

## Annotation

# Analysis of the molecular basis of neuropathogenesis of RNA viruses in experimental animals: relevance for human disease?

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**Annotation. Analysis of the molecular basis of neuropathogenesis of RNA viruses in experimental animals: relevance for human disease?**

RNA viruses with segmented genomes were the first model used for molecular analysis of viral neuropathogenesis, since they could be analysed genetically by reassortment. Four viruses with non-segmented genomes have been used as models of neurovirulence and demyelinating disease: JHM coronavirus, Theiler's virus, Sindbis virus and Semliki Forest virus (SFV). Virus gene expression in the central nervous system of infected animals has been measured by *in situ* hybridization and immunocytochemistry. Cell tropism has been analysed by neural cell culture. Infectious clones have been

constructed for Theiler's virus, Sindbis virus and SFV, and these allow analysis of the sequences involved in the determination of neuropathogenesis, through the construction of chimeric viruses and site-specific mutagenesis. Measles and rubella viruses have been studied in animal systems because of their importance for human disease. The importance of two recently discovered mechanisms of neuropathogenesis, antibody-induced modulation of virus multiplication, and persistence of virus in the absence of multiplication, remains to be assessed.

Keywords: RNA virus, neuropathogenesis, multiple sclerosis, encephalitis, teratogenesis

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## Introduction

In the last 30 years or so, a large amount of effort devoted to elucidating the molecular mechanisms of animal virus multiplication has been rewarded with considerable success. This work has been based on the use of cell culture systems, although our knowledge of some viruses, such as papilloma viruses and hepatitis B

virus, is based on sequence analysis only. In contrast, molecular mechanisms of multiplication in the animal host, and the interactions of the virus with the host to produce disease (i.e. pathogenesis) have been largely neglected. Often, viruses which exhibit similar mechanisms of multiplication in cell culture differ in the type of disease induced in the animal host. An example is the picornavirus family; although foot-and-mouth disease, hepatitis A, polio and rhino (common cold) viruses are similar in their multiplication strategies in cell cultures, the diseases produced in their animal hosts are quite

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different. Until recently, studies of viral pathogenesis were largely descriptive but, due to the introduction of new techniques, it is now possible to approach the problem of the genetic control of pathogenesis at the molecular level. For RNA viruses, these techniques have been the use of nucleic acid and antibody probes, complementary DNA (cDNA) sequencing and the construction of infectious clones.

As with earlier studies of virus multiplication, the first advances are being made with model systems. Such systems have been utilized in the analysis of the molecular control of neurovirulence, immune-mediated demyelination and teratogenicity. It is our intention here to review current work being carried out on the pathogenesis of RNA virus infection of the central nervous system (CNS); we intend to concentrate on the advances being made in determination of the genetic and molecular basis of pathogenicity for the CNS by RNA viruses. Detailed consideration of the immune response to such viruses, although a fascinating subject, is beyond the scope of the present review.

The preferred hosts for molecular analysis of viral pathogenesis are laboratory mice and rats. This is because these animals are easy to breed and maintain, and well-characterized inbred strains are available. This is particularly true for laboratory mice, and the use of inbred strains minimizes variation in the response to infection.

The first RNA viruses to be utilized for molecular analysis of neuropathogenesis were those with segmented genomes, such as reovirus, arenaviruses and bunyaviruses. This is because it was possible to assign the genetic control of pathogenic characteristics to specific genome segments by performing the appropriate crosses in cell culture and analysing reassortment.

The construction of infectious clones, combined with sequence analysis, has facilitated molecular analysis of the pathogenicity of positive-stranded RNA viruses with non-segmented genomes. An infectious clone is constructed by first sequencing cDNA clones of the virus, produced by reverse transcription. The sequence of the entire virus genome is required; cDNA fragments are then joined in the correct order, to give a DNA copy of the virus genome. Using a suitable expression plasmid, a bacteriophage promoter (usually derived from SP6 or T7) is placed adjacent to the virus genome. The cDNA corresponding to the virus genome may then be transcribed *in vitro* using the appropriate RNA polymerase, to

give infectious RNA. This RNA is used to infect cultured cells, either by chemical transfection or electroporation, and infectious virus produced.

Using an infectious clone, it is possible to apply recombinant DNA techniques to an RNA virus. Thus it is possible to use site-specific mutagenesis and the construction of recombinant (chimeric) viruses, containing sequences derived from different strains, in the analysis of pathogenicity. Infectious clones to enable the molecular analysis of neuropathogenesis have now been constructed for Theiler's virus (Theiler's murine encephalomyelitis virus, a picornavirus) and the togaviruses Sindbis virus and Semliki Forest virus (SFV). Although no infectious clone has yet been reported, murine coronavirus has long been used as a model for analysis of neuropathogenesis, and this system is ripe for exploitation at the molecular level.

Model systems are usually based on animal viruses which efficiently infect laboratory mice and rats; most human viruses do not have this ability. Systems which require the use of primates, such as poliovirus and HIV, are beyond the scope of this review. However, some rodent experiments have been carried out with measles virus, and rubella virus has been studied in neural cell culture.

### Viruses with segmented genomes

The classical early experiments on the molecular basis of pathogenicity were carried out with reovirus, a double-stranded RNA virus, and with lymphocytic choriomeningitis (LCM) virus, an arenavirus, and La Crosse virus, a bunyavirus. Arenaviruses and bunyaviruses have negative or ambisense (positive and negative) single-stranded RNA genomes.

Reoviruses have a genome consisting of 10 segments. The proteins coded by each of these segments, and their function in the multiplication of the virus is known. Also, the segments may be separated on gels and recognized by their size. Hybrid viruses can be constructed by infecting cells in culture with two different reovirus strains. Reassortment then occurs, with the formation of virions containing differing numbers of segments from each of the parental viruses. Such hybrid viruses can be isolated, propagated, and their pathogenic characteristics measured. By monitoring the segments which each hybrid isolate contains, it is possible to assign pathogenic characteristics to specific genome segments.

Reovirus is naturally a respiratory and enteric pathogen; however, for molecular analysis of pathogenicity, infection of the neonatal mouse CNS is used as a model system. Two reovirus serotypes are used as models: type 1 and type 3. After intracerebral inoculation into neonatal mice, type 3 produces a lethal meningoencephalitis, which typically includes extensive damage to neurons. In contrast, type 1 does not damage neurons, but infects the ependymal cells lining the ventricles and produces ependymitis. Infected animals also frequently develop hydrocephalus. Analysis of reassortment has demonstrated that the cell tropism of reovirus (i.e. ability to infect neurons or ependymal cells) is determined by the virus S1 gene, which is a distinct genome segment. This gene codes for the sigma outer capsid protein, and is also the protein which recognizes the reovirus cell receptor [87, 88]

The S1 gene also determines the mechanism of spread of the virus following inoculation into the hindlimb of neonatal mice. Both type 1 and type 3 reovirus spread to the spinal cord following hindlimb inoculation. However, for type 3 this spread is through axonal transport, whereas for type 1 it is haematogenous [82].

Although the S1 gene is the main pathogenicity determinant for reovirus, experiments on temperature-sensitive (ts) mutants of this virus illustrate an important point. Ts mutants will multiply at a permissive temperature, but multiplication is restricted at a higher, non-permissive temperature. The basis of such mutations is usually a missense substitution of an amino acid in the affected protein, resulting from a single base change. Such mutations usually attenuate the pathogenicity of the virus. Thus for virulent virus, such mutation may allow survival of the affected animals due to reduced multiplication rate of the virus. Such mutations may occur in several virus genes [65]. However, one effect of ts mutations may be to unmask pathogenic characteristics which are obscured by death in the case of the more virulent wild-type strain.

LCM virus is an arenavirus whose genome consists of two segments, designated small and large. It has long been used as a model of persistent infection in mice. Infection of neonatal mice leads to life-long persistent infection, which is due to immune tolerance of the virus. For adult mice, infection leads either to death or viral clearance. Persistence occurs in most tissues of the mouse: in the CNS, persistent infection of the pituitary

results in stunting of growth due to inhibition of growth hormone secretion. The infection of the pituitary is not cytolytic and can only be detected through the use of nucleic acid and antibody probes. This has led to a new concept in viral pathogenicity; inhibition of 'household' functions, such as protein and nucleic acid synthesis, leads to cell death; however, inhibition of 'luxury' functions, such as growth hormone synthesis, allows cell survival but affects the viability of the animal [32]. Although this may be a general disease mechanism, this concept should be interpreted with caution, as LCM virus causes an unusual infection, and there is as yet no evidence that such mechanisms operate in human disease.

The establishment of persistence and associated suppression of the antiviral cytotoxic T-lymphocyte response has been mapped to the small RNA segment, as has the suppression of growth hormone [67]. Indeed, persistence has been associated with a single amino acid change in the envelope glycoprotein of the virus [70].

Although LCM virus causes persistent infection in mice, it can be neurovirulent in guinea-pigs. Neurovirulence in guinea-pigs maps to the large RNA segment; however, neurovirulence for guinea-pigs is directly related to the rate of virus multiplication [67] and not to differences in cell tropism.

La Crosse virus is a bunyavirus whose genome consists of three segments, labelled large, medium and small. It is a mosquito-transmitted arbovirus, and can therefore infect both vertebrate and invertebrate hosts. Reassortment experiments involving parental viruses of differing pathogenicity have shown that both neurovirulence and ability to infect mosquitoes are determined by the medium segment. These markers vary independently and experiments with monoclonal antibodies have shown that they are probably determined by sites within the G1 envelope glycoprotein. However, as with other systems, mutations in other segments may modulate virulence by influencing multiplication rate [22].

### JHM coronavirus

JHM coronavirus is a strain of mouse hepatitis virus type 4. It is an enveloped virus whose genome consists of a single molecule of single-stranded RNA of positive polarity. In susceptible mouse strains it causes a virulent systemic infection which involves the CNS as well as

the liver. However, attenuated mutants such as temperature-sensitive (ts) mutants can produce an altered CNS disease [25, 33]. The wild-type virus produces a lethal encephalitis in most infected mice due to infection of neurons. However, mutants such as ts8 produce chronic and recurrent demyelination, which involves both virus persistence and infection of oligodendrocytes [33].

In rats, infection of susceptible strains such as Lewis rats leads to chronic demyelination and virus persistence. This is associated with sensitization of T-lymphocytes to myelin basic protein [86]. In primary glial cell cultures derived from rats, type 1 astrocytes and microglial cells are the initial target cells, whereas oligodendrocytes are rarely infected [47].

Although no infectious clone for JHM coronavirus is yet available, two approaches have indicated that virion structural proteins are important determinants of pathogenicity. In the first approach, monoclonal antibodies were raised against the E2 envelope protein. Some of these neutralized the infectivity of the virus, and when passively administered to mice, the animals developed chronic demyelination rather than lethal encephalitis [12]. Escape mutants which were resistant to neutralization could also be selected using these antibodies. Such mutants had an altered E2 protein and caused chronic demyelination in mice rather than lethal encephalitis [11].

The second approach involved utilizing the low level of recombination which occurs between virus genomes in coronavirus-infected cells. JHM coronavirus produces panencephalitis in mice but mild hepatitis, whereas the A59 strain of mouse hepatitis virus produces focal encephalitis and severe hepatitis. A panel of recombinant viruses derived from the JHM and A59 strains, and containing different proportions of RNA from the two parental strains was constructed. It was found that the main determinants of pathogenesis were localized to the 3' portion of the genome (about 25%), which encodes the viral structural proteins [35].

### Theiler's virus

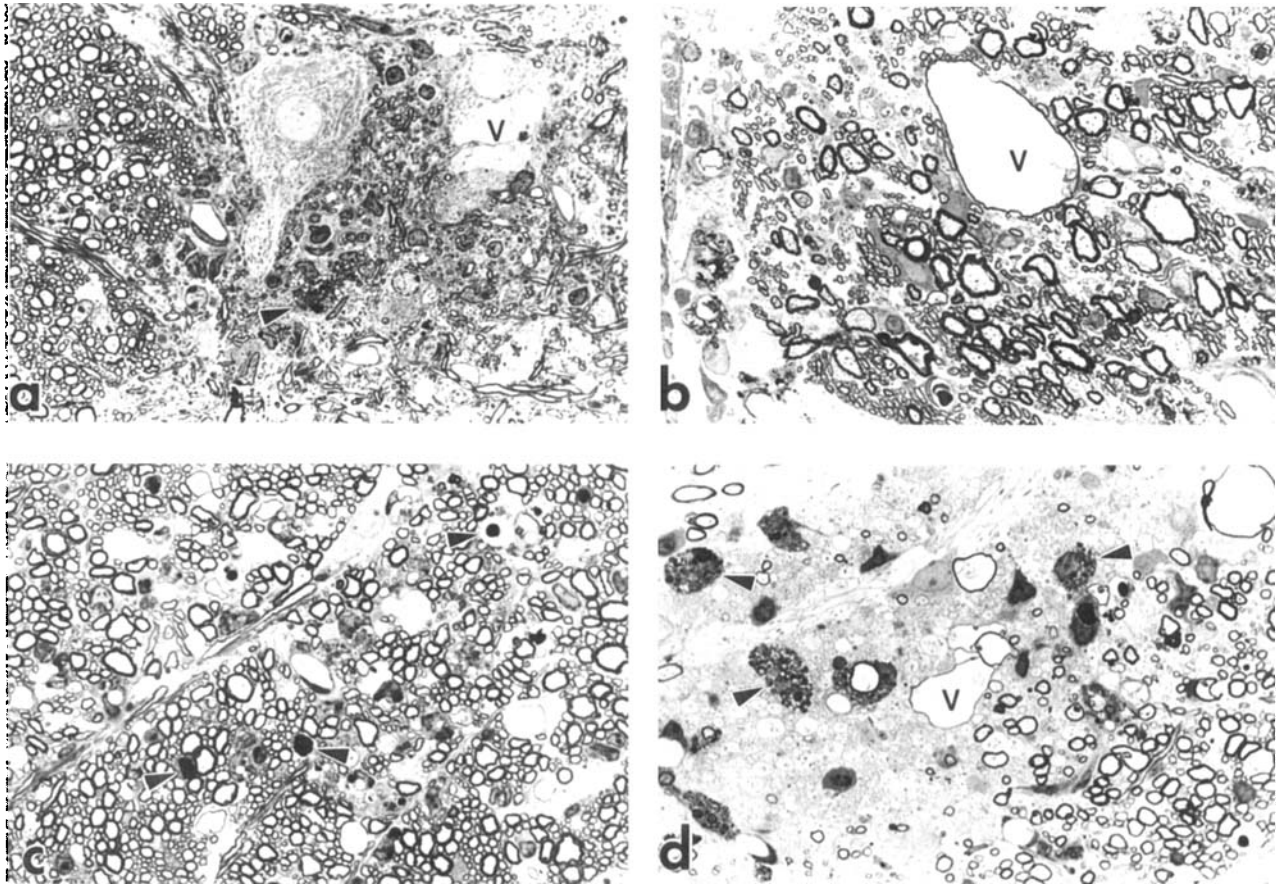
Theiler's murine encephalomyelitis (abbreviated here to Theiler's virus) is a non-enveloped picornavirus. Its genome consists of a single molecule of positive-sense single-stranded RNA. Virulent strains of Theiler's virus such as GDVII induce a lethal encephalitis when given

intracerebrally to adult mice. However, avirulent strains such as DA or BeAn induce a biphasic disease. In the first few days following infection, the virus infects neurons and induces an acute encephalomyelitis. The amount of damage is small, and most mice survive; however, in the second phase of the disease virus is found in glial cells of the white matter, and virus persists in these cells. This persistence is associated with immune-mediated demyelination (Figure 1), so Theiler's virus has been used as a model for human multiple sclerosis (MS) [89].

The cell tropism of Theiler's virus for the murine CNS has been investigated by two methods. In brain cell cultures, Theiler's virus lytically infects neurons and oligodendrocytes and persistently infects astrocytes and macrophages [14, 23, 53]. Using *in situ* hybridization, infection of these cells can also be shown in the animal [5]. There appears to be no difference in cell tropism between virulent and avirulent strains.

In terms of the genetic determinants of pathogenicity of Theiler's virus, investigations have centred on the molecular control of virulence, persistence and demyelination. The complete nucleotide sequences of the DA, BeAn and GDVII strains of Theiler's virus have been determined [54, 56, 59, 60]. Using this sequence information, infectious clones have been constructed for these three strains of virus [18, 29, 45, 68, 80]. These infectious clones have been used to construct recombinant (chimeric) virus and hence to map viral genes controlling neurovirulence, persistence and demyelination, by fragment exchange. The results indicate that all three phenotypes are determined by the capsid proteins of the virus [6, 10, 13, 17, 18, 46, 79]. The observation that the 5' non-translated region is important in the determination of neurovirulence first appeared to be due to an inadvertent base deletion which occurred during the construction of chimeric virus [64]. A three-nucleotide insertion in this region which inhibits translation also reduces neurovirulence [8] and other results also suggest that the 5' non-translated region influences neurovirulence and persistence through its effect on translation [77].

That growth rate of the virus may alter CNS disease has been shown by analysis of a neutralization escape mutant of the DA strain. This has a single amino acid change which maps to amino acid 101 of the VP1 protein. This mutant replicates poorly in the CNS and causes only mild demyelination [91–93].



**Figure 1.** Appearance of RNA virus-induced lesions in the CNS of weanling mice. **a**, Neuronal vacuolar degeneration (v) and debris-laden macrophages (arrow) in the ventral horn of the spinal cord of an SJL mouse, 9 days after intracerebral infection with the BeAn strain of Theiler's virus. **b**, Myelin vacuolation (v), loss of myelinated fibres, naked and remyelinated axons, and leptomeningeal leucocytic infiltration (left) in the spinal cord of an SJL mouse, 73 days after intracerebral infection with the BeAn strain of Theiler's virus. **c**, Myelin vacuolation and putative necrotic oligodendrocytes (arrows) in the spinal cord of a BALB/c mouse, 5 days after intraperitoneal infection with the M9 mutant of Semliki Forest virus. **d**, Myelin vacuolation (v), numerous naked axons and macrophages laden with myelin debris (arrows) in the medulla oblongata of a BALB/c mouse, 21 days after intraperitoneal infection with the M9 mutant of Semliki Forest virus. Toluidine blue stained 1  $\mu\text{m}$  resin sections.  $\times 511$ .

### Sindbis virus

Sindbis virus is an RNA-containing, enveloped togavirus, which is mosquito-transmitted in its natural state. Its genome consists of a single molecule of positive-stranded RNA. It has been used as a model for many years for the study of the molecular biology of virus multiplication in cell culture [71], mainly in the USA (a different togavirus, Semliki Forest virus, has been used in Europe, see below). Laboratory strains of Sindbis virus are avirulent for adult mice, although a neurovirulent strain has been isolated [41]. Most laboratory strains are lethal for neonatal mice [75]. In pathogenicity experiments in mice, the virus is usually given by intracerebral inoculation. A neuroadapted strain, virulent for adult mice, has

been isolated by one group of workers [24]. Other studies have utilized neonatal mice [55]. Using *in situ* hybridization and immunocytochemistry, it has been shown that neurons are the main target cells in the CNS [28], and immune-mediated demyelination is not induced. However, the basis of a paralytic disease induced in SJL mice [50] has not so far been characterized. Some temperature-sensitive mutants of Sindbis virus are avirulent for neonatal mice, although weight gain is inhibited [9].

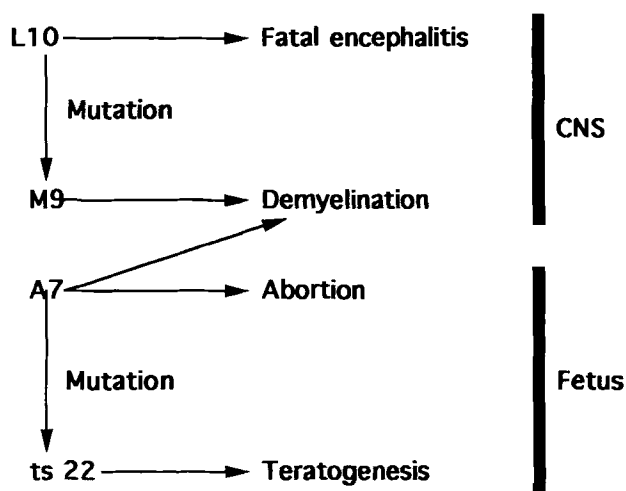
The importance of the E2 protein in the determination of pathogenesis was first shown using rapidly penetrating mutants of Sindbis virus, which are also attenuated. Such mutants have an altered E2 glycoprotein, as shown

by changes in monoclonal antibody binding [58, 69], and a pathogenesis domain has been defined on the E2 protein [58, 63, 74]. The E2 protein is one of the two Sindbis virus envelope glycoproteins, and probably recognizes the host cell receptor(s). The receptor shows a prevalence in the CNS of mice which is age-dependent [83], and one receptor at least has been identified as high affinity laminin receptor [85]. Whether the receptors described in these two studies are the same is not yet clear. However, both the E1 and E2 proteins are involved in Sindbis virus infectivity, since monoclonal antibodies to both proteins can neutralize virus infectivity; neutralizing and non-neutralizing antibodies to both proteins can passively protect mice against lethal challenge, showing that both are important determinants of pathogenicity [48].

In initial experiments, it was shown that both infectious virus and viral RNA were cleared from the CNS within 3 weeks after infection. This was not due to the action of cytotoxic T-lymphocytes, but to antibody-mediated restriction of virus gene expression in neurons [37]. The technique originally used to detect the virus genome was *in situ* hybridization. However, it was later shown using the polymerase chain reaction, a more sensitive method, that low levels of Sindbis virus RNA do persist in the CNS after clearance of infectious virus [36]. This low-level persistence does not appear to be associated with any detectable clinical signs, and the significance of this finding is not clear at present.

A laboratory strain of Sindbis virus has been sequenced and an infectious clone produced [66]. A single amino acid change in the E2 protein controls virulence. This change affects the binding of the virus to mouse brain cells [81]. Changes in both the E1 and E2 proteins reduce virulence, and a gradient of virulence exists involving changes in both proteins [42, 61, 62]. One amino acid change in the E2 protein, previously defined using monoclonal antibodies, has a marked effect on virulence. However, changes in this site which decrease virulence do not decrease binding of the virus to mouse brain cells [62, 74].

The 3' and 5' non-coding regions of the Sindbis virus genome may also play a role in pathogenesis [78]. These regions are involved in the binding of the viral polymerase for the synthesis of negative and positive RNA strands, and the 5' region is also involved in the initiation of translation. Host proteins may be important in these processes, and cellular proteins have been shown



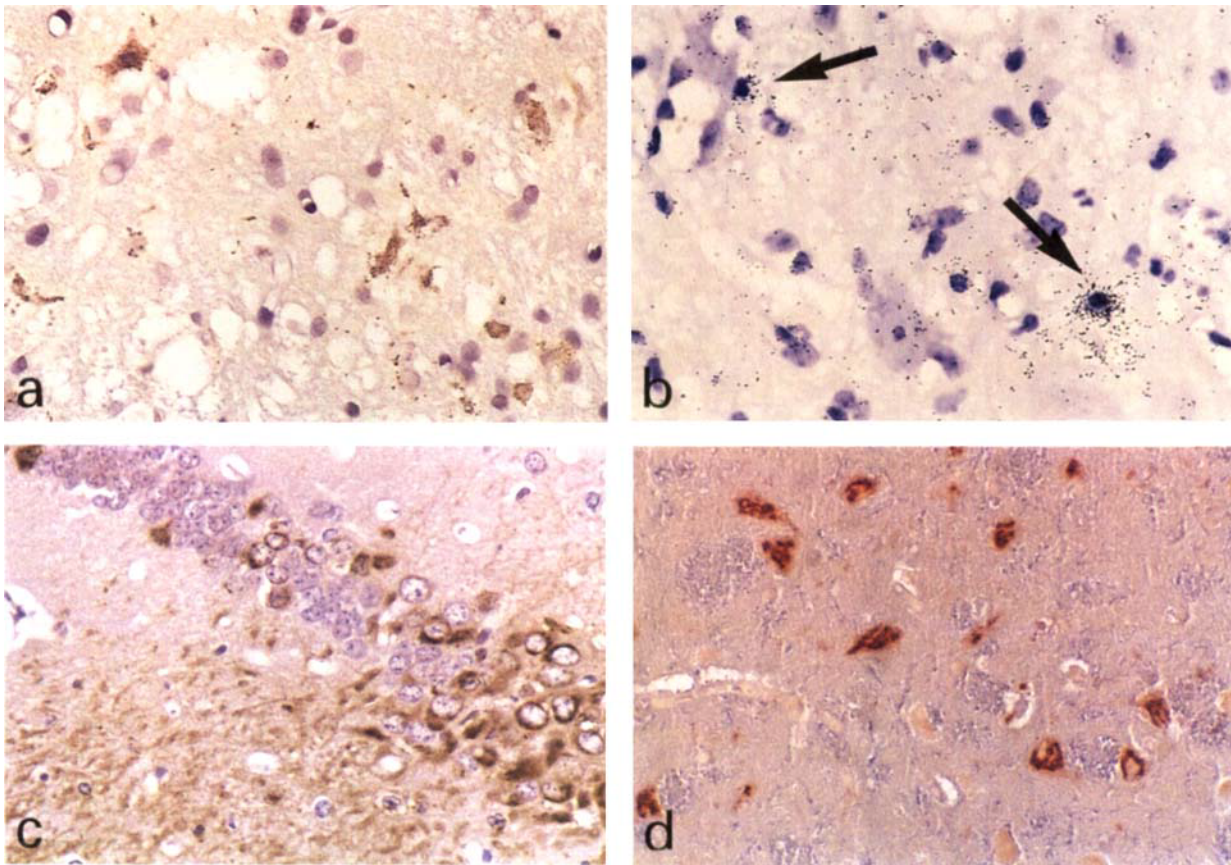
**Figure 2.** Relationship of strains and mutants of Semliki Forest virus and their pathogenic characteristics. L10 and A7 are virulent and avirulent natural isolates respectively of SFV.

to bind to the 3' region of the negative strand [57]. The 3' non-coding region of the genome may be important in controlling the cell tropism of the virus [34].

### Semliki Forest virus

Like Sindbis virus, Semliki Forest virus (SFV) is an RNA-containing togavirus. Its genome consists of a single molecule of positive-stranded RNA and it is mosquito-transmitted in its natural state. It has been used for many years in European laboratories as a model for the study of the molecular biology of virus multiplication [29]. Some naturally occurring strains of SFV are virulent for adult mice when given peripherally, and avirulent strains or mutants often induce demyelination in the CNS which is immune-mediated (Figure 1). Avirulent strains or mutants also may infect the developing fetus and induce fetal death or teratogenesis [3]. Figure 2 summarizes the relationships between strains and mutants of SFV.

Both virulent and avirulent strains of SFV induce a viraemia following peripheral infection of adult mice, and may enter the CNS via vascular endothelial cells [15]. Alternatively, the virus may spread by axonal transport to the CNS from peripheral sites [30]. Demyelination is immune-mediated and involves the action of T-lymphocytes [16, 19]. Mice infected with the avirulent A7 strain of SFV have been shown to develop

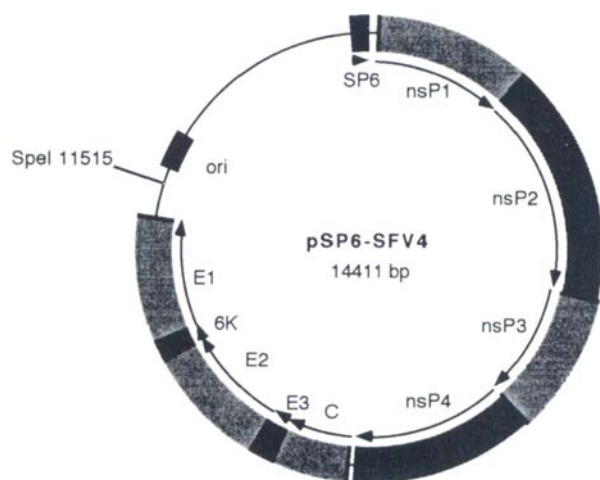


**Figure 3.** Immunocytochemistry and *in situ* hybridization on CNS tissue from Semliki Forest virus (SFV) infected weanling BALB/c mice. **a.** Viral antigen in putative oligodendrocytes and cell processes in an area of spongiform degeneration in the spinal cord, 6 days after intraperitoneal infection with the M9 mutant of SFV. Immunogold silver staining using SFV specific IgG. **b.** Viral RNA in putative oligodendrocytes (arrows) in an area of spongiform degeneration in the white matter of the cerebellum, 6 days after intraperitoneal infection with the M9 mutant of SFV. *In situ* hybridization using a [<sup>35</sup>S]-labelled RNA probe complementary to SFV RNA. **c.** Viral antigen in neurons and in neuronal processes in the hippocampus, 4 days after intranasal infection with the virulent SFV4 strain (derived from an infectious clone). Immunoperoxidase staining using SFV-specific IgG. **d.** Viral RNA in neurons between bundles of myelinated fibres in the thalamus, 4 days after intranasal infection with the virulent SFV4 strain (derived from an infectious clone). *In situ* hybridization using a biotinylated cDNA probe specific for SFV (red) and alkaline phosphatase immunocytochemistry using anti-myelin basic protein (MBP) antibody (blue). All illustrations  $\times 364$ .

T-cell mediated autoimmunity to myelin basic protein [51]. In immune-competent mice infectious virus is cleared from the CNS, although there is one report of the persistence of viral antigen [31]. In nude mice, demyelination is reduced in severity [16, 19], and the A7 strain persists in the CNS [16]. In SJL mice, which have a T-suppressor defect, lesions of demyelination persist in the CNS of a proportion of infected mice after infection with an avirulent mutant, but infectious virus is cleared [76].

By light and electron microscopy it has been shown that both avirulent strains and mutants infect both oligodendrocytes and neurons in the CNS. For virulent

strains, damage to neurons exceeds a lethal threshold, but for avirulent strains immune intervention occurs before a lethal threshold of damage is reached [3]. *In situ* hybridization and immunocytochemistry (examples are shown in Figure 3) show that virulent and avirulent strains have similar cell tropism for the CNS; they both infect oligodendrocytes and neurons, but not astrocytes. Virulent strains are, however, more cytopathic for neurons than avirulent strains or mutants [7]. This has also been shown by experiments involving neural cell culture. In mixed glial cell cultures, rapid destruction of oligodendrocytes occurs. Virulent strains such as L10 multiply faster in cultured neurons and induce a faster



**Figure 4.** Diagram of the Semliki Forest virus infectious clone. The origin (ori) of replication of the plasmid is shown. To produce infectious RNA, the plasmid is linearized with the restriction enzyme SpeI, and RNA transcribed using bacteriophage SP6 polymerase. The location of the SP6 promoter is shown. The SFV non-structural genes nsP1, nsP2, nsP3 and nsP4, and the structural genes E1, E2, E3 (the three envelope protein genes) and C (the core protein gene) are shown in order of transcription. 6K is a small protein which is not incorporated into virions.

cytopathic effect than an avirulent strain such as A7 or an avirulent mutant such as M9 [4, 20].

Defective interfering (DI) particles have also been shown to modulate SFV virulence [3]. These are deletion mutants which can only replicate in the presence of a wild-type helper virus, but which interfere with its multiplication. Different DI clones vary in their ability to protect mice from lethal infection (M. Thomson and N. Dimmock, personal communication).

SFV is one of the few good models of viral teratogenesis. Avirulent strains such as A7 induce rapid fetal death when given peripherally to pregnant mice [1]. The mutant ts22, derived from A7, is teratogenic [27] and induces skeletal, skin and CNS defects [43]. For the CNS, ts22 induces open neural tube defects in a minority of infected fetuses; the majority of fetuses show abnormal development of the neuroepithelium. These neural tube defects are indirectly induced by infection of mesenchymal cells adjacent to the neural tube, and not by infection of the neuroepithelium itself [44].

The genome of a laboratory strain of SFV has been completely sequenced and an infectious clone produced [40] (Figure 4). The infectious virus produced from this clone is virulent when given intranasally to adult mice. Two mutants in the E2 glycoprotein have been shown to

be attenuated; for one mutant, infection of developing fetuses occurs, and for the other immune-mediated damage to the CNS occurs [21].

## Measles and rubella viruses

Measles and rubella viruses have been studied in animals because of their importance to human disease. Measles virus is a negative-stranded paramyxovirus. Rubella virus is a togavirus like Sindbis virus and SFV; however, in its natural state it infects only humans and no vector is involved in its transmission cycle.

When inoculated intracerebrally with measles virus, neonatal rats die from encephalopathy. However, adult Lewis rats develop a subacute encephalomyelitis which is associated with virus persistence [39]. Restricted expression of the viral envelope protein genes occurs both in the CNS of infected rats [73] and in cultured rat glial cells [72], and may be associated with the regulation of persistence. Antibody-induced restriction of measles virus gene expression also occurs in infected rats [38] and in cell culture [90].

Rubella virus does not multiply well in experimental animals. However, it does infect cultured mouse embryos [26] and cultured rat neural cells. In neural cell culture, oligodendrocytes only are infected and the virus does not multiply in neurons or astrocytes [2, 52].

## Conclusions

One of the main reasons for the use of animal model systems is to gain insight into mechanisms of human disease. Animals are used to perform experiments which would be difficult, unethical or impossible using humans. However, caution must be exercised in interpreting results from animal models in terms of human disease, since a comparison only can be made and no definitive conclusions reached. In terms of RNA virus models, comparisons have been made to two human diseases which affect the CNS: multiple sclerosis (MS) and viral encephalitis. Teratogenesis has also been studied for SFV.

MS is an autoimmune, demyelinating disease of the human CNS. Its aetiology is unknown, although viruses and genetic factors have been implicated. Evidence implicating viruses in MS has been equivocal [84], but if a virus is involved, two mechanisms could operate: the disease could be triggered by a virus, which then



disappears, or a persistent virus infection could be involved. The animal models described here indicate how such mechanisms could operate in MS. Two of the models, Theiler's virus and JHM coronavirus, induce autoimmune demyelination which is linked to virus persistence. For SFV, persistence of infectious virus may not be involved but the virus may act as a trigger.

Two mechanisms of RNA virus pathogenesis have been described recently which may be of relevance to MS and other CNS diseases. These are antibody-induced modulation and persistence of the virus genome in neurons in the absence of virus multiplication. Further work with model systems is necessary to gain further insight into the relevance of these mechanisms for virus persistence and pathogenesis of human neurological disease.

The use of infectious clones is advancing our understanding of the molecular determination of neurovirulence. The main model systems used so far have been Theiler's virus, Sindbis virus and SFV. The use of these systems may enhance our understanding of pathogenic mechanisms in human encephalitis, and may lead to improved virus vaccines. The use of adult animals in infection experiments, and avoidance of intracerebral inoculation, allows the study of the interaction of the virus with a fully developed immune system and the use of natural routes of infection, but this may not be possible for some systems. So far, the work has been confined to sequence analysis. Two types of mutation have been analysed. Mutations which impair the function of a viral protein attenuate the virus, but may lead to the unmasking of pathogenic mechanisms which are obscured by death for virulent viruses. Mutations which do not impair virus multiplication but determine cell tropism and hence disease syndromes have also been described, and are of fundamental importance in understanding the molecular control of pathogenicity. Such mutations have so far only been described in genes coding for viral structural proteins. The study of structure-function relationships between viral and cellular nucleic acids and proteins, and their rôle in pathogenesis, is just beginning.

### Acknowledgements

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### Addendum

For a detailed review of the pathogenesis of virus-induced demyelination, particularly immunological aspects, the reader is referred to Fazakerley JK and Buchmeier MJ *Adv Virus Res* 1993; **42**: 249–324.

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