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REVIEW ARTICLE

The molecular pathogenesis of Semliki Forest virus: a model virus made useful?

Gregory J. Atkins,[1] Brian J. Sheahan[2] and Peter Liljeström[3,4]

[1] Department of Microbiology, Moyne Institute of Preventive Medicine, Trinity College, Dublin 2, Ireland
[2] Department of Veterinary Pathology, Faculty of Veterinary Medicine, University College Dublin, Dublin 4, Ireland
[3] Microbiology and Tumorbiology Center, Karolinska Institute, S-171 77 Stockholm, Sweden
[4] Department of Vaccine Research, Swedish Institute for Infectious Disease Control, S-171 82 Solna, Sweden

Introduction

Semliki Forest virus (SFV) has for many years been used as a model to study the molecular biology of RNA virus multiplication, since it grows well and to high titre in cultured cells such as BHK cells and chick embryo cells. Initially, studies on the pathogenesis of SFV were descriptive, but recent advances have facilitated analysis of the pathogenesis of SFV at the molecular level. As with several other viruses, it has become apparent that some characteristics of SFV, which normally contribute to pathogenesis, may be manipulated to treat disease rather than cause it.

Semliki Forest virus is a positive-stranded RNA virus of the genus *Alphavirus* of the family *Togaviridae*. It was first isolated from mosquitoes in Uganda in 1944 (Smithburn & Haddow, 1944) and there have been a number of subsequent isolations (Bradish *et al.*, 1971). It is a relatively simple virus, encoding only nine functional proteins of unique sequence. The four nonstructural proteins are concerned with viral RNA synthesis, whereas the structural proteins form the capsid (the C protein) and the envelope (the E1, E2 and E3 proteins). A small 6 kDa protein encoded by the structural region of the genome is not incorporated into virions. The structural proteins are encoded by a subgenomic RNA species, usually labelled 26S, whereas the nonstructural proteins are translated from the genomic 42S RNA. The structural and nonstructural proteins are formed from precursors by separate post-translational cleavage pathways (Strauss & Strauss, 1994).

By historical accident, most work on SFV has been carried out in Europe, whereas a different model alphavirus, Sindbis virus, has been studied in the USA. Here we intend to concentrate on reviewing recent advances in the molecular pathogenesis of SFV, but to refer to work on Sindbis virus where relevant. We last reviewed the molecular pathogenesis of SFV in 1985 (Atkins *et al.*, 1985), and since then infectious clones of SFV have been constructed and developed into vectors, and there have been advances in understanding immune mechanisms in SFV infection and virus–cell interactions.

Author for correspondence: Gregory Atkins.
Fax +353 1 6799294. e-mail gatkins@tcd.ie

Strains of SFV

The original isolate of SFV is designated L10, and is neurovirulent for mice, causing lethal encephalitis by infection of the central nervous system (CNS). Subsequently, an avirulent strain designated A7 was isolated from mosquitoes in Mozambique (McIntosh *et al.*, 1961). Most SFV strains used for laboratory studies are derived from these two isolates. The avirulent A7[74] strain was derived from A7 by further selection for avirulence (Bradish *et al.*, 1971). The prototype strain, from which the original infectious clone of SFV was derived, appears to be derived from the L10 strain. However, it has lost some of its virulence, possibly due to an indeterminate number of passages in cell culture (Glasgow *et al.*, 1991). The original infectious clone of SFV, constructed from the prototype strain, is designated pSP6-SFV4; the virus produced by transcription of this infectious clone is labelled SFV4.

Laboratory safety

SFV does infect humans and although an outbreak of a mild febrile illness among French soldiers serving in the Central African Republic was shown to be due to SFV (Mathiot *et al.*, 1990), SFV is considered less of a hazard to laboratory personnel than many other togaviruses (Winkler & Blenden, 1995). However, there has been one death suspected to be due to laboratory infection by SFV (Willems *et al.*, 1979). For this reason, SFV is classified as a Biosafety Level 3 virus in the USA, but with the caveat that most activities can be carried out at level 2 (U. S. HHS Publication no. (CDC) 93-8395, 1993). In the European Union, SFV is classified as a Biosafety Level 2 virus (E. C. Council Directive 93/88/EEC, 1993). The SFV expression vectors, which lack the structural genes, are classified as Biosafety Level 2 in the EU and USA (Federal Register, 1993).

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The pathogenesis of SFV infection

The original L10 isolate of SFV shows complete virulence in inbred mouse strains such as BALB/c. Following peripheral infection, all infected mice die, and the LD_{50} is 1 p.f.u.; benign multiplication resulting in immune protection against virulent challenge does not occur. However, the virulent virus may be attenuated by mutation. The first such mutations studied were induced by chemical mutagenesis (Barrett *et al.*, 1980) and affected defined stages in virus multiplication; for example, the M9 mutant, derived from the virulent L10 strain, has a defect in efficiency of viral RNA synthesis (Atkins & Sheahan, 1982). These defects allowed the survival of the majority of mice following peripheral infection, and allowed the consequences of avirulent virus multiplication to be detected (mainly CNS demyelination and teratogenesis). The avirulent A7 strain, however, multiplies in standard BHK cells at least as efficiently as the virulent L10 strain. All adult mice survive infection with A7, but neonatal mice are killed by infection with either L10 or A7. Infection with A7 does, however, induce CNS demyelination and foetal death in pregnant mice.

Analysis of virulence

As indicated above, initial attempts to analyse the virulence of SFV utilized chemically induced attenuated mutants of the virulent L10 strain. Such mutants are partially defective in the efficiency of virus multiplication (Barrett *et al.*, 1980; Atkins & Sheahan, 1982; Atkins *et al.*, 1985). Perhaps more interesting is analysis of the avirulent A7 strain, which is attenuated but multiplies at least as efficiently as L10 in standard cultured cells such as BHK cells (Atkins, 1983; Glasgow *et al.*, 1997). Initial studies analysed virulence following intraperitoneal (i.p.) infection, but it was found subsequently that intranasal (i.n.) infection gives more consistent results, and is a more direct route of infection to the CNS. Intranasal infection also targets the olfactory bulb, allowing analysis of early events following CNS infection (Sheahan *et al.*, 1996). It is a more sensitive indicator of virulence than i.p. infection, although a higher dose is required to produce initial infection. Male mice appear to be more sensitive to SFV infection than female mice (Santagati *et al.*, 1998).

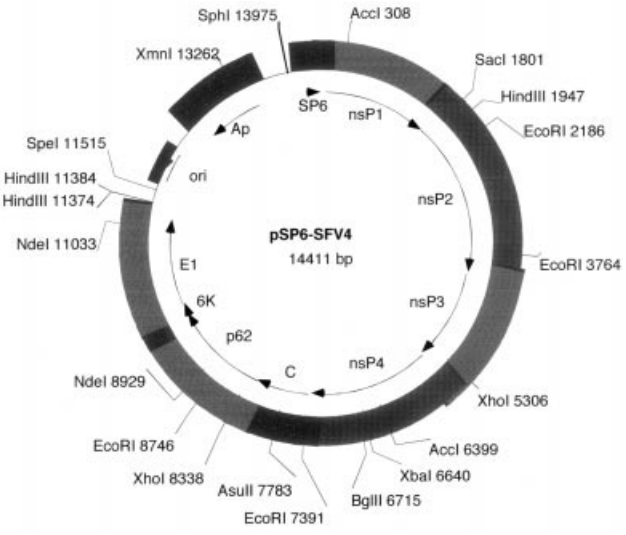


Fig. 1. The infectious clone pSP6-SFV4 showing some of the restriction sites which can be used for genetic manipulation. The SFV genome (11445 nt) has been cloned under the control of the SP6 promoter; p62 is the precursor to the E2 and E3 envelope proteins which is cleaved during virus maturation. To produce infectious virus, the plasmid is linearized with *Spe*I, then transcribed with SP6 polymerase to produce infectious RNA, which is then transfected into BHK cells by electroporation.

Analysis of the molecular basis of SFV virulence was facilitated by the construction of an infectious cDNA clone of SFV, derived from the prototype strain (Fig. 1; Liljeström *et al.*, 1991). The SFV4 virus, produced by transcription of the plasmid encoding the SFV sequence, kills about 60–70% of BALB/c mice when 10^6 p.f.u. is given i.p., and 100% when the same dose is given i.n. (Glasgow *et al.*, 1991).

The A7 strain has been completely sequenced and the sequence compared to the more virulent prototype strain (Glasgow *et al.*, 1994; Santagati *et al.*, 1994, 1995; Tarbatt *et al.*, 1997). The most striking feature of the A7 sequence is the presence of a long untranslated sequence containing multiple repeats at the 3′ end of the genome. In A7 this region is 334 nucleotides longer than that of the prototype (and L10) strains (Glasgow *et al.*, 1994; Santagati *et al.*, 1994). There are multiple mutations throughout the A7 genome compared to the prototype strain, in all virus genes and in the 5′ untranslated region. A large proportion of the nucleotide substitutions in the translated region result in amino acid substitutions. Analysis of chimeras constructed between the avirulent A7 and A7[74] strains, and the more virulent SFV4 strain, shows that determination of virulence is polygenic. However, three regions of the genome appear to be important: the E2 gene, the nsP3 gene and the 5′ untranslated region (Santagati *et al.*, 1995, 1988; Santagati, 1998; Tarbatt *et al.*, 1997). Surprisingly, the 3′ untranslated region is unimportant in the determination of virulence (Tarbatt *et al.*, 1997; Santagati *et al.*, 1998). Control of virulence by the E2 gene has been described for Sindbis virus (Pence *et al.*, 1990; Tucker *et al.*, 1993; Ubol *et al.*, 1994) and Ross River virus (Meek *et al.*, 1989), and by the 5′ untranslated region in combination with the E2 gene for Venezuelan equine encephalitis virus (Kinney *et al.*, 1993). For the A7[74] strain of SFV, which has been further selected for avirulence compared to A7 (Bradish *et al.*, 1971), further changes have occurred in the nsP3 gene. First, a 21 nucleotide in-frame deletion has occurred in the portion of the nsP3 gene corresponding to the C-terminal domain of the nsP3 protein; this deletion is flanked by repeated sequences. Secondly, an opal codon is present near the 3′ end of the nsP3 gene (Santagati, 1998). These changes are attenuating, and a chimeric virus containing the nsP3 and nsP4 genes from A7[74]

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and the rest of the genome from SFV4 is attenuated when given by the i.p. route. However, when given by the i.n. route, virus having the nsP3 deletion is still virulent. Changes in other regions of the genome appear to be required to fully attenuate the virus (G. Atkins, unpublished results). For the virulent SFV4 strain, a nuclear targeting signal is present in the nsP2 gene, which has the sequence P^{648}RRRV (Rikkonen *et al.*, 1992; Kujala *et al.*, 1997), and whose function is unknown. Modification of this signal by site-directed mutagenesis does not affect the multiplication of the virus in cell culture, but does affect its pathogenicity for mice (Rikkonen, 1996). In the avirulent A7[74] strain, the nuclear targeting function is abrogated by mutation to the sequence PQRKF (G. Atkins, unpublished results). Thus this appears to be a further pathogenicity determinant which contributes to the avirulence of the A7[74] strain.

Early work on SFV virulence indicated that virulent strains induced more extensive damage to neurons in the CNS than avirulent strains (Atkins *et al.*, 1985). Multiplication of avirulent strains in the CNS is slower than virulent strains, and this is linked to the ability to multiply in neurons, which is partially restricted in avirulent strains (Gates *et al.*, 1985; Balluz *et al.*, 1993; Fazakerley *et al.*, 1993). In cultured rat cerebellar granule cells (a type of neuron, Fig. 2*c*, *d*), virulent strains multiply faster and to higher titre than avirulent strains. However, the restricted multiplication is more marked at low m.o.i., when the virus has to undergo several rounds of multiplication to infect every cell in the culture (Atkins *et al.*, 1990). Thus the crucial difference between virulent and avirulent strains is the rapidity of spread of neuronal damage in the CNS (Balluz *et al.*, 1993), leading to a lethal threshold in the case of virulent strains before the immune system can intervene.

It is clear that virulent strains of SFV spread rapidly in the CNS, probably by axonal transport (Fig. 2*a*; Kaluza *et al.*, 1987). However, following i.p. infection, the avirulent strain A7[74] of SFV crosses the blood–brain barrier by infection of vascular endothelial cells (Dropulic & Masters, 1990; Soiluhänninen *et al.*, 1994), but then fails to spread rapidly in neurons (Fazakerley *et al.*, 1993). Thus virulent and avirulent strains of SFV differ in their ability both to multiply in neurons and to cause extensive neuronal damage by rapid spread. As yet, the virus properties which control these functions are unknown.

Virus-induced demyelination

Mice surviving infection by avirulent SFV show virus-induced demyelination. This demyelination occurs in discrete areas of the CNS called plaques and avirulent SFV has therefore been suggested as a model for human demyelinating disease such as multiple sclerosis (MS; Atkins *et al.*, 1994). Any mutation or genotype which partially restricts multiplication of SFV in the CNS, and allows survival, results in CNS demyelination (Atkins *et al.*, 1985, 1994; Glasgow *et al.*, 1991). This suggests that both virulent and avirulent strains of SFV have the capability to induce demyelination, but that this is obscured by death for virulent strains.

Demyelination is reduced in severity in nude mice, and $CD8^+$ lymphocytes are involved in mediating this acute demyelination (Gates *et al.*, 1984; Fazakerley & Webb, 1987; Subak-Sharpe *et al.*, 1993). In BALB/c mice demyelination is maximal at about 14 days after i.p. infection, at a time when infectious virus has been cleared from the CNS, and is then repaired (Fig. 2*b*). In SJL mice, however, lesions of demyelination persist up to a year after i.p. infection with the M9 mutant; a minority of such lesions show active inflammatory demyelination (Smyth *et al.*, 1990; Donnelly *et al.*, 1997). SJL mice have a defect in innate suppression of immune responses, probably due to decreased secretion of the negative regulatory cytokine TGF-β (Mokhtarian *et al.*, 1994). Although persistence of the virus genome can be detected in individual mice of both the BALB/c and SJL strains up to 3 months after infection, this does not correlate in individual mice with the presence of lesions of demyelination, or synthesis in the CNS of the pro-inflammatory cytokines interferon-γ or TNF-α. Messenger RNA for the latter two cytokines persists in the CNS of SJL mice up to a year after infection, but does not persist in BALB/c mice beyond 1 month after infection (Donnelly *et al.*, 1997). Thus SFV infection of SJL mice represents a model of human MS where immune-mediated demyelination is triggered by virus infection of the CNS, but is not linked to virus persistence. It thus differs from other virus models of MS such as Theiler's murine encephalitis virus and murine coronavirus, where demyelination is linked to persistence (Atkins *et al.*, 1994).

This is further illustrated by studies of the relationship between experimental autoimmune encephalomyelitis (EAE) and SFV infection. EAE is a demyelinating disease induced by injecting mice with myelin components, and is a model of MS which does not involve virus infection. In its chronic relapsing form it is similar to most cases of human MS. Prior infection of mice with avirulent SFV exacerbates EAE (Mokhtarian & Swoveland, 1987); similarly, subsequent infection of mice with SFV after EAE induction exacerbates the demyelination (Erälinna *et al.*, 1994). Infection with SFV induces T-cell-mediated immunity to myelin basic protein (Mokhtarian & Swoveland, 1987; Mokhtarian *et al.*, 1994), raising the possibility that demyelination induced by SFV could be due to anti-myelin autoimmunity like EAE. A possible mechanism of induction of immune-mediated demyelination by SFV is indicated in Fig. 3.

Immune mechanisms in SFV infection

As discussed above, it is clear that the resistance of mice to infection by avirulent SFV is not due to differential induction of immunity, but is due to differences in cytopathogenicity for

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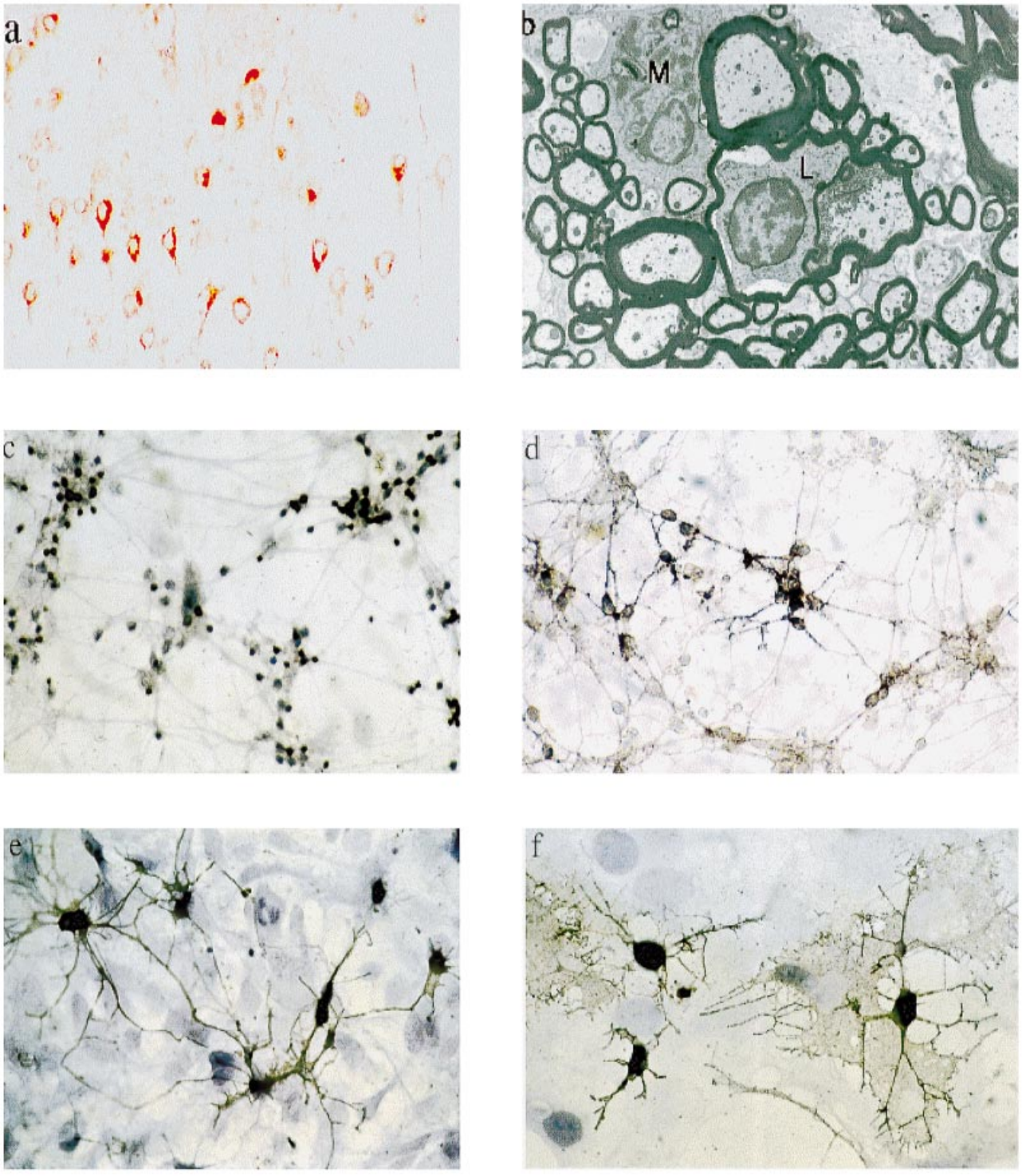


Fig. 2. Micrographs illustrating SFV neuropathogenesis. (*a*) *In situ* hybridization using a specific biotinylated SFV cDNA probe on SFV4-infected BALB/c mouse brain at 5 days after i.n. infection, showing viral RNA in neurons and axons (stained red). Contrast-enhanced image without counterstain. (*b*) Electron micrograph showing myelin sheaths in the mouse spinal cord undergoing immune-mediated demyelination at 5 days after i.p. infection with the M9 mutant of SFV. Note a lymphocyte (L) in a vacuole in a myelin sheath and an adjacent macrophage (M). (*c*) Cultured rat cerebellar granule cells (neurons). (*d*) Cultured granule cells 24 h after infection with the L10 strain of SFV at low m.o.i. (10^{-2}); immunogold silver staining with polyclonal anti-SFV antiserum (brown/black granules). Note immunostaining of neurons and processes. (*e*) Rat mixed glial cell culture immunostained with anti-GC, an oligodendrocyte marker (brown/black). Note the immunostained upper layer of oligodendrocytes and the lower counterstained astrocyte layer. (*f*) Rat mixed glial cell culture 24 h after infection with the M9 mutant of SFV. Note oligodendrocytes immunostained brown/black with polyclonal anti-SFV antiserum. The infection is beginning to spread to the lower astrocyte layer.

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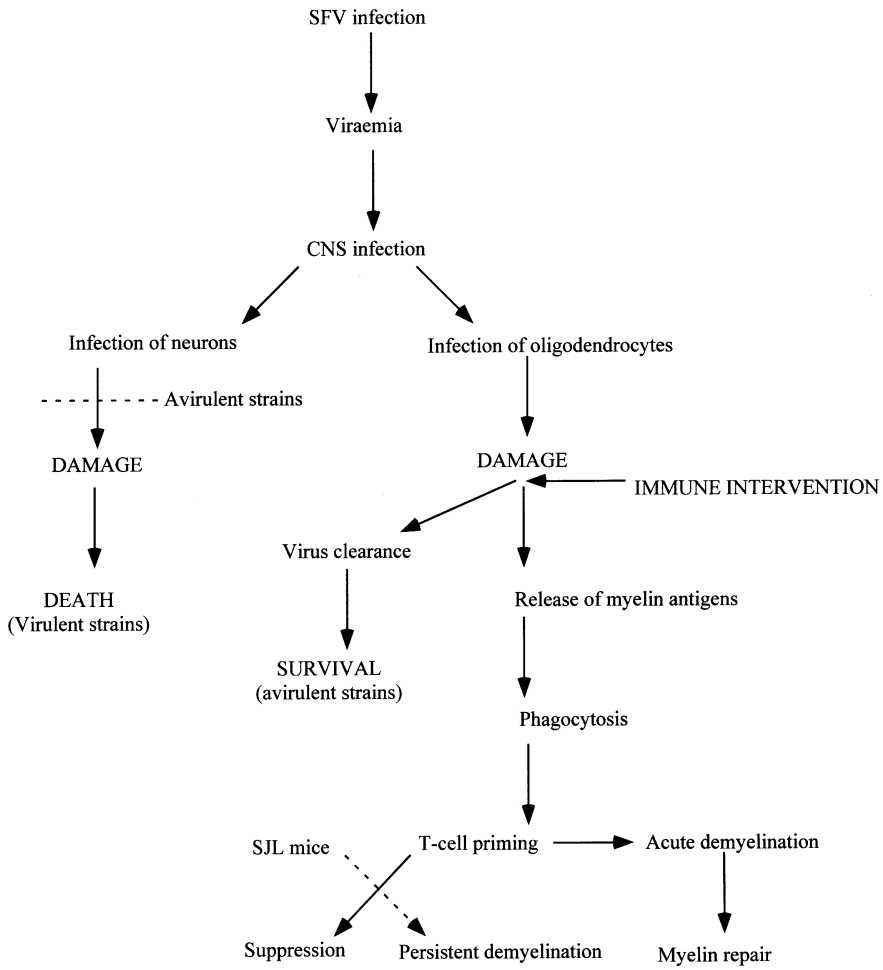


Fig. 3. General scheme to explain the mechanism of SFV pathogenesis; modified from Atkins *et al.* (1990). M9 and A7 are avirulent strains of SFV which induce less neuronal damage than virulent strains. Death following infection with a virulent strain such as L10 is due to a lethal threshold of neuronal damage. Dotted lines represent partial inhibition. The damage to oligodendrocytes could be directly induced by virus infection, or be induced as a result of the immune reaction to virus-infected oligodendrocytes.

neurons, speed of multiplication in neurons and spread of virus within the CNS. However, clearance of virus from the nervous system after avirulent SFV infection is due to immune mechanisms, and SCID mice do eventually succumb to infection (Amor *et al.*, 1996). The immune response to SFV infection is complex, and it is probable that both humoral neutralizing and non-neutralizing antibodies, and T-cell-mediated immunity, play a role (Amor *et al.*, 1996). In addition, SFV infection induces the expression of adhesion molecules on both splenic lymphocytes and brain endothelial cells (Soiluhänninen *et al.*, 1997). Both T- and B-cell epitopes have been detected in the E2 envelope protein of SFV, and this knowledge has been used to design effective peptide vaccines which protect against SFV infection (Snijders *et al.*, 1992; Fernandez *et al.*, 1993). The cytokine profile induced in the CNS following SFV infection is complex, with no bias towards either a Th1 or Th2 response (Morris *et al.*, 1997), and is influenced by the genetic background of the mouse (Mokhtarian *et al.*, 1996).

Cell death mechanisms in SFV infection

As for other viruses, for many years it was thought that the death of cells from alphavirus infection resulted from inhibition of cell macromolecule synthesis and general disruption of cell metabolism and synthetic processes. It is now known that, like many other viruses (Razvi & Welsh, 1995), death of most cells following SFV infection results from induction of apoptosis or programmed cell death (Fig. 4; Glasgow *et al.*, 1997; Scallan *et al.*, 1997). SFV infection of the mouse or rat CNS results in infection of mainly oligodendrocytes (myelin-forming cells in the CNS) and neurons, both in the animal (Atkins *et al.*, 1985; Smyth *et al.*, 1990; Balluz *et al.*, 1993; Fazakerley *et al.*, 1993) and in culture (Fig. 2*e*, *f*; Gates *et al.*, 1985; Atkins *et al.*, 1990).

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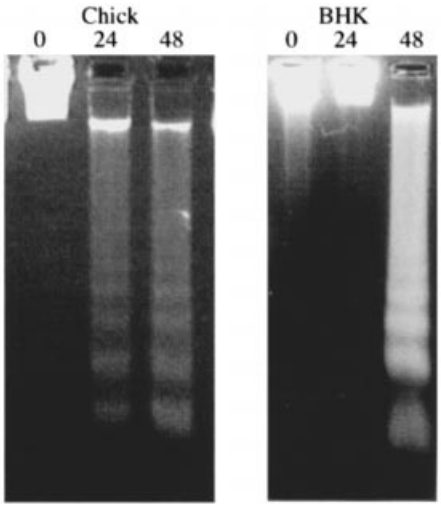


Fig. 4. Internucleosomal DNA bands indicative of apoptosis formed at 24 and 48 h after infection with the SFV4 strain, in BHK cells and chick embryo cells. The 0 h sample represents uninfected cells. Apoptosis is induced faster in chick cells than in BHK cells. Courtesy of Rosalind Lacy.

For culture of CNS cells, rats have been used because rat CNS cell cultures are easier to prepare and better characterized than those from mice. The pathogenicity of SFV for rats and mice is similar. In rat mixed glial cell cultures (mainly astrocytes and oligodendrocytes), oligodendrocytes are preferentially infected for both virulent and avirulent strains of SFV, and apoptosis is induced in these cells. However, astrocytes in glial cell cultures do eventually become infected (Fig. 2*e*, *f*). For cultured rat cerebellar granule cells (neurons which have differentiated in culture), virulent strains multiply more rapidly and cause more rapid cytopathic effect (CPE) than avirulent strains, particularly when the cells are infected at low m.o.i. (Fig. 2*c*, *d*). Death of such infected neurons occurs by necrosis rather than apoptosis (Atkins *et al.*, 1990; Glasgow *et al.*, 1997).

Neonatal mice infected by virulent or avirulent strains of SFV always succumb to infection; this is associated with extensive induction of apoptosis, presumably of immature neurons, and the virus spreads throughout the CNS by axonal transport (Allsopp *et al.*, 1997; Oliver *et al.*, 1997; Oliver & Fazakerley, 1998). In adult animals, infection of the CNS by the avirulent A7[74] strain is focal (Fazakerley *et al.*, 1993), although for virulent strains a spreading infection occurs (Smyth *et al.*, 1990; Balluz *et al.*, 1993). The difference between the widespread infection seen in neonates for all SFV strains and the widespread infection seen in adult mice for virulent strains only is that the cell death associated with neuronal infection in neonates occurs by apoptosis, whereas that occurring in adults following infection by virulent strains is not apoptosis, but a mechanism which resembles necrosis. The dependence of cell death mechanisms on maturity of neurons is illustrated by intranasal infection of adult rats. Cells in the rostral migratory stream of the olfactory bulb are continuously being replaced and undergoing differentiation, unlike the majority of neurons in the adult animal. Such cells undergo apoptosis when infected by SFV, unlike other neurons which undergo necrosis which is more extensive for virulent strains (Sammin *et al.*, 1999).

It has been shown that expression of the anti-apoptotic gene *bcl-2* restricts SFV multiplication in cultured rat AT3 cells, and also delays the onset of SFV-induced apoptosis (Scallan *et al.*, 1997). A similar result has been obtained for the SFV vector in RIN cells expressing *bcl-2* (Lundström *et al.*, 1997). However, in contrast to this, it has been shown that three cell lines overexpressing bcl-2 underwent apoptosis after SFV infection as efficiently as control cells which did not express bcl-2. This was associated with cleavage of bcl-2 within a caspase target site, thus removing the N-terminal region of bcl-2 known to be essential for the death-protective activity of this protein. The viral function which induces this caspase-mediated inactivation of bcl-2 is as yet unknown (Grandgirard *et al.*, 1998). In rats infected intranasally with SFV, most neurons do not express bcl-2, so the resistance of mature neurons to SFV-induced apoptosis is not due to bcl-2 expression (Sammin *et al.*, 1999).

Cultured oligodendrocytes in mixed glial cell cultures infected with SFV undergo apoptosis (Glasgow *et al.*, 1998). However, in the animal it has been shown that oligodendrocytes damaged by SFV infection may survive and become capable of carrying out remyelination, which is characteristic of mice recovering from avirulent SFV infection (Butt *et al.*, 1996). Earlier studies show that extensive damage of oligodendrocytes occurs following avirulent SFV infection of the CNS (Atkins *et al.*, 1985), but the type of damage occurring, and whether apoptosis is involved, has not yet been established. Oligodendrocytes in the animal could either undergo cell death as a direct result of virus infection, or be targeted by cytotoxic T-cells following virus infection and expression of viral antigens.

Use of the SFV infectious clone has shown that the nonstructural region of the SFV genome is necessary for the induction of apoptosis, and the structural region may be deleted without affecting apoptosis. Deletion of part of the nonstructural nsP2 gene abrogates both apoptosis induction and viral RNA synthesis (Glasgow *et al.*, 1998). This is in contrast to the situation for Sindbis virus, where apoptosis induction, viral virulence and counteraction of the effects of the bcl-2 gene are all functions of the viral E2 envelope protein gene (Ubol *et al.*, 1994). However, it has also been shown that the induction of CPE by Sindbis virus, including the inhibition of cellular macromolecule synthesis, can proceed in the absence of viral structural protein synthesis, although expression of the structural proteins is required for the full and rapid CPE shown by the wild-type virus (not recognized at the time as apoptosis; Atkins, 1976, 1977; Frolov & Schlesinger, 1994). Apoptosis induction by SFV is not dependent on p53, and therefore probably does not result from damage to cellular DNA (Glasgow *et al.*, 1998). One possible mechanism of induction of apoptosis by SFV is activation of the double-stranded RNA-dependent protein kinase PKR; alternatively, apoptosis could

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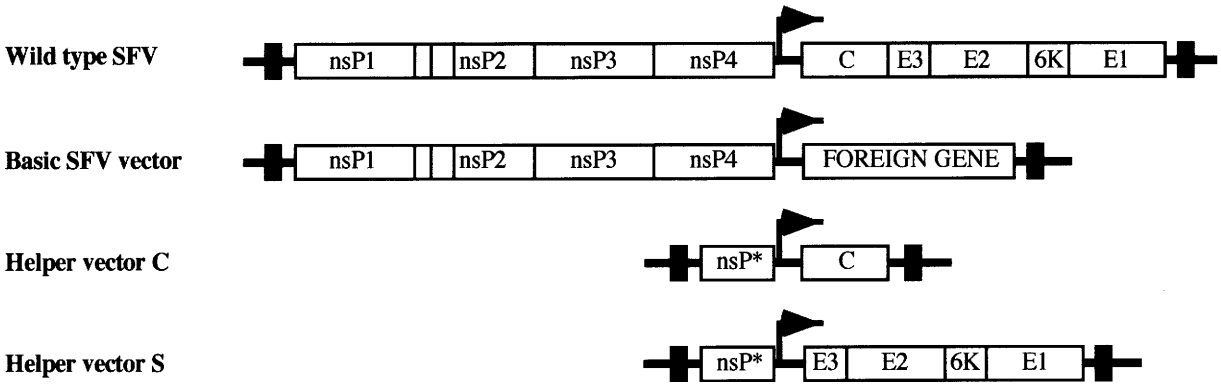


Fig. 5. The SFV vector system. The organization of the wild-type SFV genome is shown at the top. The right-handed arrow denotes the promoter which is recognized by the viral replicase for transcription of the subgenomic RNA encoding the structural proteins. In the vector, the foreign gene to be expressed is inserted into a multiple cloning site adjacent to this promoter. The helper RNAs encode all the structural proteins of SFV, but RNAs encoding the capsid and envelope proteins are transcribed from separate plasmids. The helper RNAs have nearly the complete replicase region deleted, including the packaging signal. Black boxes at the ends denote sequences used by the replicase for amplification of the RNAs.

result from leakage of cytochrome *c* from mitochondria and subsequent activation of caspase 3 (Lee & Esteban, 1994; Kibler *et al.*, 1997). Since the H358a cells used to demonstrate p53-independent apoptosis by SFV and the SFV vector (Glasgow *et al.*, 1998) are human lung cancer cells carrying a p53 deletion (Fujiwara *et al.*, 1994), this offers the intriguing possibility of the use of SFV and its derived vector to induce apoptosis in cancer cells.

Modulation of SFV pathogenicity by defective interfering (DI) particles

DI particles of SFV are produced by high multiplicity passage of virus in cell culture. They contain RNA which has a deletion of the wild-type or standard virus genome, and interfere with the multiplication of the standard virus in cell culture. For SFV, DI particle RNA always contains the ends of the virus genome, and the packaging signal which is located in the nsP2 gene (Thomson & Dimmock, 1994; White *et al.*, 1998). DI particles are able to protect mice against virulent virus administered i.n. (Barrett & Dimmock, 1984). However, for two molecularly cloned DI particle isolates, labelled DI-6 and DI-19, DI-19 protected mice whereas DI-6 did not. This was linked to the ability of DI-19 but not DI-6 to be replicated in the brain in the presence of standard virus. Since all the sequence of DI-19 (1244 nt) is contained in DI-6 (2146 nt), this result is difficult to explain at present (Thomson *et al.*, 1998). However, it is clear that these DI particles differ in their ability to interfere with the standard virus in different cell environments, and optimum interference may depend on a particular cell type–DI genome sequence permutation (C. L. White & N. J. Dimmock, unpublished data).

Teratogenesis

Although it is avirulent for adult mice, the A7 strain of SFV is lethal for the developing foetus. Following peripheral infection, it rapidly infects the developing foetus and causes foetal death and resorption, although the mother survives. Virulent strains also have the capacity to infect the foetus, since attenuated mutants derived from the virulent L10 and SFV4 strains have this property, but the consequences of this are obscured by death (Atkins *et al.*, 1982; Hearne *et al.*, 1986). The avirulent A7 strain of SFV can be further attenuated by mutation; in this case the mutants can be teratogenic (Hearne *et al.*, 1987). Thus foetal death induced by A7 infection of the mother probably results from a lethal threshold of damage to the foetus before immune intervention can occur (i.e. the transfer of maternal immunity to the foetus). For teratogenic mutants such as ts22, the foetus survives in some cases, so the consequences of foetal infection (i.e. teratogenesis) can be observed. Teratogenic effects induced by SFV include skeletal, skin and neural tube defects. They probably result in all cases from infection of undifferentiated mesenchymal cells (Mabruk *et al.*, 1988, 1989). The mechanism of teratogenesis by SFV and other viruses has been reviewed by Atkins *et al.* (1995).

SFV as a vector

Vector development

Following the construction of the first full-length infectious clone of SFV (Liljeström *et al.*, 1991), a vector system was developed which induces high-level transient expression of cloned genes in transfected cells. During the multiplication of SFV, a subgenomic 26S RNA species is formed which encodes the structural proteins of the virus only, and is a gene amplification mechanism. In the vector system, a foreign gene is inserted into the infectious clone in this region. RNA transcribed from this vector *in vitro* is transfected into cultured cells, and viral RNA replication and expression occur as in a productive infection. In the original vector system, a helper clone was constructed by deletion of the packaging signal, which is the RNA sequence recognized by the capsid protein for encapsidation. The viral structural proteins, which are

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deleted in the vector, were supplied by the helper. Dual transfection of helper and vector constructs resulted in the packaging of vector RNA into particles and their subsequent release from the cell (Liljeström & Garoff, 1991; Berglund *et al.*, 1993; Liljeström, 1994). In a more recent modification of this system, the structural proteins are encoded by a split-helper system which increases biosafety by preventing the formation of wild-type virus by recombination between helper and vector. To further increase biosafety, an S219A substitution has been made in the capsid sequence encoded by the helper vector C to prevent self-cleavage of the capsid protein and hence to prevent the production of infectious virus in the unlikely event that a complete coding sequence is generated by recombination (Fig. 5; Smerdou & Liljeström, 1999*a*). Packaged particles are infectious and contain RNA encoding the cloned gene. Although the RNA will be expressed on infection, progeny particles will not be produced because the packaged vector RNA lacks the viral structural protein genes.

A subsequent modification of this vector system is the layered DNA/RNA vector. Here the SFV vector is cloned as cDNA under the control of a cytomegalovirus (CMV) immediate early promoter. When such DNA is transfected into cells, the CMV promoter stimulates the transcription of the SFV sequence along with any cloned gene, and high-level transient expression occurs (Berglund *et al.*, 1998*b*). Both types of SFV vector induce apoptosis late in infection, so persistence of the virus genome is unlikely to occur (Glasgow *et al.*, 1998; Kohno *et al.*, 1998).

Recombinant SFV vectors as prototype vaccines

SFV vectors have several advantages as vectors to construct new prototype vaccines (Zhou *et al.*, 1994; Atkins *et al.*, 1996; Tubulekas *et al.*, 1997; Berglund *et al.*, 1998*a*; Smerdou & Liljeström, 1999*b*). RNA replication is cytoplasmic, so there is no risk of integration into the chromosome, few viral proteins are expressed (only low-level expression of the nonstructural proteins) and cell death occurs so the virus genome does not persist in tissue. Most people and animals have no pre-existing immunity against the vector and SFV has a broad host-range, infecting most types of cell. Good immune responses can be generated against the NP protein of influenza virus using naked vector RNA (Zhou *et al.*, 1994), but since RNA is unstable, recombinant particles or the DNA/RNA vector is a more practical approach. Both types of vector have been successful in inducing humoral and cell-mediated immunity against the HA and NP proteins of influenza virus (Zhou *et al.*, 1995; Berglund *et al.*, 1998*a*, *b*, 1999). In monkeys, recombinant SFV particles expressing the envelope glycoproteins of human and simian immunodeficiency viruses (SIV) have been shown to induce immunity, which in the case of SIV was protective (Berglund *et al.*, 1997; Mossman *et al.*, 1996). The broad host-range of SFV vectors is illustrated by their possible potential for the construction of new vaccines for fish (Vaughan *et al.*, 1998; Phenix *et al.*, 1999).

Louping ill virus (LIV) infection of mice and sheep is being developed as a model to test the efficacy and biosafety of SFV-based vaccines. LIV is a tick-transmitted flavivirus which infects sheep in upland pastures in the British Isles (the UK and Ireland). Virulent strains of the virus are rapidly lethal for both mice and sheep, and therefore represent a good challenge model to test vaccine constructs. The structural prME and nonstructural NS1 flavivirus proteins have previously been shown induce protective immunity; SFV vectors expressing these antigens have been constructed. Recombinant SFV particles expressing these proteins have been shown to induce protective immunity (both humoral and cell-mediated) in mice against both the prototype LI/31 strain of LIV, and the LI/I strain, which is a naturally occurring antibody-escape variant (Fleeton *et al.*, 1999).

Future work

In our last review, published in 1985, SFV was considered as a model for the molecular analysis of viral pathogenicity (Atkins *et al.*, 1985). SFV remains such a model which will contribute to our understanding of the molecular basis of viral multiplication and pathogenesis. However, in the intervening period, SFV has also become important in advancing our understanding of disease processes, and as a vector for the construction of new viral vaccines.

In the area of viral pathogenesis, it is important to understand the virus functions which control virulence, which in turn is related to the ability of the virus to efficiently infect neurons and to spread through the CNS. Understanding of the death mechanism induced in neurons by virulent SFV may contribute to our knowledge of mechanisms of neuro-degeneration. Also, avirulent SFV infection may be an important model of MS in man which is different and more relevant than other viral models such as Theiler's murine encephalitis virus and murine coronavirus, since it does not involve persistence of infectious virus in the CNS. At present the mechanism of SFV-induced teratogenesis, particularly the cell death mechanism in infected foetuses, is not understood at the molecular level. It is still not understood how DI particles may modulate pathogenicity, but understanding this may facilitate the utilization of the antiviral activity of DI particles in the treatment of disease.

The conflicting results concerning apoptosis induction by SFV and the role of the bcl-2 gene need to be resolved. In particular, it is important to know the viral function that leads to the caspase-mediated cleavage of bcl-2. Understanding the mechanisms of apoptosis induction by SFV may lead to the development of SFV vectors as agents to treat cancer by apoptosis induction.

It remains to be seen whether effective and safe vaccines can be constructed using SFV vectors. Certainly SFV vectors have potential in this area, because of their relative simplicity, high level of expression, efficient immune stimulation and

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suicidal nature. SFV is thus one of the best examples of a virus which was once studied mainly because of its academic interest as a model, but which can now be exploited to provide tangible benefits to molecular medicine.

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References

Allsopp, T. E., Scallan, M. F., Williams, A. & Fazakerley, J. K. (1997). Virus infection induces neuronal apoptosis: a comparison with trophic factor withdrawal. *Cell Death and Differentiation* **5**, 50–59.

Amor, S., Scallan, M. F., Morris, M. M., Dyson, H. & Fazakerley, J. K. (1996). Role of immune responses in protection and pathogenesis during Semliki Forest virus encephalitis. *Journal of General Virology* **77**, 281–291.

Atkins, G. J. (1976). The effect of infection with Sindbis virus and its temperature-sensitive mutants on cellular protein and DNA synthesis. *Virology* **71**, 593–597.

Atkins, G. J. (1977). Cytopathogenicity of temperature-sensitive mutants of Sindbis virus. *FEMS Microbiology Letters* **2**, 51–55.

Atkins, G. J. (1983). The avirulent A7 strain of Semliki Forest virus has reduced cytopathogenicity for neuroblastoma cells compared to the virulent L10 strain. *Journal of General Virology* **64**, 1401–1404.

Atkins, G. J. & Sheahan, B. J. (1982). Semliki Forest virus neurovirulence mutants have altered cytopathogenicity for central nervous system cells. *Infection and Immunity* **36**, 333–341.

Atkins, G. J., Carter, J. & Sheahan, B. J. (1982). Effect of alphavirus infection on mouse embryos. *Infection and Immunity* **38**, 1285–1290.

Atkins, G. J., Sheahan, B. J. & Dimmock, N. J. (1985). Semliki Forest virus infection of mice: a model for genetic and molecular analysis of viral pathogenicity. *Journal of General Virology* **66**, 395–408.

Atkins, G. J., Sheahan, B. J. & Mooney, D. A. (1990). Pathogenicity of Semliki Forest virus for the rat central nervous system and primary rat neural cell cultures – possible implications for the pathogenesis of multiple sclerosis. *Neuropathology and Applied Neurobiology* **16**, 57–68.

Atkins, G. J., Balluz, I. M., Glasgow, G. M., Mabruk, M. J. E. M. F., Natale, V. A. I, Smyth, J. M. B. & Sheahan, B. J. (1994). Analysis of the molecular basis of neuropathogenesis of RNA viruses in experimental animals: relevance for human disease? *Neuropathology and Applied Neurobiology* **20**, 91–102.

Atkins, G. J., Mabruk, M. J. E. M. F., Glasgow, G. M., Griffin, A. M. & Sheahan, B. J. (1995). Mechanisms of viral teratogenesis. *Reviews in Medical Virology* **5**, 75–86.

Atkins, G. J., Sheahan, B. J. & Liljeström, P. (1996). Manipulation of the Semliki Forest virus genome and its potential for vaccine construction. *Molecular Biotechnology* **5**, 33–38.

Balluz, I. M., Glasgow, G. M., Killen, H. M., Mabruk, M. J. E. M. F., Sheahan, B. J. & Atkins, G. J. (1993). Virulent and avirulent strains of Semliki Forest virus show similar cell tropism for the murine central nervous system but differ in the severity and rate of induction of cytolytic damage. *Neuropathology and Applied Neurobiology* **19**, 233–239.

Barrett, A. D. T. & Dimmock, N. J. (1984). Modulation of Semliki Forest virus infection of mice by defective-interfering virus. *Journal of Infectious Diseases* **150**, 98–104.

Barrett, P. N., Sheahan, B. J. & Atkins, G. J. (1980). Isolation and preliminary characterization of mutants of Semliki Forest virus with altered virulence. *Journal of General Virology* **49**, 141–148.

Berglund, P., Sjöberg, M., Atkins, G. J., Sheahan, B. J., Garoff, H. & Liljeström, P. (1993). Semliki Forest virus expression system: production of conditionally infectious recombinant particles. *Bio/Technology* **11**, 916–920.

Berglund, P., Quesada-Rolander, M., Putkonen, P., Biberfeld, G., Thorstensson, R. & Liljeström, P. (1997). Outcome of immunization of cynomolgus monkeys with recombinant Semliki Forest virus encoding human immunodeficiency virus type 1 envelope protein and challenged with a high dose of SHIV-4 virus. *AIDS Research and Human Retroviruses* **13**, 1487–1495.

Berglund, P., Fleeton, M., Smerdou, C., Tubulekas, I., Sheahan, B. J., Atkins, G. J. & Liljeström, P. (1998*a*). Vaccination with recombinant suicidal DNA/RNA. In *New Developments and New Applications in Animal Cell Technology*, pp. 583–590. Edited by O. Merten, P. Perrin & B. Griffiths. Dordrecht: Kluwer Academic Publishers.

Berglund, P., Smerdou, C., Fleeton, M. N., Tubulekas, I. & Liljeström, P. (1998*b*). Enhancing immune responses using suicidal DNA vaccines. *Nature Biotechnology* **16**, 562–565.

Berglund, P., Fleeton, M. N., Smerdou, C. & Liljeström, P. (1999). Immunization with recombinant Semliki Forest virus induces protection against influenza challenge in mice. *Vaccine* **17**, 497–507.

Bradish, C. J., Allner, K. & Maber, H. B. (1971). The virulence of original and derived strains of Semliki forest virus for mice, guinea-pigs and rabbits. *Journal of General Virology* **12**, 141–160.

Butt, A. M., Tutton, M. G., Kirvell, S. L., Amor, S. & Jenkins, H. G. (1996). Morphology of oligodendrocytes during demyelination in optic nerves of mice infected with Semliki Forest virus. *Neuropathology and Applied Neurobiology* **22**, 540–547.

Donnelly, S. M., Sheahan, B. J. & Atkins, G. J. (1997). Long-term effects of Semliki Forest virus infection in the mouse central nervous system. *Neuropathology and Applied Neurobiology* **23**, 235–241.

Dropulic, B. & Masters, C. L. (1990). Entry of neurotropic arboviruses into the central nervous system: an *in vitro* study using mouse brain endothelium. *Journal of Infectious Diseases* **161**, 685–691.

E. C. Council Directive 93/88/EEC (1993). Amending directive 90/679/EEC on the protection of workers from risks related to exposure to biological agents at work. *Official Journal of the European Communities* **268**, 71–82.

Erälinna, J. P., Soiluhänninen, M., Roytta, M., Ilonen, J., Mäkelä, M., Salmi, A. & Salonen, R. (1994). Facilitation of experimental allergic encephalomyelitis by irradiation and virus infection: role of inflammatory cells. *Journal of Neuroimmunology* **55**, 81–90.

Fazakerley, J. K. & Webb, H. E. (1987). Semliki Forest virus-induced, immune-mediated demyelination: adoptive transfer studies and viral persistence in nude mice. *Journal of General Virology* **68**, 377–386.

Fazakerley, J. K., Pathak, S., Scallan, M., Amor, S. & Dyson, H. (1993). Replication of the A7(74) strain of Semliki Forest virus is restricted in neurons. *Virology* **195**, 627–637.

Federal Register 58 no. 19, 9 (1993). Addition of Appendix DL-X to the NIH guidelines regarding Semliki Forest virus. *Human Gene Therapy* **5**, 401.

Fernandez, I. M., Snijders, A., Benaissatrouw, B. J., Harmsen, M., Snippe, H. & Kraaijeveld, C. A. (1993). Influence of epitope polarity and adjuvants on the immunogenicity and efficacy of a synthetic peptide vaccine against Semliki Forest virus. *Journal of Virology* **67**, 5843–5848.

Fleeton, M. N., Sheahan, B. J., Gould, E. A., Atkins, G. J. & Liljeström,

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P. (1999). Recombinant Semliki Forest virus particles encoding the prME or NS1 proteins of louping ill virus protect mice from lethal challenge. *Journal of General Virology* **80**, 1189–1198.

Frolov, I. & Schlesinger, S. (1994). Comparison of the effects of Sindbis virus and Sindbis virus replicons on host cell protein synthesis and cytopathogenicity in BHK cells. *Journal of Virology* **68**, 1721–1727.

Fujiwara, T., Cai, D. W., Georges, R. N., Mukhopadhyay, T., Grimm, E. A. & Roth, J. A. (1994). Therapeutic effect of a retroviral wild-type p53 expression vector in an orthotopic lung cancer model. *Journal of the National Cancer Institute* **86**, 1458–1462.

Gates, M. C., Sheahan, B. J. & Atkins, B. J. (1984). The pathogenicity of the M9 mutant of Semliki Forest virus in immune compromised mice. *Journal of General Virology* **65**, 73–80.

Gates, M. C., Sheahan, B. J., O'Sullivan, M. A. & Atkins, G. J. (1985). The pathogenicity of the A7, M9 and L10 strains of Semliki Forest virus for weanling mice and primary mouse brain cell cultures. *Journal of General Virology* **66**, 2365–2373.

Glasgow, G. M., Sheahan, B. J., Atkins, G. J., Wahlberg, J. M., Salminen, A. & Liljeström, P. (1991). Two mutations in the envelope glycoprotein-E2 of Semliki Forest virus affecting the maturation and entry patterns of the virus alter pathogenicity for mice. *Virology* **185**, 741–748.

Glasgow, G. M., Killen, H. M., Liljeström, P., Sheahan, B. J. & Atkins, G. J. (1994). A single amino acid change in the E2 spike protein of a virulent strain of Semliki Forest virus attenuates pathogenicity. *Journal of General Virology* **75**, 663–668.

Glasgow, G. M., McGee, M. M., Mooney, D. A., Sheahan, B. J. & Atkins, G. J. (1997). Death mechanisms in cultured cells infected by Semliki Forest virus. *Journal of General Virology* **78**, 1559–1563.

Glasgow, G. M., McGee, M. M., Tarbatt, C. J., Mooney, D. A., Sheahan, B. J. & Atkins, G. J. (1998). The Semliki Forest virus vector induces p53-independent apoptosis. *Journal of General Virology* **79**, 2405–2410.

Grandgirard, D., Studer, E., Monney, L., Belser, T., Fellay, I., Borner, C. & Michel, M. R. (1998). Alphaviruses induce apoptosis in Bcl-2 overexpressing cells: evidence for a caspase-mediated, proteolytic inactivation of Bcl-2. *EMBO Journal* **17**, 1268–1278.

Hearne, A. M., O'Sullivan, M. A. & Atkins, G. J. (1986). Infection of cultured early mouse embryos with Semliki Forest and rubella viruses. *Journal of General Virology* **67**, 1091–1098.

Hearne, A. M., O'Sullivan, M. A. & Atkins, G. J. (1987). Isolation and preliminary characterization of Semliki Forest virus mutants with altered pathogenicity for mouse embryos. *Journal of General Virology* **68**, 107–113.

Kaluza, G., Lell, G., Reimacher, M., Stitz, L. & Willems, W. R. (1987). Neurogenic spread of Semliki Forest virus in mice. *Archives of Virology* **93**, 97–110.

Kibler, K. V., Shors, T., Perkins, K. B., Zeman, C. C., Banaszak, M. P., Biesterfeldt, J., Langland, J. O. & Jacobs, B. L. (1997). Double-stranded RNA is a trigger for apoptosis in vaccinia virus-infected cells. *Journal of Virology* **71**, 1992–2003.

Kinney, R. M., Chang, G.-J., Tsuchiya, K. R., Sneider, J. M., Roerig, J. T., Woodward, T. M. & Trent, D. W. (1993). Attenuation of Venezuelan equine encephalitis strain TC-83 is encoded by the 5′ noncoding region and the E2 envelope glycoprotein. *Journal of Virology* **67**, 1269–1277.

Kohno, A., Emi, N., Kasai, M., Tanimoto, M. & Saito, H. (1998). Semliki Forest virus-based DNA expression vector – transient protein production followed by cell death. *Gene Therapy* **5**, 415–418.

Kujala, P., Rikkonene, M., Ahola, T., Kelve, M., Saarma, M. & Kääriäinen, L. (1997). Monoclonal antibodies specific for Semliki Forest virus replicase protein nsP2. *Journal of General Virology* **78**, 343–351.

Lee, S. B. & Esteban, M. (1994). The interferon-induced double-stranded RNA-activated protein kinase induces apoptosis. *Virology* **199**, 491–496.

Liljeström, P. (1994). Alphavirus expression systems. *Current Opinion in Biotechnology* **5**, 495–500.

Liljeström, P. & Garoff, H. (1991). A new generation of animal cell expression vectors based on the Semliki Forest virus replicon. *Bio/Technology* **9**, 1356–1361.

Liljeström, P., Lusa, S., Huylebroeck, D. & Garoff, H. (1991). In vitro mutagenesis of a full-length cDNA clone of Semliki Forest virus: the small 6,000-molecular weight membrane protein modulates virus release. *Journal of Virology* **65**, 4107–4113.

Lundström, K., Pralong, W. & Martinou, J.-C. (1997). Anti-apoptotic effect of Bcl-2 overexpression in RIN cells infected with Semliki Forest virus. *Apoptosis* **2**, 189–191.

Mabruk, M. J. E. M. F., Flack, A. M., Glasgow, G. M., Smyth, J. M. B., Folan, J. C., Bannigan, J. G., O'Sullivan, M. A., Sheahan, B. J. & Atkins, G. J. (1988). Teratogenicity of the Semliki Forest virus mutant ts 22 for the foetal mouse: induction of skeletal and skin defects. *Journal of General Virology* **69**, 2755–2762.

Mabruk, M. J. E. M. F., Glasgow, G. M., Flack, A. M., Folan, J. C., Bannigan, J. G., Smyth, J. M. B., O'Sullivan, M. A., Sheahan, B. J. & Atkins, G. J. (1989). Effect of infection with the ts 22 mutant of Semliki Forest virus on development of the central nervous system in the fetal mouse. *Journal of Virology* **63**, 4027–4033.

McIntosh, B. M., Brookworth, C. & Kokernot, R. H. (1961). Isolation of Semliki Forest virus from *Aëdes* (*Aëdimorphus*) *argenteopunctatus* (Theobald) collected in Portuguese East Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **55**, 192–198.

Mathiot, C. C., Grimaud, G., Garry, P., Bouquety, J. C., Mada, A., Daguisy, A. M. & Georges, A. J. (1990). An outbreak of human Semliki Forest virus infection in Central African Republic. *American Journal of Tropical Medicine and Hygiene* **42**, 386–393.

Meek, A. D. J., Faragher, S. G., Weir, R. C. & Dalgarno, L. (1989). Genetic and phenotypic studies on Ross River virus variants of enhanced virulence selected during mouse passage. *Virology* **172**, 399–407.

Mokhtarian, F. & Swoveland, P. (1987). Predisposition to EAE induction in resistant mice by prior infection with Semliki Forest virus. *Journal of Immunology* **138**, 3264–3268.

Mokhtarian, F., Shi, Y., Zhu, P. F. & Grob, D. (1994). Immune responses, and autoimmune outcome, during virus infection of the central nervous system. *Cellular Immunology* **157**, 195–210.

Mokhtarian, F., Wesselingh, S., Choi, S., Maeda, A., Griffin, D. E., Sobel, R. A. & Grob, D. (1996). Production and role of cytokines in the CNS of mice with acute viral encephalomyelitis. *Journal of Neuroimmunology* **66**, 11–22.

Morris, M. M., Dyson, H., Baker, D., Harbige, L. S., Fazakerley, J. K. & Amor, S. A. (1997). Characterization of the cellular and cytokine response in the central nervous system following Semliki Forest virus infection. *Journal of Neuroimmunology* **74**, 185–197.

Mossman, S., Bex, F., Berglund, P., Arthos, J., O'Neill, S. P., Riley, D., Maul, D. H., Bruck, C., Momin, P., Burny, A., Fultz, P. N., Mullins, J. I., Liljeström, P. & Hoover, E. A. (1996). Protection against lethal SIVsmmPBj14 disease by a recombinant Semliki Forest virus gp160 vaccine and by gp120 subunit vaccine. *Journal of Virology* **70**, 1953–1960.

Oliver, K. R. & Fazakerley, J. K. (1998). Transneuronal spread of Semliki Forest virus in the developing mouse olfactory system is determined by neuronal maturity. *Neurosciences* **82**, 867–877.

Oliver, K. R., Scallan, M. F., Dyson, H. & Fazakerley, J. K. (1998).

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BioNTech SE and Pfizer Inc. v. ModernaTX, Inc. IPR2023-01358
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Susceptibility to a neurotropic virus and its changing distribution in the developing brain is a function of CNS maturity. *Journal of Neurovirology* **3**, 38–48.

Pence, D. F., Davis, N. L. & Johnston, R. E. (1990). Antigenic and genetic characterization of Sindbis virus monoclonal antibody escape mutants which define a pathogenesis domain on glycoprotein E2. *Virology* **175**, 41–49.

Phenix, K. V., McKenna, B., Fitzpatrick, R., Vaughan, L., Atkins, G., Liljeström, P. & Todd, D. (1999). Evaluation of the Semliki Forest virus expression system as a novel approach for antigen delivery and expression in fish. *Marine Microbiology* (in press).

Razvi, E. S. & Welsh, R. M. (1995). Apoptosis in viral infections. *Advances in Virus Research* **45**, 1–60.

Rikkonen, M. (1996). Functional significance of the nuclear-targeting and NTP-binding motifs of Semliki Forest virus nonstructural protein nsP2. *Virology* **218**, 352–361.

Rikkonen, M., Peränen, J. & Kääriäinen, L. (1992). Nuclear and nucleolar targeting signals of Semliki forest virus nonstructural protein nsP2. *Virology* **189**, 462–473.

Sammin, D. J., Butler, D., Atkins, G. J. & Sheahan, B. J. (1999). Cell death mechanisms in the olfactory bulb of rats infected intranasally with Semliki Forest virus. *Neuropathology and Applied Neurobiology* (in press).

Santagati, M. G. (1998). *Virulence determinants of Semliki Forest virus*. PhD thesis, University of Turku, Finland.

Santagati, M. G., Itäranta, P. V., Koskimies, P. R., Määttä, J. A., Salmi, A. A. & Hinkkanen, A. E. (1994). Multiple repeating motifs are found in the 3′-terminal nontranslated region of Semliki Forest virus A7 variant genome. *Journal of General Virology* **75**, 1499–1504.

Santagati, M. G., Määttä, J. A., Itäranta, J. A., Salmi, A. A. & Hinkkanen, A. E. (1995). The Semliki Forest virus E2 gene as a virulence determinant. *Journal of General Virology* **76**, 47–52.

Santagati, M. G., Määttä, J. A., Röyttä, M., Salmi, A. A. & Hinkkanen, A. E. (1998). The significance of the 3′-nontranslated region and E2 amino acid mutations in the virulence of Semliki Forest virus in mice. *Virology* **243**, 66–77.

Scallan, M. F., Allsopp, T. E. & Fazakerley, J. K. (1997). *bcl*-2 acts early to restrict Semliki Forest virus replication and delays virus-induced programmed cell death. *Journal of Virology* **71**, 1583–1590.

Sheahan, B. J., Ibrahim, M. A. & Atkins, G. J. (1996). Demyelination of olfactory pathways in mice following intranasal infection with the avirulent A7 strain of Semliki Forest virus. *European Journal of Veterinary Pathology* **2**, 117–125.

Smerdou, C. & Liljeström, P. (1999*a*). Two-helper RNA system for production of recombinant Semliki Forest virus particles. *Journal of Virology* **73**, 1092–1098.

Smerdou, C. & Liljeström, P. (1999*b*). Non-viral amplification systems for gene transfer: vectors based on alphaviruses. *Current Opinion in Molecular Therapy* (in press).

Smithburn, K. C. & Haddow, A. J. (1944). Semliki Forest virus. I. Isolation and pathogenic properties. *Journal of Immunology* **49**, 141–157.

Smyth, J. M. B., Sheahan, B. J. & Atkins, G. J. (1990). Multiplication of virulent and demyelinating Semliki Forest virus in the mouse central nervous system: consequences in BALB/c and SJL mice. *Journal of General Virology* **71**, 2575–2583.

Snijders, A., Benaissatrouw, B. J., Snippe, H. & Kraaijeveld, C. A. (1992). Immunogenicity and vaccine efficacy of synthetic peptides containing Semliki Forest virus B-cell and T-cell epitopes. *Journal of General Virology* **73**, 2267–2272.

Soiluhänninen, M., Erälinna, J. P., Hukkanen, V., Röyttä, M., Salmi, A. A. & Salonen, R. (1994). Semliki Forest virus infects mouse brain endothelial cells and causes blood–brain barrier damage. *Journal of Virology* **68**, 6291–6298.

Soiluhänninen, M., Röyttä, M., Salmi, A. A. & Salonen, R. (1997). Semliki Forest virus infection leads to increased expression of adhesion molecules on splenic T-cells and on brain vascular endothelium. *Journal of Neurovirology* **3**, 350–360.

Strauss, J. H. & Strauss, E. G. (1994). The alphaviruses: gene expression, replication and evolution. *Microbiological Reviews* **58**, 491–562.

Subak-Sharpe, I., Dyson, H. & Fazakerley, J. K. (1993). In vivo depletion of CD8+ T cells prevents lesions of demyelination in Semliki Forest virus infection. *Journal of Virology* **67**, 7629–7633.

Tarbatt, C. J., Glasgow, G. M., Mooney, D. A., Sheahan, B. J. & Atkins, G. J. (1997). Sequence analysis of the avirulent, demyelinating A7 strain of Semliki Forest virus. *Journal of General Virology* **78**, 1551–1557.

Thomson, M. & Dimmock, N. J. (1994). Sequences of defective interfering Semliki Forest virus necessary for antiviral activity *in vivo* and interference and propagation *in vitro*. *Virology* **199**, 354–365.

Thomson, M., White, C. L. & Dimmock, N. J. (1998). The genomic sequences of defective interfering Semliki Forest virus (SFV) determines its ability to be replicated in mouse brain and to protect against a lethal SFV infection *in vivo*. *Virology* **241**, 215–223.

Tubulekas, I., Berglund, P., Fleeton, M. & Liljeström, P. (1997). Alphavirus expression vectors and their use as recombinant vaccines – a minireview. *Gene* **190**, 191–195.

Tucker, P. C., Strauss, E. G., Kuhn, R. J., Strauss, J. H. & Griffin, D. E. (1993). Viral determinants of age-dependent virulence of Sindbis virus for mice. *Journal of Virology* **65**, 4605–4610.

Ubol, S., Tucker, P. C., Griffin, D. E. & Hardwick, J. M. (1994). Neurovirulent strains of alphavirus induce apoptosis in bcl-2-expressing cells: role of a single amino acid change in the E2 glycoprotein. *Proceedings of the National Academy of Sciences, USA* **91**, 5202–5206.

U.S. Department of Health & Human Services (1993). Biosafety in Microbiological and Biomedical Laboratories. *HHS Publication no.* (*CDC*) 93-8395, 3rd edn.

Vaughan, L. M., Fitzpatrick, R. M., McKenna, B. & Atkins, G. J. (1998). Molecular approaches to fish vaccine design. In *Methodology in Fish Disease Research*, pp. 161–166. Edited by A. Barnes, G. Davidson, M. Hiney & D. McIntosh. Aberdeen: Albion Press.

White, C. L., Thomson, M. & Dimmock, N. J. (1998). Deletion analysis of a defective interfering Semliki Forest virus RNA genome defines a region in the nsP2 sequence that is required for efficient packaging of the genome into virus particles. *Journal of Virology* **72**, 4320–4326.

Willems, W. R., Kaluza, G., Boschek, C. B., Bauer, H., Hager, H., Schütz, H.-J. & Feistner, H. (1979). Semliki Forest virus: cause of a fatal case of human encephalitis. *Science* **203**, 1127–1129.

Winkler, W. G. & Blenden, D. C. (1995). Transmission and control of viral zoonoses in the laboratory. In *Laboratory Safety: Principles and Practices*, 2nd edn. Edited by D. O. Fleming, J. H. Richardson, J. L. Tulis & D. Vesley. Washington, DC: American Society for Microbiology.

Zhou, X. P., Berglund, G., Rhodes, G., Parker, S. E., Jondal, M. & Liljeström, P. (1994). Self-replicating Semliki Forest virus RNA as recombinant vaccine. *Vaccine* **12**, 1510–1514.

Zhou, X. P., Berglund, G., Zhao, H., Liljeström, P. & Jondal, M. (1995). Generation of cytotoxic and humoral immune responses by non-replicative recombinant Semliki Forest virus. *Proceedings of the National Academy of Sciences, USA* **92**, 3009–3013.

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