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Pathogenesis of Semliki Forest virus encephalitis

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This article provides a review of the pathogenesis of Semliki Forest virus (SFV) encephalitis. In mice, outcome of infection varies according to age of the mouse and strain of the virus and can include acute encephalitis, subacute demyelinating meningoencephalomyelitis, and persistent subclinical central nervous system (CNS) infection. All strains of virus are virulent in mice infected <12 days of age. The L10 strain is also virulent in mice >14 days age, whereas the A7(74) strain is avirulent. The genetic difference between these strains maps to the nsp3 gene. For A7(74) virus, age-related virulence correlates with ability of CNS neurons to replicate virus and undergo apoptotic cell death. Immature developing neurons support complete virus replication but as neuronal populations and circuits mature in the postnatal brain, virus infection becomes progressively restricted and nonproductive. This restricted replication can be overcome by gold I compounds, which may function by inducing neuronal dedifferentiation to a state permissive for virus replication. Biochemical pathways associated with membrane biogenesis may be an important determinant of this effect. Infection of some developing neuronal populations results in apoptosis, whereas infection of mature neurons results in persistent infection. An active type-I interferon system prevents virus spread in extraneural tissues. An initial high-titer plasma viremia is controlled by immunoglobulin M (IgM) antibodies. Virus enters the brain across cerebral endothelial cells and initiates scattered foci of perivascular infection. The blood-brain barrier is disrupted. Neurons and oligodendrocytes are the cell types most frequently infected. Infectivity in the brain can be eliminated by IgG antibodies, though an active T-cell response is required for virus elimination. Lesions of inflammatory demyelination require the presence of $CD8^+$ T lymphocytes and probably result from destruction by these cells of virally infected oligodendrocytes. *Journal of NeuroVirology* (2002) **8(suppl. 2),** 66–74.

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Strains of SFV

Semliki Forest virus (SFV), an alphavirus of the Togaviridae family, is naturally found in sub-Saharan Africa where it is spread principally by *Aedes africanus* and *Aedes aegypti* mosquitoes. SFV is most closely related to Chikungunya, Getah, and Mayaro alphaviruses. SFV was first isolated in 1942 from a squash of 130 female, *Aedes abnormalis* mosquitoes captured in the Semliki Forest in Uganda (Smithburn and Haddow, 1944). The natural vertebrate host remains unknown but infections have been documented in horses, monkeys, and man. The virus was associated with an epidemic of equine encephalitis in Senegal (Robin *et al*, 1974). In a 1987 survey in the Central African Republic, SFV was isolated from 22 patients with fever, severe persistent headaches, myalgia, and arthralgia (Mathiot *et al*, 1990). Virus was also isolated from locally caught *Aedes aegypti* mosquitoes. There is one reported case of the death of a scientist working with the Osterrieth strain of the virus (Willems *et al*, 1979). The patient had neurological disease and at postmortem examination, a typical viral meningoencephalomyelitis was observed. SFV was isolated from both the cerebrospinal fluid and the brain. It is known that the scientist was working with virus supernatant from baby hamster kidney (BHK) cells but the route and dose of infection and the genotype of this virus are unknown. Seroconversion in laboratory workers is common (Willems *et al*, 1979). SFV

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is relatively stable in aerosols of tissue homogenates (Benbough, 1969), although aerosols of purified virus are rapidly inactivated (de Jong *et al*, 1976).

Various strains of virus have been used in laboratories around the world and have been shown experimentally to infect voles, mice, guinea pigs, rabbits, and rats (Seamer *et al*, 1967a, 1967b; Bradish *et al*, 1971). Different strains have been designated as virulent or avirulent according to their virulence in adult mice. The L10, V13, and Osterrieth strains and the strain designated prototype are virulent (Bradish *et al*, 1971; Glasgow *et al*, 1991). The A8, A7, A7(74), and MRS MP 192/7 strains are avirulent (Bradish *et al*, 1971; Henderson *et al*, 1970). Virulence in mice is age-related because all strains are virulent in neonatal and suckling mice. The L10 strain was derived from the original isolate (Smithburn and Haddow, 1944) by eight intracerebral passages through adult and two through baby mouse brains (Bradish *et al*, 1971). A7(74) was derived from the AR2066 strain by seven passages through neonatal mouse brain and colony selection on chick embryo fibroblasts. AR2066 was isolated from *Aedes argenteopunctatus* mosquitoes in Namancurra, Mozambique (McIntosh *et al*, 1961). A panel of 15 E1 or E2 monoclonal antibodies raised to MRS MP 192/7 can distinguish between some of the virus strains (Boere *et al*, 1984, 1986). Several series of SFV mutants, including temperature-sensitive (ts) mutants, have been described (Tan *et al*, 1969; Keranen and Kaariainen, 1974; Hearne *et al*, 1987; Barrett *et al*, 1980).

A number of strains including prototype, which is related to L10, A7, and A7(74), have been cloned and sequenced (Garoff *et al*, 1980; Takkinen, 1986; Glasgow *et al*, 1994; Santagati *et al*, 1995, 1998; Tarbatt *et al*, 1997; Tuittila *et al*, 2000). The molecular clone of the prototype strain, known as SFV4, has been engineered to form a much used viral expression and vaccine system (Liljestrom and Garoff, 1991; Tubulekas *et al*, 1997). Isolated SFV RNA is infectious (Friedman *et al*, 1966). As with many positive-stranded RNA viruses, studies of site-directed mutants have proved to be a powerful approach to mapping areas of the genome responsible for individual phenotypic characteristics. Among the SFV4, A7, and A7(74) strains, there are multiple changes scattered throughout the genome (Tuittila *et al*, 2000). The difference in virulence between SFV4 and A7 or A7(74) appears to be polygenic and changes in the E2 gene, the 5′ untranslated region, and the carboxy-terminal end of the nsP3 gene have been shown to be determinants of virulence between these particular strains (Santagati *et al*, 1995, 1998; Tarbatt *et al*, 1997; Tuittila *et al*, 2000).

SFV replication

SFV is a relatively simple virus, it has an approximately 12-kb genome of single-stranded positive-sense RNA. Replication is via a negative-sense intermediate that gives rise to full-length genomic RNA and a subgenomic message, which represents the 3′ third of the genomic RNA and contains the genes for the viral structural proteins. Both the genomic and subgenomic RNAs are capped and polyadenylated. From 5′ to 3′ along the genomic RNA, there are four nonstructural replicase proteins, designated nsP1 to nsP4, followed by the capsid protein C and the envelope glycoproteins E2, E3, and E1. Many of the functions of the replicase proteins have been elucidated. NsP1 is involved in capping the viral RNAs and has methyl and guanylyl transferase activities; it participates in initiation of negative-strand RNA synthesis and targets the replicase complex to membranes (Ahola and Kaariainen, 1995; Laakkonen *et al*, 1994; Ahola *et al*, 1997; Peranen and Kaariainen, 1991). The large nsP2 protein has single-stranded RNA-stimulated ATPase and GTPase activities, RNA triphosphatase activity, RNA helicase activity, regulates synthesis of the 26S subgenomic RNA, is involved in the cessation of negative-strand synthesis, and contains a papain-like proteinase domain responsible for processing the nonstructural polyprotein (Rikkonen *et al*, 1994; Vasiljeva *et al*, 2000; Gomez de Cedron *et al*, 1999; Suopanki *et al*, 1998; Merits *et al*, 2001). In infected cells, about half the nsP2 synthesized is translocated to the nucleus (Kujala *et al*, 1997; Peranen *et al*, 1990; Rikkonen *et al*, 1992). The protein contains a nuclear-targeting sequence that is present in all three cloned and sequenced strains of the virus. Disruption of this sequence has minimal effect on virus replication in continuously cultured cells but severely attenuates replication in mouse brain (Fazakerley *et al*, 2002). The role of nsP3 is less well known. It is a phosphoprotein, with a role in the regulation of RNA synthesis (Peranen *et al*, 1988; Wang *et al*, 1991). NsP4 is the catalytic subunit of the RNA polymerase (Keranen and Kaariainen, 1979). The polymerase complex is associated with plasma membrane and endo/lysosomes by specific interactions of nsP1 and nsP2. SFV virions are enveloped and on budding have a spike composed of three E1-E2 heterodimers. Within the lipid envelope, the RNA is associated with the capsid protein, which is in contact with the C-terminal domain of the E2 protein. Budding of virions is driven by spike capsid interactions and does not require the presence of full-length genomic RNA (Suomalainen *et al*, 1992).

The receptor remains undetermined but virus entry and fusion are via the endosomal system and have been much studied (Helenius *et al*, 1986). Fusion of the viral and endosomal membranes is a function of the E1 glycoprotein and is dependent upon the presence of cholesterol and reorganization of the envelope spike at acid pH (Omar and Koblet, 1988; Helenius *et al*, 1980; Kielian and Helenius, 1984; Wahlberg and Garoff, 1992). Upon fusion, the capsid is liberated into the cytoplasm. RNA replication is

associated with smooth membrane structures termed cytopathic vacuoles (CPVs), types I and II (Grimley *et al*, 1968; Grimley and Friedman, 1970; Kujala *et al*, 2001). These structures can be separated from other subcellular structures on sucrose gradients, and biochemical markers indicate they are derived from late endosomes and lysosomes (Friedman *et al*, 1972; Froshauer *et al*, 1988; Mehta *et al*, 1990; Kujala *et al*, 2001). Confocal microscopy and electronmicroscopy (EM) studies reveal that the surfaces of CPV-I are covered by small invaginations termed spherules, which contain the viral nsP's and are the likely sites of viral RNA replication (Grimley *et al*, 1968; Kujala *et al*, 2001). *In vitro* studies of cells in continuous culture have generally observed virus budding at the plasma membrane. However, EM observations on brain sections from SFV-infected neonatal mice indicate that in neurons, virions frequently bud into the lumen of type-II vacuoles, which then fuse with the cell membrane to release mature virions (Pathak *et al*, 1976). In brain sections, clumps of electron-dense material are observed throughout the cytoplasm early in infection. These contain capsid protein and are probably RNA capsid complexes. They migrate to underlie membranes, including the plasma membrane at sites of virus budding (Pathak *et al*, 1976; Pathak and Webb, 1978, 1983a, 1988).

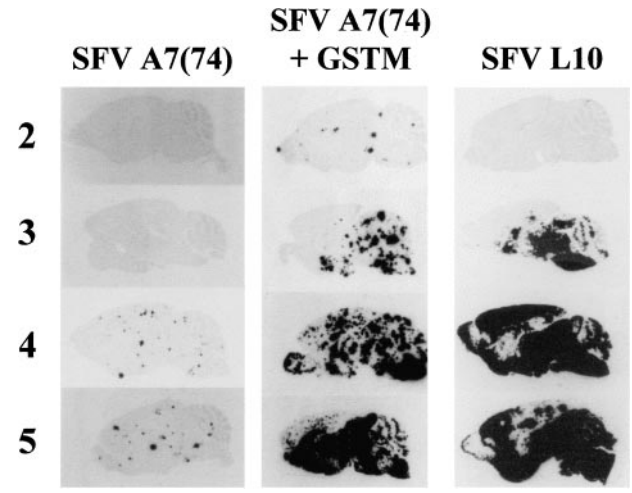

Figure 1 Autoradiographic images showing distribution of SFV RNA by *in situ* hybridization in brains of 3- to 4-week-old BALB/c mice at 2, 3, 4, and 5 days post inoculation (IP). Mice were inoculated with 5000 PFU of SFV A7(74) or L10. One group of mice also received 10 mg of sodium aurothiomalate (GSTM) IP 3 to 4 hours prior to virus infection. Infection with SFV A7(74) results in discrete scattered foci of perivascular infection first apparent here at 4 days post infection. These foci do not enlarge with time even in SCID mice (not shown). Prior treatment with GSTM results in rapid spread of A7(74) infection to produce a panencephalitis similar to that seen with the L10 strain of the virus. Modified from Fazakerley *et al* (1993) and Scallan *et al* (1999), with permission.

Neuroinvasion, CNS tropism, and age-related virulence

Following intraperitoneal (IP) inoculation into adult mice, SFV replicates in muscles, including skeletal, smooth, and cardiac muscle (Pusztai *et al*, 1971; Amor *et al*, 1996). A high-titer plasma viremia is detectable within 24 h. This usually falls after 48 h and in the blood, virus is generally undetectable by infectivity assay by 4 days (Pusztai *et al*, 1971; Fazakerley *et al*, 1993). All strains that have been studied (L10, A7, A7(74)) are neuroinvasive. The much studied A7(74) and L10 strains have been shown to replicate in and probably enter the brain across cerebral endothelial cells (Pathak and Webb, 1974; SoiluHanninen *et al*, 1994), initiating perivascular foci of infection (Fazakerley *et al*, 1993). It is likely that other strains also enter the central nervous system (CNS) by this route. Following intranasal inoculation, infection of the brain via olfactory nerves can also occur (Kaluza *et al*, 1987; Oliver and Fazakerley, 1998). Once in the CNS, all strains of the virus infect neurons and oligodendrocytes, but not astrocytes, and only very rarely meningeal, ependymal, or choroid plexus cells (Balluz *et al*, 1993; Pathak and Webb, 1983b).

From the original perivascular foci, the virulent L10 strain spreads rapidly around the brain, producing a fatal panencephalitis (Figure 1) (Fazakerley *et al*, 1993). This occurs irrespective of the age of the mice and so rapidly that the immune response has no time to intervene. In neonatal and suckling mice, many neurons are destroyed. In adult mice, at the time of death, most infected neurons have a relatively normal morphology and the exact cause of death is unclear. Destruction of a vital group of neurons, toxic levels of cytokines, neuronal dysfunction, or extraneural pathology are possibilities. In contrast, the dynamics of A7(74) infection of the CNS varies according to age of the mice. In neonatal mice, A7(74) virus spreads rapidly around the brain and infection is, as with the L10 strain, rapidly fatal. As the mice age, the A7(74) virus is less able to spread in the CNS, and there is a sharp age-related virulence: mice infected at 12 days of age or less die, whereas those infected at 14 days of age or more survive (Fleming, 1977; Oliver *et al*, 1997; Oliver and Fazakerley, 1998). In the mature adult mouse CNS, A7(74) virus has a restricted replication and remains confined to perivascular foci (Figure 1) (Pathak and Webb, 1978; Fazakerley *et al*, 1993; Oliver *et al*, 1997). This age-related virulence is not a function of the maturity of specific immune responses, as this virus also remains confined to small, predominantly perivascular foci in athymic *nu/nu* mice or mice with severe combined immunodeficiency (SCID) (Fazakerley and Webb, 1987b; Fazakerley *et al*, 1993; Amor *et al*, 1996).

The A7(74) age-related virulence is a function of the maturity of CNS cells. In the first two weeks after birth, in the mouse a number of major maturational events are ongoing in the CNS, these include

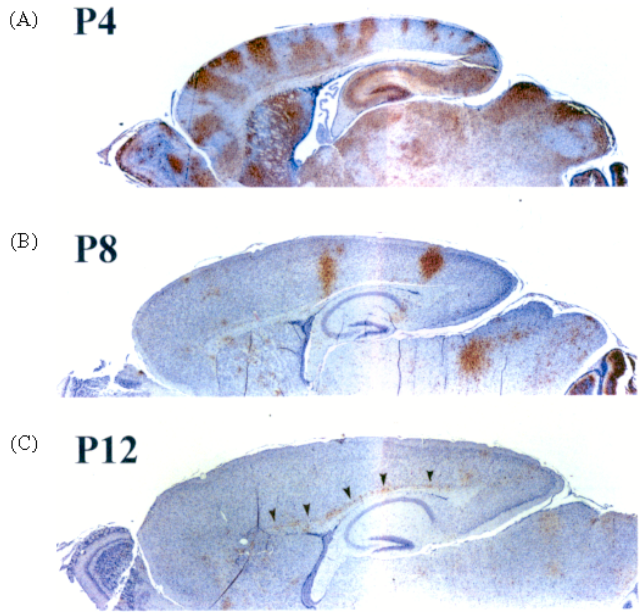

Figure 2 Distribution of SFV A7(74) RNA (brown staining) by *in situ* hybridization at 40 h post infection in the brains of mice inoculated (IP) at (**A**) P4, (**B**) P8, and (**C**) P12. In (**A**), virus can be observed to infect columns of interconnected neurons. These are broader in the frontal and fore- and hind-limb areas and narrower in the occipital cortex, consistent with the known banding patterns of these functional motor and sensory neuronal groupings (Purves *et al*, 1992). At this age (P4), there is widespread axonogenesis and synaptogenesis occurring in these interconnecting columns. This connectivity is progressively completed between P4 and P10 and is associated with a progressive curtailment in the number of columns infected by the virus (**B** and **C**). In the animals inoculated at P12 (**C**), only cells in deep layer VI of the cortex, the cingulated gyrus, and the corpus callosum are infected (*arrowheads*). Modified from Oliver *et al* (1997), with permission.

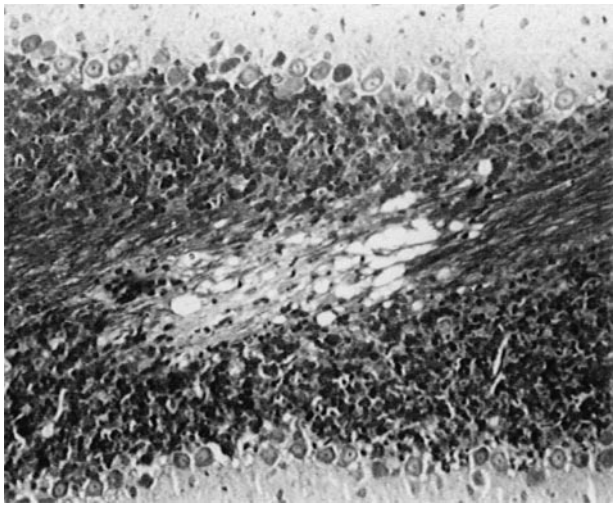

Figure 3 A discrete lesion of inflammatory demyelination in a white matter tract in the cerebellum. SFV A7(74), 18 days post infection (IP), 5-week-old BALB/c mouse.

axonogenesis, synaptogenesis, gliogenesis, and myelination (reviewed by Fazakerley, 2001). The A7(74) strain of SFV is able to replicate productively in highly active maturing neurons undergoing axonogenesis and synaptogenesis (Figure 2). As these processes are completed, a number of major changes occur that restrict virus replication (Pathak and Webb, 1978; Fazakerley *et al*, 1993; Oliver *et al*, 1997; Oliver and Fazakerley, 1998). Restriction may be linked to shutdown of metabolic processes required by the virus, for example, production and transport of smooth membrane vesicles (Oliver *et al*, 1997) or to up-regulation of inhibitory processes, for example, antiapoptotic genes (Scallan *et al*, 1997). Of relevance here is the changed course of events following treatment of infected mice with gold compounds. Treatment of adult mice with sodium aurothiomalate and other gold I compounds results in conversion of this normally avirulent infection to a virulent infection (Allner *et al*, 1974; Bradish *et al*, 1975; Mehta and Webb, 1982; Gates *et al*, 1984; Scallan and Fazakerley, 1999). The gold salt is transported to the CNS where it induces smooth membrane production in neurons (Pathak and Webb, 1983a). In mice treated with gold compounds, A7(74) virus replication is no longer restricted in neurons but is productive and spreads rapidly around the brain (Figure 1), resulting in a panencephalitis characteristic of that observed with the virulent L10 virus (Pathak and Webb, 1983a; Scallan and Fazakerley, 1999).

Infection of neurons in the neonate can result in death of specific neuronal populations by apoptosis (Allsopp *et al*, 1998), whereas infection of mature (adult) neurons is generally nondestructive and can result in virus persistence (Fazakerley *et al*, 1993; Amor *et al*, 1996). In the normal course of postnatal development of the CNS, as immature neurons of the neonatal mouse brain make their connections, they become more resistant to apoptotic death, whereas those neurons that do not form correct connections die by this process (reviewed by Fazakerley, 2001). In culture, SFV A7(74) and L10 both kill continuously growing cell lines by apoptosis, but expression of antiapoptotic genes prolongs survival (Scallan *et al*, 1997). SFV L10 and A7(74) also kill primary cultures of mouse embryonic sensory neurons by apoptosis (Allsopp *et al*, 1998), but A7(74) does not destroy primary cultures of neurons from adult mice whereas L10 does. For renewable cell populations, apoptotic cell death upon infection can be considered to be a highly successfully, altruistic antiviral defense mechanism. For mature, nonrenewable, vital cell populations such as neurons this would not be the case, and selective pressures may have dictated that mature neurons are a specialized case and do not readily undergo apoptosis (Allsopp and Fazakerley, 2000; Fazakerley and Allsopp, 2001).

Taken together, the foregoing studies suggest the following course of events: In the developing mouse brain, both strains of SFV, L10 and A7(74), replicate efficiently in immature neurons. It is likely that

these cells contain suitable membranes and biochemical pathways for complete virus replication. Given their susceptibility to apoptosis, some infected populations of neurons undergo apoptotic cell death. As neuronal circuits and cells mature in the first 2 postnatal weeks, neuronal physiology changes such that A7(74) replication becomes restricted with viral RNA replication and protein synthesis but no, or minimal, virus budding and spread of infection. These more mature neurons are also less susceptible to apoptosis and the result, in the absence of specific immunity (SCID mice), is a persistent nondestructive neuronal infection. Given that gold compounds relieve the restriction on viral replication and induce smooth membrane synthesis, and given the requirement for suitable membranes for virus replication (CPVs, spherules) and budding, it seems likely that a change in membrane synthesis, or associated biochemical pathways, may be the age-related change responsible for switching the outcome of A7(74) infection from productive and destructive to restricted and persistent. Given that replication of the L10 strain is not restricted in mature neurons, the genetic locus responsible for this phenotypic difference between the L10 and A7(74) strains may be informative as to the mechanisms involved. This difference appears to reside in the carboxy-terminus domain of the nsP3 protein (Tuittila *et al*, 2000), but the function of this remains unknown. It also remains unknown how SFV triggers the apoptotic response in cells, including neurons, and what changes occur on neuronal maturation, at least in some populations of neurons, that reduce susceptibility to apoptosis.

Apart from CNS disease, another interesting property of some strains, for example, A7 but not L10, is their ability to cross the mouse placenta and induce abortions (Atkins *et al*, 1982; Milner and Marshall, 1984). The A7 ts22 mutant is teratogenic, inducing skeletal, skin, and neural tube defects in developing fetuses (Mabruk *et al*, 1988, 1989). This is of interest given the teratogenic effects of the related rubella virus. As with most RNA viruses, SFV readily generates defective interfering particles on high-multiplicity passage in culture (Bruton and Kennedy, 1976; Barrett *et al*, 1984). In a series of experiments, defective interfering (DI) particles administered either intraperitoneally or intranasally at the same time as virulent virus have been shown to inhibit virus replication, delay the time of death, completely protect animals, or convert the infection from lethal to persistent (Dimmock and Kennedy, 1978; Barrett and Dimmock, 1984; Atkinson *et al*, 1986).

Role of immune response

Infection of SCID mice with SFV A7(74) results in persistent CNS infection without any apparent neuronal loss (Amor *et al*, 1996). In contrast, in immunocompetent mice, virus is eliminated from the CNS. This comparison demonstrates two important points, firstly there is no, or at most only rare, direct A7(74) virus-induced death of infected cells in the adult mouse brain, and secondly, specific immune responses are required to eliminate this infection. In immunocompetent mice, infectious virus cannot be detected by plaque assay after day 8, by the more sensitive $ICLD_{50}$ after day 11, and by *in situ* hybridization after day 14 (Suckling *et al*, 1978; Jagelman *et al*, 1978; Fazakerley *et al*, 1993). Reverse transcriptase–polymerase chain reaction (RT-PCR) indicates that viral RNA can be detected at later time points (Donnelly *et al*, 1997). After the clearance of detectable infectivity, from day 14 onwards, immunocompetent mice develop lesions of inflammatory demyelination in the white matter. These lesions are apparent throughout the CNS (Suckling *et al*, 1978; Kelly *et al*, 1982). No demyelinating lesions are observed in SCID mice (Amor *et al*, 1996), despite CNS virus persistence. Specific immune responses therefore have a role both in clearing this infection and in the generation of lesions of demyelination.

The roles of different effector functions of the immune response have been determined over the years by studying naturally arising mutant mice, genetically engineered mice, mice treated with general or selective immunosuppressive regimens, and by adoptive transfer experiments. Following IP inoculation of virus, plasma levels of type-I interferons parallel those of the viremia (Bradish *et al*, 1975). In mice with a genetic deletion of functional type-I interferon receptors (Muller *et al*, 1994), IP infection with SFV is fatal within 48 h, with widespread infection of many organs and tissues (JKF, unpublished data). Type-I interferons are therefore crucial in protecting against widespread SFV infection in numerous tissues and their absence leads to widespread infection and death. The role of other cytokines has not been investigated in detail, but interleukin (IL)-1α, IL-1β, IL-3, IL-6, IL-10, and tumor necrosis factor (TNF)α can be readily detected in the CNS following SFV A7(74) infection (Morris *et al*, 1997).

Following IP inoculation of SFV, A7(74) BALB/c mice produce a rapid and neutralizing serum immunoglobulin M (IgM) antibody response followed rapidly by an IgG2a response and a much slower IgG1 response (Fazakerley *et al*, 1993). During the normal course of infection in immunocompetent mice, the blood-brain barrier is leaky from days 4 to 10, allowing passage of immunoglobulins (Parsons and Webb, 1982a; SoiluHanninen *et al*, 1994). Antibody-producing plasma cells can be detected in the brain and intrathecal antibodies can be detected for months post infection (Parsons and Webb, 1984). SCID mice, which have no serum antiviral antibodies, have both a persistent viremia and a persistent CNS infection (Amor *et al*, 1996), whereas *nu/nu* mice control the viremia but have a persistent CNS infection. The *nu/nu* mice produce serum antiviral IgM but not IgG (Suckling *et al*, 1982), suggesting that this may be

controlling the viremia but unable to eliminate the CNS infection. That this is the case is confirmed by resolution of the persistent viremia but not the CNS infection on transfer of day 7 *nu/nu* mouse serum to infected SCID mice (Amor *et al*, 1996). In contrast, transfer of high-titer IgG sera from immunocompetent BALB/c mice to SCID mice was able to abolish both the viremia and titers of infectious virus from the CNS (Amor *et al*, 1996). Whether this was complete eradication of all viral sequences was not checked by more sensitive techniques such as RT-PCR. Antibodies are highly effective in protecting mice from challenge with a lethal dose of SFV. A number of B-cell epitopes have been mapped on the viral glycoproteins (Boere *et al*, 1984; Snijders *et al*, 1991) and even a single monoclonal antibody directed to the E2 envelope glycoprotein has been shown to protect mice against a virulent infection (Boere *et al*, 1983). Furthermore, a non-neutralizing anti-E2 monoclonal has been shown to be able to clear A7(74) virus from persistently infected SCID mouse brains (Amor *et al*, 1996). Combinations of linear B-cell epitopes and B- and T-cell epitopes have been tested as potential vaccine candidates (Snijders *et al*, 1991).

Following IP infection of BALB/c mice with SFV A7(74), perivascular and infiltrating mononuclear cells are apparent in the CNS from 3 days onwards (Parsons and Webb, 1982b). Histopathological studies show that the areas of inflammatory infiltrates correspond to the areas of infection (Subak-Sharpe *et al*, 1993). Perivascular cuffs are a prominent feature of the neuropathology and are maximal between 7 and 10 days. Cerebral endothelial cells upregulate adhesion molecule expression after infection (SoiluHanninen *et al*, 1997; Morris *et al*, 1997), and some of these, ependymal cells, meningeal cells, most infiltrating mononuclear cells and some parenchymal cells with a dendritic morphology, are major histocompatibility complex (MHC)-I$^+$ (Morris *et al*, 1997). The parenchymal MHC-I$^+$ cells form a network surrounding the foci of infection in which the level of MHC-I staining decreases with distance from the infected cells. MHC-II$^+$ cells, probably pericytes, can be observed adjacent to blood vessels in areas of infection, and MHC-II$^+$ astrocytes and microglial cells are present in the areas of infection and inflammation (Morris *et al*, 1997). A few of the infiltrating inflammatory cells are MHC-II$^+$, the majority are not. CD45/B220$^+$ B cells are also present in the inflammatory lesions, including those showing demyelination.

Mice infected with SFV have good delayed-type hypersensitivity responses to the virus (Kraaijeveld *et al*, 1983), and T-cell epitopes for some haplotypes have been mapped (Snijders *et al*, 1991, 1992a, 1992b). Adoptive-transfer studies in SCID and *nu/nu* mice and studies in mice immunosuppressed with total body irradiation, cyclophosphamide, cyclosporine, or cycloleucine all demonstrate that T-cell responses are pathogenic and are required to generate the lesions of demyelination (Fazakerley and Webb, 1987a, 1987c; Amor and Webb, 1987; Amor *et al*, 1996). Depletion of CD8$^+$ cells with a monoclonal antibody greatly reduces demyelination, whereas depletion of CD4$^+$ cells reduces the extent of inflammation but not of demyelination (Subak-Sharpe *et al*, 1993). Given the requirement for CD8$^+$ cells for demyelination and the tropism of A7(74) virus for oligodendrocytes, the most likely mechanism of demyelination would seem to be a CD8$^+$ T cell–mediated destruction of virally infected oligodendrocytes, though this remains to be proven. Mice infected with SFV A7(74) generate CNS antigen-specific autoantibodies, are more sensitive to the induction of experimental allergic encephalomyelitis, and have T cells that cross-react between viral and CNS epitopes (Amor and Webb, 1988; Mokhtarian and Swoveland, 1987), though any role for autoimmune responses in the generation of SFV A7(74)-induced demyelinating lesions remains unclear.

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