyelination, axonal injury, and disability [226].

**Table 1** Characteristics of viral murine models that mimic different aspects of MS

Characteristics	MS	TMEV - DA	MHV - JHM	SFV
Clinical course	Progressive Relapsing, remitting [1]	Progressive [146]	Monophasic – no recovery [10, 147]	Monophasic – recovery [149, 150]
Disability – Movement disorder	Limb weakness Ataxia Impaired ambulation [7]	Flaccid hind limb paralysis Spastic gait [130, 144]	Flaccid hind limb paralysis Incoordination Tetraplegia [10, 79, 147]	Hind limb paralysis [149, 150]
Optic nerve involvement w/demonstrable visual defects	Yes [199]	No	No	Yes [7, 188, 228]
Demyelination – Prominent location Type	Brain & Spinal cord [7, 79] Acute/Chronic [1]	Spinal cord [17, 130, 144, 167] Chronic [80, 130, 144]	Spinal cord [82] Acute/Chronic [10, 99, 147]	Brain & Spinal cord [22, 184, 185] Acute (25% of SFV A7 infected) [149, 150]
Lesions	Focal lesions – early Multifocal sclerotic plaques – late In areas of inflammation [7, 79]	Large plaques in white matter [17] In areas of inflammation [80, 130]	Small, widespread lesions in white matter [177, 178] In areas of inflammation [82]	Focal lesions [184, 185] In areas of inflammation [31, 79]
Cause	Immunopathological [173]	Immunopathological [130, 146, 168, 170]	Direct viral – acute [179, 180] Immunopathological – chronic [181]	Immunopathological [186, 190]
Neuropathology – Inflammation	Yes	Yes	Yes	Yes
Meningeal inflammation	Yes	Yes	Yes	Yes
Perivascular cuffs	lymphocytes	lymphocytes; plasma cells; Monocyte/Macrophage	Lymphocytes	Lymphocytes
Axon damage/neuron loss	Yes [199-203]	Yes [3, 11, 17, 80, 93, 144]	Yes [3, 82, 174-176]	Yes (original isolate) [19, 22, 79, 149, 150, 184, 185]
Immune responses – Intrathecal antibody	Yes – oligoclonal bands [199-206]	Yes [97]	Yes [105]	Yes [107]
Potential autoimmunity – Myelin specific portion	MBP; PLP; MOG [205, 210, 211]	PLP <sub>139-151</sub> ; PLP <sub>56-70</sub> ; PLP <sub>178-191</sub> ; MOG <sub>92-106</sub> [172, 173]	No [183]	MBP; MBP <sub>54-68</sub> ; MOG <sub>18-32</sub> ; PLP <sub>89-104</sub> [192, 193]
Cell death – Neurons	Necrosis (chronic) [209]	Apoptosis	Apoptosis [155]	Apoptosis – immature Necrosis – mature [28, 157, 158]
Oligodendrocytes	Apoptosis (new lesions) [208]	Apoptosis [154]	Necrosis – early Apoptosis – late [156]	Apoptosis [25]
Remyelination	New lesion, early disease [173]	No [144, 173]	Yes [177, 178]	Yes [31, 186, 187]
Viral persistence	Unknown	Infectious: virus & RNA [11, 17, 96]	Antigen &/or RNA [79, 99, 100]	Infectious RNA [13, 84, 109]

MS, multiple sclerosis; TMEV, Theiler’s murine encephalomyelitis virus; DA, Daniels strain; MHV, mouse hepatitis virus; JHM, John Howard Mueller strain; SFV, Semliki Forest virus; CNS, central nervous system; MBP, myelin basic protein; PLP, myelin proteolipid protein; MOG, myelin oligodendrocyte glycoprotein

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**Compliance with ethical standards**

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