

Semliki Forest virus induced, immune mediated demyelination: the effect of irradiation

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Summary. Intraperitoneal infection with the avirulent A7(74) strain of the alphavirus Semliki Forest virus (SFV) induces an immune mediated demyelinating encephalomyelitis. The blood and brain virus titres, the serum antibody titres and the histopathological changes in the brains of normal mice and mice immunosuppressed with 5.0 or 8.0 Gy total body irradiation (TBX) were determined. SFV infection of immunosuppressed mice resulted in persistently high blood and brain virus titres, neuronal pycnosis, paralysis and death. No demyelination or central nervous system (CNS) inflammatory response occurred in these immunosuppressed mice despite high and persistent brain virus titres. The CNS inflammatory response and associated demyelination could be restored to infected immunosuppressed mice by adoptive transfer of spleen cells, and these changes were brought forward if the donor spleen cells were from mice previously sensitized to SFV. The results indicate that the immune response following SFV A7(74) infection is both protective and pathogenic, and that the demyelination is immune mediated and does not result from direct viral destruction of oligodendrocytes, or any other direct effect of the virus.

Keywords: demyelination, Semliki Forest virus, immunosuppression, irradiation

Immunosuppression as a means of investigating the relationship between viral infection, immune response and pathology has been used for many years and studied in many experimental viral infections (reviewed by Nathanson & Cole 1971). Immunosuppression by irradiation has been used in the study of several infections including: lymphocytic choriomeningitis virus (Rowe 1954), encephalomyocarditis virus (Murphy & Glasgow 1968), Langat virus (Webb *et al.* 1968), and Sindbis virus (Park *et al.* 1980).

Semliki Forest virus (SFV) is an alphavirus of the Togaviridae. Intraperitoneal infection of the mouse with the avirulent A7(74) strain (Bradish *et al.* 1971) results in; a

viraemia (Fleming 1977), replication of virus in several tissues including the brain (Pusztai *et al.* 1971), a transient disturbance of the blood brain barrier (Parsons & Webb 1982a) and production of antibody within the CNS (Parsons & Webb 1984). Histopathological examination of the brain reveals a demyelinating meningo-encephalo-myelitis (Chew-Lim 1975; Mackenzie *et al.* 1978; Kelly *et al.* 1982), with an associated pleocytosis (Parsons & Webb 1982b). The spinal cord (Pathak *et al.* 1983) and optic nerves (Illavia *et al.* 1982) are also involved, and there are demyelination associated neurophysiological abnormalities of the optic nerve (Tremain & Ikeda 1983; Pessoa & Ikeda, 1984).

Several investigations have attempted to

determine the cause of the demyelination, and whether this results directly from viral damage or from the inflammatory response initiated by the infection. Chew-Lim *et al.* (1977), in a complicated experiment, studied the effect of 5.0 Gy total body irradiation on the outcome of A7(74) infection following three inoculations of the virus, and concluded that demyelination was related directly to viral activity and not to the immune response. Contrary to this finding, Jagelman *et al.* (1978) determined that, despite prolonged brain virus in infected athymic nude mice, demyelination did not occur, indicating that demyelination is immune mediated. That demyelination is immune mediated is supported by the production of demyelination in infected nude mice reconstituted with normal or with SFV sensitized spleen cells (Fazakerley *et al.* 1983). In consideration of the finding that demyelination in the nude mouse is immune mediated and is not a direct result of infection, it was decided to investigate in more detail the immunosuppressive effect of whole body irradiation on the course of SFV A7(74) infection.

Materials and methods

Mice. Inbred male mice, 4 to 6 weeks old were from a colony of Swiss/A2G mice bred at St Thomas' Hospital Medical School, London.

Virus and virus titres. The avirulent A7(74)/C2 strain of SFV (Bradish *et al.* 1971) was used. On killing of the mice, blood samples were diluted 1:10 in phosphate buffered saline containing 0.75 bovine serum albumin (BAPS) and half brains for assay were stored at -70°C until use. The virus infectivity of a sample was determined by preparing serial 10-fold dilutions in sterile BAPS and inoculating 0.02 ml of an appropriate range of dilutions intracerebrally (i.c.) into groups of four to six suckling mice, 0-4 days old. The i.c. 50% lethal dose/0.02 ml (ICLD₅₀) was calculated by the method of Reed and Muench (1938). Mice were inoculated intra-

peritoneally 24 h after irradiation with $10^{4.5}$ ICLD₅₀ of virus, in 0.1 ml of BAPS.

Antibody titres. An enzyme-linked immunosorbent assay (ELISA) was used to measure anti-SFV IgG levels. The assay was based on that of Voller *et al.* (1976). Virus was purified by a modification of the method of Bruton and Kennedy (1976), and coated to the bottom of Dynatech PVC microtitre plates (Dynatech Laboratories Ltd, Daux Road, Billingham, Sussex). Serum samples were diluted 1/200 in PBS + 5% FCS (fetal calf serum) and run in triplicate. Goat anti-mouse IgG (Fc IgG1/2a/2b/3) linked to horse radish peroxidase, was used as the conjugate (Nordic Immunological Reagents, Maidenhead, Berks). The substrate was *o*-phenylenediamine (Sigma Chemicals, Poole, Dorset). The difference in absorbance at 492 nm and 690 nm was measured and expressed relative to that of a known positive - a pooled post inoculation day (PID) 21, anti-SFV serum, which was run in triplicate on each plate. The intraplate coefficient of variation was 8% and the interplate 12%.

Histology. Half brains were placed immediately after removal in 5% formol saline, and processed using standard histological techniques. The brains were sectioned sagittally and 5 μm sections were cut from three different areas of each brain. Two sections from each of these three areas were stained with haematoxylin and eosin and two sections with luxol fast blue. A total of 12 sections was thus examined from each brain. Sections were coded, examined in random order and scored on a scale of + to + + + + according to increasing degree of severity of the lesions, for meningitis (MEN), perivascular cuffing (PVC), microcystic change (MCC) and demyelination (DEM).

Irradiation. Mice were placed in a perspex box specially designed to ensure an even dose of irradiation to all the mice within. The mice each received 5.0 or 8.0 Gy total body gamma irradiation (TBX) from a ^{60}Co Mobal-

tron radiotherapy machine. The machine had two opposed sources, with a 90 cm source-to-axis distance, a beam size of 24×24 cm, and a dose rate of approximately 0.65 Gy/min.

All mice given 8.0 Gy were given 2×10^7 bone marrow cells intravenously within 4 h of irradiation. The bone marrow cells were prepared from the femurs of syngeneic mice.

Irradiated mice were kept in a filtered isolator and provided with sterile feed and water. The water contained antibiotics, 10 mg/ml neomycin (mycifradin sulphate) and 1 mg/ml polymyxin (aerosporin), and was acidified to pH 3.5 by the addition of a few drops of 2M HCl.

Adoptive transfer of cells. Spleen cell suspensions were prepared from syngeneic mice. These cells had a viability $> 90\%$ and each mouse received $5-10 \times 10^7$ nucleated splenocytes in 0.5 ml of RPMI-1640 media (Flow Laboratories) intraperitoneally 24 h post-infection. Mice were hyperimmunized to the virus by giving three doses of $10^{4.5}$ ICLD₅₀ of SFV at 0, 14 and 21 days. Spleen cells were removed from hyperimmune mice 7 days after the last inoculation of virus.

Determination of mean survival time. The mean survival time (t) was calculated according to the formula of Semenov *et al.* (1975).

$$1/t = \frac{1/t_1 + 1/t_n}{N}$$

where ' t_1 ' is the time of death of the first mouse and ' t_n ' that of the last mouse. N is the total number of animals. Animals surviving 21 days were deemed to have survived and were not included in the calculation.

Results

The normal infection of S/A2G mice

Mice were inoculated with virus and two mice sampled on each post-inoculation day (PID), 1 to 12. The blood and brain virus

titres and the serum antibody levels are recorded in Fig. 1.

Histological examination of the brains of these mice demonstrated by PID 4, a mononuclear cell meningitis and mononuclear cell perivascular cuffing of some of the vessels. From PID 4 onwards the extent of the cuffing around the vessels, and the number of vessels involved increased. Mononuclear cells could be seen leaving some of the vessels or cuffs and invading the surrounding tissues, this was most obvious from PID 7 onwards and

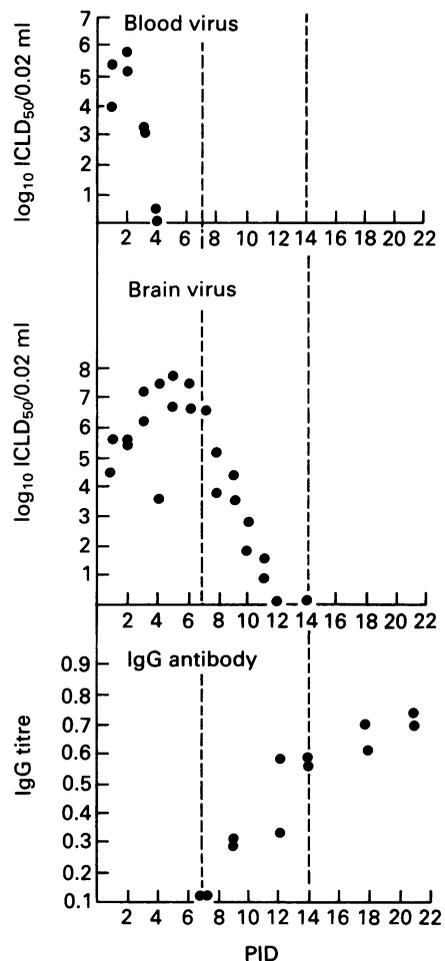


Fig. 1. Blood and brain virus titres and serum IgG anti-viral antibody titres following intraperitoneal inoculation of SFV.

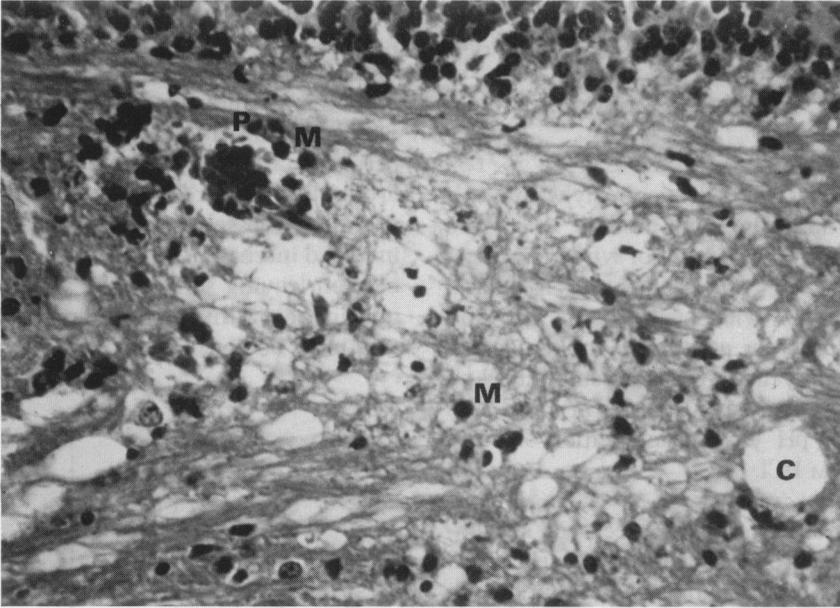


Fig. 2. Perivascular cuffing (P), microcystic changes (C), invading mononuclear cells (M) and demyelination in a cerebellar tract. PID 12 after SFV. Luxol fast blue, cresyl violet, $\times 360$.

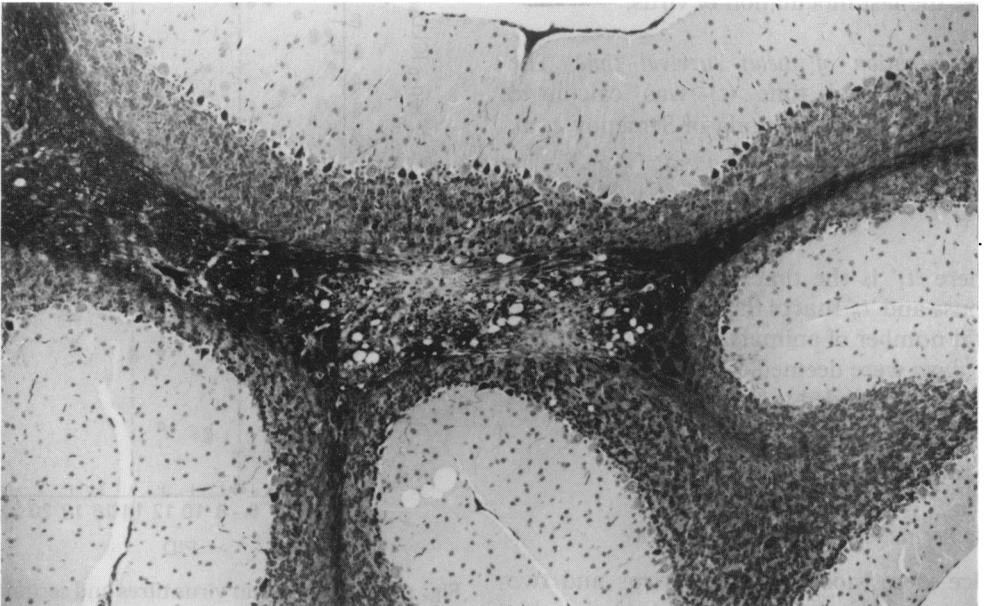


Fig. 3. Lesions of demyelination in the cerebellum. PID 14 after SFV. Luxol fast blue, cresyl violet, $\times 96$.

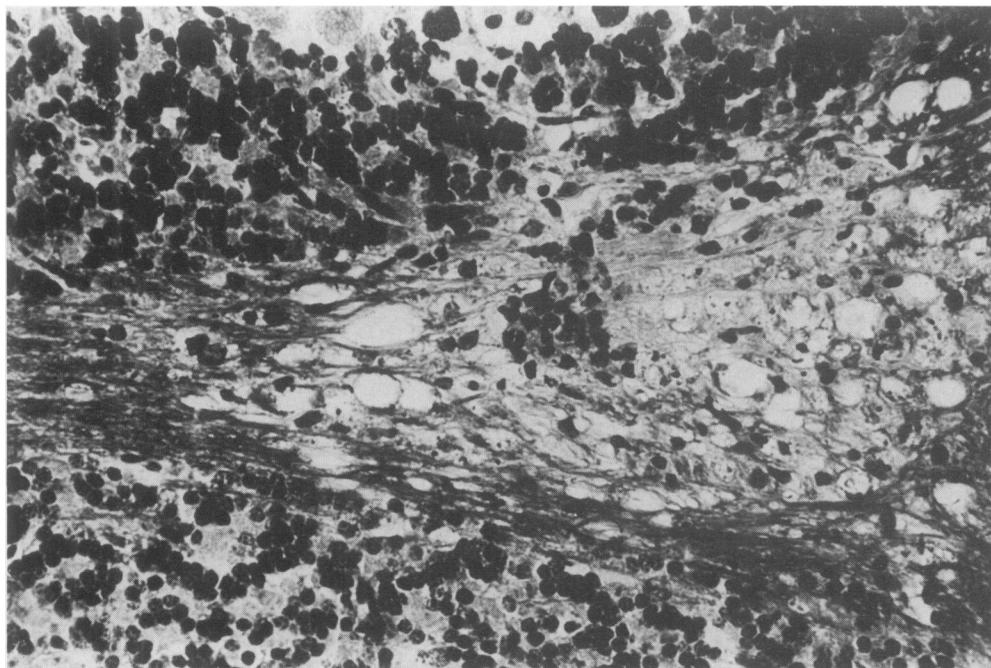


Fig. 4. Lesion of demyelination in the white matter of the cerebellum, with invading mononuclear cells. Luxol fast blue, cresyl violet, $\times 360$.

was associated with a microcystic degeneration of the invaded white matter (Fig. 2). Demyelination was occasionally seen by PID 9, but was most apparent between PID 14 and 21 (Fig. 3). The lesions of demyelination were always associated with invading mononuclear cells (Fig. 4). No pycnosis or loss of granule cells (Fig. 5) or oligodendrocytes (Fig. 6) was apparent in the non-inflammatory areas of the brains of these mice. In the areas of microcystic change and inflammation it was impossible by light microscopy to determine the integrity of these cells, and it is possible that loss of neurones and oligodendrocytes occurs in such areas (Figs 2 & 4).

The effect of 5.0 Gy irradiation

Forty-five mice were given 5.0 Gy TBX, 24 h before SFV A7(74) infection. Five mice were sampled for determination of the blood, brain and antibody titres, as shown in Fig. 7.

Table 1 summarizes the histopathological changes.

Meningitis and perivascular cuffing, apparent in all the control mice by PID 4, were not observed in the infected mice given 5.0 Gy until PID 9, and then only in 2/5 of these mice. The development of the microcystic changes was also delayed, however by PID 18 these were at least as extensive as those seen in the control infection. Few inflammatory cells were associated with the microcystic change. In some areas of the white matter microcysts ran in lines between and parallel to the fibres (Fig. 8). Following 5.0 Gy TBX the onset of demyelination was not delayed in all the mice, but when present was less intense between PID 12 and 18 than in the mice given virus alone; demyelination comparable to that seen in the control mice was not seen in the 5.0 Gy irradiated mice until PID 21. Slight pycnosis of the granule cells of the cerebellum was apparent from

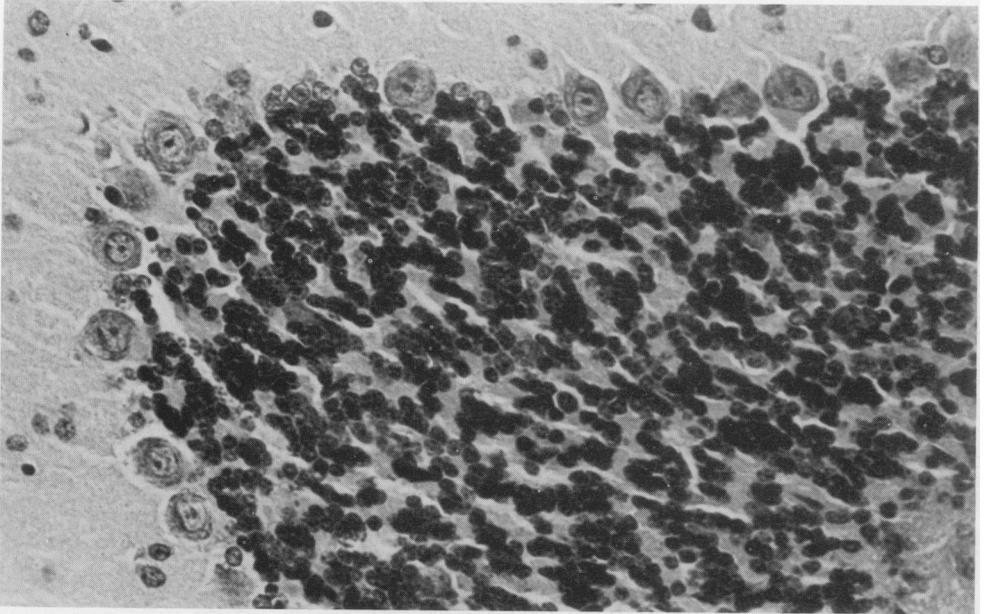


Fig. 5. Granule cells of the cerebellum, are not damaged during the normal infection. PID 7 after SFV. H & E, $\times 360$.

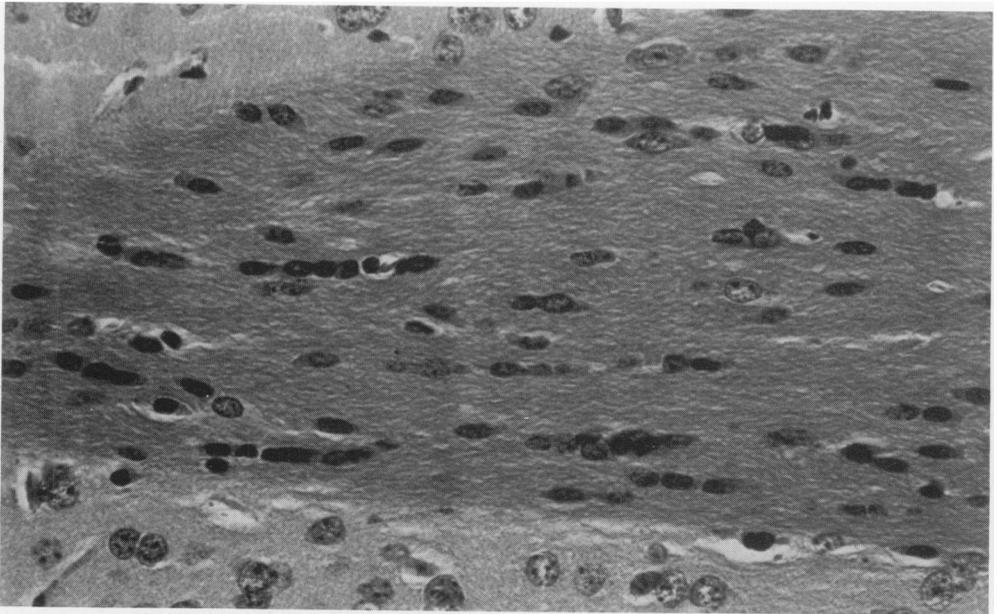


Fig. 6. Chains of normal healthy oligodendrocytes in the white matter. PID 7 after SFV. H & E, $\times 360$.

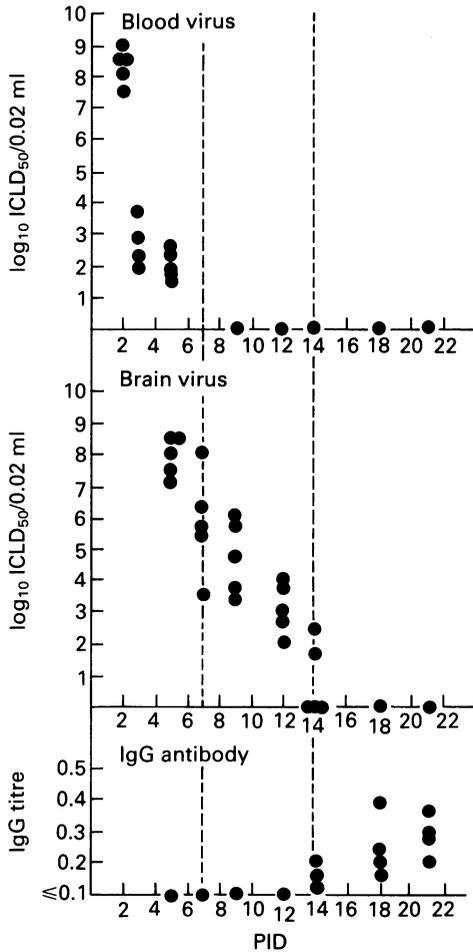


Fig. 7. Blood and brain virus titres and serum IgG anti-viral antibody titres, in mice given 5.0 Gy TBX, 24 h before SFV infection.

PID 3 onwards, but was most obvious on days, 5, 7 and 9.

The effect of 8.0 Gy irradiation

Twenty-five mice were given 8.0 Gy TBX and syngeneic bone marrow cells. Fifteen mice were infected with virus 24 h later, the remaining 10 mice remained uninfected. Two of these uninfected mice died (one on PID 9, and one on PID 13), two mice were sampled on each of days 5, 7 and 9 post-

Table 1. Pathological changes in the CNS following 5.0 Gy TBX

PID	MEN	PVC	MCC	DEM
2	-	-	-	-
3	-	-	-	-
5	-	-	-	-
7	-	-	-	-
9	+(2/5)	+(2/5)	+(1/5)	-
12	++	++	++	+(3/5)
14	+	+(3/5)	++	+(2/5)
18	-	+	+++	+(3/5)
21	+	++	++	++

Each figure represents the mean of five mice. Figures in parentheses indicate the number of mice in the group that were positive, if not shown, all the mice in the group were positive/negative (as indicated).

MEN, meningitis; PVC, perivascular cuffing; MCC, microcystic change; DEM, demyelination.

irradiation, the remaining two mice survived for at least 8 weeks after irradiation. The mice sampled were the most sick looking mice on each day. The infected irradiated mice looked thin, hunched with ruffled fur and had paresis of the limbs by PID 7. By PID 9 some of the mice, and by PID 12 all of the mice, had complete paralysis of the front and hind limbs and were moribund. Three mice died (two on PID 15 and one on PID 20) and two mice were sampled on each of PID 4, 5, 7, 9, 12 and 14; on each day the most sick looking mice were sampled.

Figure 9 shows the blood and brain virus titres. No antiviral IgG antibodies were detectable by ELISA, and no inflammation, microcysts or demyelinations were present in any of the brains; with the exception of a slight meningitis in 1/2 mice on PID 12 and 1/2 mice on PID 14. This can probably be attributed to a partial recovery of the immune system by this time. Pycnosis and loss of granule cells (Fig. 10, cf. Fig. 5), was apparent in the brains of all the infected mice examined on PID 7, 9, 12 and 14, and was

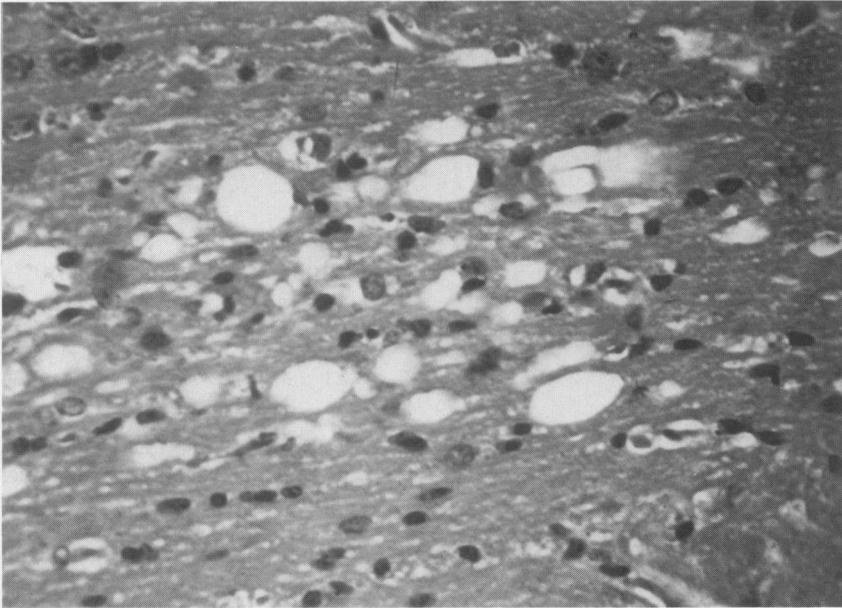


Fig. 8. Microcysts in the white matter. PID 9 after SFV. H & E, $\times 360$.

much more extensive than that seen following 5.0 Gy TBX. Neuronal loss was also apparent in other areas, such as the hippocampus. Loss of neurones was rarely seen in the mice given irradiation alone, and this was minimal compared to that seen in the infected irradiated mice.

Adoptive transfer of cells to irradiated mice

Mice were infected with virus 24 h after 8.0 Gy TBX and bone marrow transfer, and reconstituted with spleen cells from syngeneic donors 24 h after infection. The mice received spleen cells from either, normal uninfected mice (group A), or from mice hyperimmunized to the virus (group B). Two mice were sampled from each group on each of PID 7, 9 and 14. The results are shown in Table 2.

The SFV sensitized cells produced high levels of antiviral IgG (Table 2) which probably prevented the brain virus titres from rising to lethally high levels. The brain virus was cleared in these mice by PID 9, earlier

even than in normal infection (Fig. 1). Following transfer of normal spleen cells 24 h post-infection, by PID 14 no antiviral IgG was detectable in the sera the viraemia persisted until PID 9 and the brain virus titres were only cleared in 1/2 mice on PID 14. It is likely that the transferred spleen cells were not sufficiently active or numerous to prevent brain virus titres rising to lethally high titres.

Average day of death

Table 3 shows the average day of death of 't' for groups of mice given various treatments. In all cases irradiation was given 24 h pre-infection and spleen cells 24 h pre-infection and spleen cells 24 h post-infection. None of the mice in these groups were sampled for other studies.

SFV infection of the irradiated mice was 100% lethal with an average day of death of 8.0. Transfer of normal spleen cells 24 h post infection did not alter this outcome, though transfer of virally sensitized spleen cells

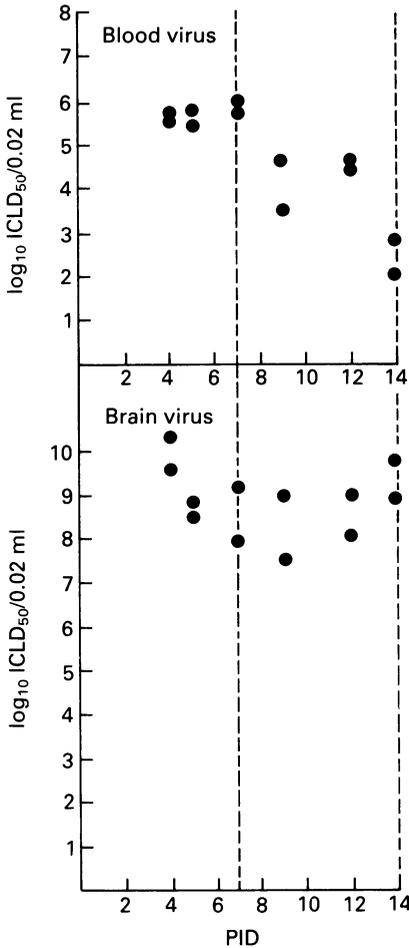


Fig. 9. Blood and brain virus titres in mice given 8.0 Gy TBX, 24 h before SFV infection. No serum anti-viral IgG was detectable.

reduced the mortality to 19% and slightly delayed the average day of death.

Discussion

The A7(74) strain of SFV has been shown to replicate predominantly in neurones (Zlotnik & Harris 1970), but also in oligodendrocytes (Pathak *et al.* 1983). Damage to neurones has been observed following infection with the virulent L10 strain of SFV (Pathak *et al.* 1976; Barrett *et al.* 1980), and the avirulent

A8 strain (Zlotnik *et al.* 1972), but not following infection with the A7 or A7(74) strains. The A7 strain seems to have reduced cytopathogenicity for neurones (Atkins 1983). The integrity of the oligodendrocytes following A7(74) infection of normal control mice has not been previously studied, though probable loss of these cells has been shown in the brains of mice infected with the mutant M9 and M136 strains (Sheahan *et al.* 1981; 1983). In the present study, outside the areas of inflammation, no loss or pycnosis of oligodendrocytes was seen in mice infected with the A7(74) strain of SFV, and none was apparent in an electron microscopic study of these brains (personal communication Dr S. Pathak).

Blood and brain virus titres in the control infected mice were similar to those found by other workers using the A7(74) strain of SFV (Oaten *et al.* 1976; 1980; Suckling *et al.* 1976; 1977). Histopathological study of the brains of these mice confirmed the findings of the previous studies by Chew-Lim (1975), Chew-Lim *et al.* (1977), Suckling *et al.* (1978), Mackenzie *et al.* (1978) and Kelly *et al.* (1982).

Compared to the infection of the normal non-immunosuppressed Swiss/A2G mice (Fig. 1), the blood and brain virus titres (Fig. 7) of the infected mice given 5.0 Gy TBX were prolonged, whilst the production of viral specific IgG and the onset of the CNS inflammatory response (Table 1) were delayed. No deaths resulted in these mice. These findings are in agreement with the irradiation studies of Smillie *et al.* (1973) and Chew-Lim *et al.* (1977). Chew-Lim *et al.* (1977) also examined the brain pathology and observed an increase in the incidence of demyelination and occasional foci of degenerate neurones in the cerebellum. Though not directly comparable to those of Chew-Lim *et al.* (1977), the experiments reported here demonstrate that a single dose of 5.0 Gy irradiation delays and decreases both the inflammation and the demyelination resulting from a single inoculation of virus.

Immunosuppression with 8.0 Gy TBX

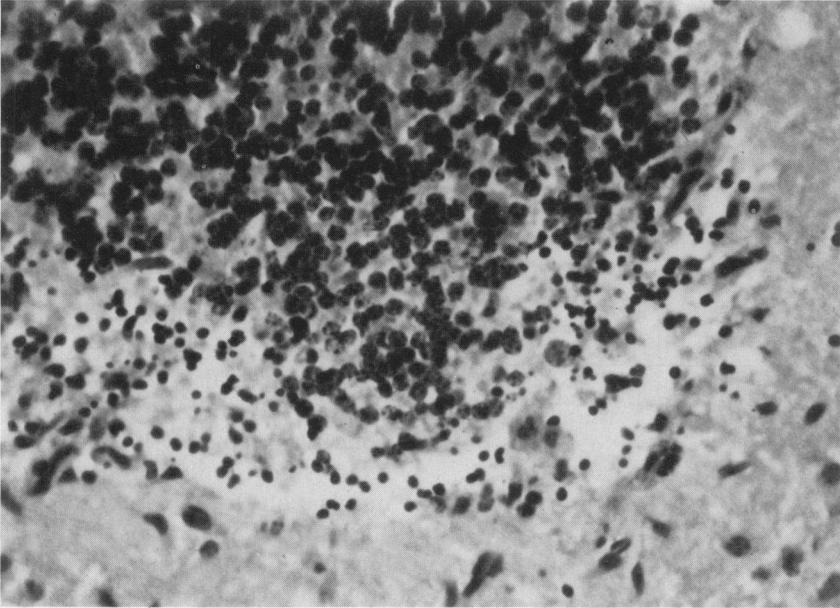


Fig. 10. Pycnosis of granule cells in the cerebellum. PID 7 after SFV, mouse received 8.0 Gy TBX, 24 h before infection. H & E, $\times 360$ (cf. Fig. 5).

Table 2. Adoptive transfer of normal and hyperimmune spleen cells to mice immunosuppressed with 8.0 Gy TBX

Mouse	PID	Blood virus	Brain virus	Serum IgG	MEN	PVC	MCC	DEM
A1	7	4.0	9.0	0	+	+	-	-
A2	7	3.8	8.2	0	+	+	+	-
A3	9	4.2	6.0	0	+	+	+	-
A4	9	4.0	5.5	0	++	++	+	-
A5	14	0	0	0	+	++	+	+
A6	14	0	1.7	0	++	+	+	+
B1	7	0	5.0	1.336	+	-	-	-
B2	7	0	4.6	0.727	+	+	-	-
B3	9	0	0	1.391	+++	+++	++	+
B4	9	0	0	0.438	+++	+++	++	+
B5	14	0	0	0.461	+	++	+	-
B6	14	0	0	0.703	+	+	-	-

Group A: 8.0 Gy TBX, A7(74) and normal spleen cells.

Group B: 8.0 Gy TBX, A7(74) and hyperimmune spleen cells.

MEN, meningitis; PVC, perivascular cuffing; MCC, microcystic change; DEM, demyelination.

Table 3. Average day of death

Treatment	Deaths	t
8.0 Gy TBX(bmc)	8/25	10.4 (8-13)*
8.0 Gy TBX(bmc)+SFV	25/25	8.0 (5-19)
8.0 Gy TBX(bmc)+SFV + normal spleen cells	16/16	7.0 (5-14)
8.0 Gy TBX(bmc)+SFV + hyperimmune spleen cells	3/16	10.2 (9-12)*

t, Average day of death (days post-inoculation).
bmc, Bone marrow cells (2×10^7 /mouse).

Figures in parentheses indicate the days between which the animals died.

before infection with the avirulent A7(74) strain of SFV resulted in a rapid rise of the brain virus titres to a high level (10 logs by PID 4, Fig. 9). This is to be compared with a normal maximum of 8 logs by PID 5 (Fig. 1). The rapid rise in brain virus titres following 8.0 Gy TBX is similar to that seen following infection with the virulent L10 or V13 strains of SFV. (Puszta *et al.* 1971; Oaten *et al.* 1980). This finding suggests that one difference determining the rate of replication of these two strains of SFV may be a difference in their interaction with the hosts immune system. For a review of this subject see Atkins *et al.* (1985).

The effects of irradiation on immune cells have been reviewed by Anderson and Warner (1976) and Doria *et al.* (1982). Radiation results in a rapid interphase death of lymphocytes, 8.0 Gy also destroys the bone marrow cells but at a dose of 5.0 Gy these cells are spared, and the population of circulating T and B lymphocytes is soon replenished from their precursors in the bone marrow. The latter accounts for the temporary delay in both antibody production and the onset of the inflammatory response, following 5.0 Gy TBX. As expected, the immunosuppressive effect of 8.0 Gy TBX was longer lasting and more dramatic on both blood and brain virus titres, which remained high over the 2 weeks studied. No anti-viral IgG was detectable. Some reduction of the

blood virus titres did occur from PID 7 onwards, but even 14 days after infection there were over 2 logs of virus in the blood (Fig. 9). In the normal infection the viraemia was cleared by PID 4 (Fig. 1).

Examination of the brains of mice given 8.0 Gy irradiation alone (uninfected) demonstrated no, or only very occasional loss of, or damage to neurones. Some pycnosis of neurones was apparent in the brains of the mice given 5.0 Gy and this was more apparent in those given 8.0 Gy TBX before infection. CNS cells are generally highly radioresistant, except for some occasional long-term effects, no radiation damage is apparent in the brains of rodents given less than 15 Gy irradiation (Hubbard 1980), and loss of oligodendrocytes is seen only several weeks after irradiation in rats given 40 Gy (Hubbard & Hopewell 1979). The loss and pycnosis of neurones in the irradiated and infected mice is thus most likely to result, following immunosuppression, from the greater spread of virus within the brains of these mice to involve more cells, or even susceptible subpopulations of cells. It is likely that the neuronal destruction following high virus titres is first apparent as paralysis and is then responsible for the death of the infected irradiated (8.0 Gy) mice. Paresis was apparent in the 8.0 Gy treated infected mice by PID 7, and some of the mice were paralysed by PID 9.

The absence of demyelination in the brains of the 8.0 Gy immunosuppressed mice, despite the higher and prolonged brain virus titres, clearly demonstrates that the demyelination does not result from direct viral damage but is dependent upon the immune response. This is confirmed by the results of the reconstitution experiments. The results are similar to those obtained by adoptive transfer of cells to athymic nude mice infected with the A7(74) strain of SFV (Fazakerley *et al.* 1983).

The experimental findings presented in this paper indicate that following immunosuppression with 8.0 Gy TBX, the A7(74) strain of SFV replicates to high titres within

the brain. These high titres result in occasional loss of neurones and foci of granule cell pycnosis, such mice become paralysed and die. No such changes and no deaths occur in normal immunocompetent mice infected with this virus, demonstrating the protective aspect of the immune response. Despite the very high and persistent virus titres in the brains of the irradiated (8.0 Gy) immunosuppressed mice, no demyelination occurs. In contrast infection of normal immunocompetent mice with this virus results in a demyelinating encephalomyelitis; this aspect of the immune response is thus pathogenic. It is likely that anti-viral antibodies are protective, preventing high, damaging and lethal brain virus titres, and that T lymphocytes are pathogenic producing the demyelination. T lymphocytes in close contact with macrophages, astrocytes and demyelinating axons have been described in the spinal cord following SFV A7(74) infection (Pathak *et al.* 1983).

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References

- ANDERSON R.E. & WARNER N.L. (1976) Ionizing radiation and the immune response. *Adv. Immunol.* **24**, 216-335.
- ATKINS G.J. (1983) The avirulent A7 strain of Semliki Forest virus has reduced cytopathogenicity for neuroblastoma cells compared to the virulent L10 strain. *J. Gen. Virol.* **64**, 1401-1404.
- 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. *J. Gen. Virol.* **66**, 395-408.
- BARRETT P.N., SHEAHAN B.J. & ATKINS G.J. (1980) Isolation and characterization of Semliki Forest virus mutants with altered virulence. *J. Gen. Virol.* **49**, 141-147.
- BRADISH C.J., ALLNER K. & MABER H.M. (1971) The virulence of original and derived strains of Semliki Forest virus for mice, guinea-pigs and rabbits. *J. Gen. Virol.* **12**, 141-160.
- BRUTON C.J. & KENNEDY S.I.T. (1976) Defective interfering particles of Semliki Forest virus: structural differences between standard virus and defective interfering particles. *J. Gen. Virol.* **31**, 383-395.
- CHEW-LIM M. (1975) Mouse encephalitis induced by avirulent Semliki Forest virus. *Vet. Pathol.* **12**, 387-393.
- CHEW-LIM M., WEBB H.E. & JAGELMAN S. (1977) The effect of irradiation on demyelination induced by avirulent Semliki Forest virus. *Br. J. exp. Path.* **58**, 459-464.
- DORIA G., AGAROSI G. & ADORINI L. (1982) Selective effects of ionizing radiations on immunoregulatory cells. *Immunol. Rev.* **65**, 23-54.
- FAZAKERLEY J.K., AMOR S. & WEBB H.E. (1983) Reconstitution of Semliki Forest virus infected mice, induces immune mediated pathological changes in the CNS. *Clin. exp. Immunol.* **52**, 115-120.
- FLEMING P. (1977) Age-dependent and strain related differences of virulence of Semliki Forest virus in mice. *J. Gen. Virol.* **37**, 93-105.
- HUBBARD B.M. & HOPEWELL J.W. (1979) Changes in the neurological cell populations of the rat spinal cord after local X-irradiation. *Br. J. Radiol.* **52**, 816-821.
- HUBBARD B.M. (1980) Irradiation of the central nervous system, In *Progress in Neurology (Animal Models of Neurological Disease)*, Eds F.C. Rose & P.O. Behan. Tunbridge Wells: Pitman Medical.
- ILLAVIA S.J., WEBB H.E. & PATHAK S. (1982) Demyelination induced in mice by avirulent Semliki Forest virus. I. Effects on optic nerve. *Neuropath. Appl. Neurobiol.* **8**, 35-42.
- JAGELMAN S., SUCKLING A.J., WEBB H.E. & BOWEN E.T.W. (1978) The pathogenesis of avirulent Semliki Forest virus infections in athymic nude mice. *J. Gen. Virol.* **41**, 599-607.
- KELLY W.R., BLAKEMORE W.F., JAGELMAN S. & WEBB H.E. (1982) Demyelination induced in mice by avirulent Semliki Forest virus. II. An ultrastructural study of focal demyelination in the brain. *Neuropath. Appl. Neurobiol.* **2**, 43-53.
- MACKENZIE A., SUCKLING A.J., JAGELMAN S. & WILSON A.M. (1978) Histopathological and enzyme histochemical changes in experimental Semliki Forest virus infection in mice and their relevance to Scrapie. *J. Comp. Path.* **88**, 335-344.
- MURPHY B.R. & GLASGOW L.A. (1968) Factors modifying host resistance to viral infection. III. Effect of whole body X-irradiation on encephalomyocarditis virus infection in mice. *J. exp. Med.* **127**, 1035-1052.
- NATHANSON N. & COLE G.A. (1971) Immunosuppression: a means to assess the role of the

- immune response in acute virus infection. *Fed. Proc.* **30**, 1822-1830.
- OATEN S.W., WEBB H.E. & BOWEN E.T.W. (1976) Enhanced resistance of mice to infection with Langat (TP 21) virus following pre-treatment with Sindbis or Semliki Forest virus. *J. Gen. Virol.* **33**, 381-388.
- OATEN S.W., JAGELMAN S. & WEBB H.E. (1980) Further studies of macrophages in relationship to avirulent Semliki Forest virus infections. *Br. J. exp. Path.* **61**, 150-155.
- PARK M.M., GRIFFIN D.E. & JOHNSON R.T. (1980) Studies of immune responses during recovery from Sindbis virus encephalitis in selectively reconstituted thymectomized, lethally irradiated mice. *Infect. Immun.* **34**, 306-309.
- PARSONS L.M. & WEBB H.E. (1982a) Blood brain barrier disturbance and immunoglobulin G levels in the cerebrospinal fluid of the mouse following peripheral infection with demyelinating Semliki Forest virus. *J. Neurol. Sci.* **57**, 307-318.
- PARSONS L.M. & WEBB H.E. (1982b) Virus titres and persistently raised white cell counts in the cerebrospinal fluid in mice after peripheral infection with demyelinating Semliki Forest virus. *Neuropath. Appl. Neurobiol.* **8**, 395-401.
- PARSONS L.M. & WEBB H.E. (1984) Specific immunoglobulin G in serum and cerebrospinal fluid of mice infected with the demyelinating strain of Semliki Forest virus. *Microbios Letts.* **25**, 135-140.
- PATHAK S., WEBB H.E., OATEN S.W. & BATEMAN S. (1976) An electron microscopic study of the development of virulent and avirulent strains of Semliki Forest virus in mouse brain. *J. Neurol. Sci.* **28**, 289-300.
- PATHAK S., ILLAVIA S.J. & WEBB H.E. (1983) The identification and role of cells involved in CNS demyelination in mice after SFV infection: An ultrastructural study. In *Immunology of Nervous System Infections (Progress in Brain Research)* Eds P.O. Behan, V. ter Meulen & F.C. Rose. Amsterdam: Elsevier. pp. 237-254.
- PESSOA V.F. & IKEDA H. (1984) Increase in axonal transport in demyelinating optic nerve fibers in mice infected with Semliki Forest virus. *Brain* **107**, 433-446.
- PUSZTAI R., GOULD E.A. & SMITH H. (1971) Infection patterns in mice of an avirulent and virulent strain of Semliki Forest virus. *Br. J. Exp. Path.* **52**, 669-677.
- REED L.J. & MUENCH H. (1938) A simple method of estimating fifty per cent end points. *Am. J. Hyg.* **27**, 493-497.
- ROWE W.P. (1954) Studies on pathogenesis and immunity in lymphocytic choriomeningitis infection in the mouse. *Nav. Med. Res. Inst. Rep.* **12**, 167-220.
- SEMEV B.F., KHOZINSKY V.V. & VARGIN V.V. (1975) The damaging action of cellular immunity in flavivirus infections in mice. *Med. Biol.* **53**, 331-336.
- SHEAHAN B.J., BARRETT P.N. & ATKINS G.J. (1981) Demyelination in mice resulting from infection with a mutant of Semliki Forest virus. *Acta Neuropath.* **53**, 129-136.
- SHEAHAN B.J., GATES M.C., CAFFREY J.F. & ATKINS G.J. (1983) Oligodendrocyte infection and demyelination produced in mice by the M9 mutant of Semliki Forest virus. *Acta Neuropath.* **60**, 257-265.
- SMILLIE J., PUSZTAI R. & SMITH H. (1973) Studies on the influence of host defence mechanisms on infection of mice with an avirulent or virulent strain of Semliki Forest virus. *Br. J. exp. Pathol.* **54**, 260-266.
- SUCKLING A.J., WEBB H.E., CHEW-LIM M. & OATEN S.W. (1976) Effect of inapparent viral encephalitis on the levels of lysosomal glycosidases in mouse brain. *J. Neurol. Sci.* **29**, 109-116.
- SUCKLING A.J., JAGELMAN S. & WEBB H.E. (1977) Brain lysosomal glycosidase activity in immunosuppressed mice infected with avirulent Semliki Forest virus. *Infect. Immun.* **15**, 386-391.
- SUCKLING A.J., PATHAK S., JAGELMAN S. & WEBB H.E. (1978) Virus associated demyelination a model using avirulent Semliki Forest virus infection of mice. *J. Neurol. Sci.* **36**, 147-154.
- TREMAIN K.E. & IKEDA H. (1983) Physiological deficits in the visual system of mice infected with Semliki Forest virus and their correlation with those seen in patients with demyelinating disease. *Brain* **106**, 879-895.
- VOLLER A., BIDWELL D. & BARTLETT (1976) Micropate enzyme immunoassays for the immunodiagnosis of virus infections. In *Manual of Clinical Immunology*. Eds N.R. Rose & H. Friedman. Washington DC: American Society for Microbiology.
- WEBB H.E., WRIGHT D.C.D., PLATT G.S. & SMITH C.E.G. (1968) Langat virus encephalitis in mice: II. The effect of irradiation. *J. Hyg.* **66**, 355-364.
- ZLOTNIK I. & HARRIS W.J. (1970) The changes in cell organelles of neurones in the brains of adult mice and hamsters during Semliki Forest virus and Louping Ill encephalitis. *Br. J. exp. Pathol.* **52**, 37-42.
- ZLOTNIK I., GRANT & BATTER-HATTON (1972) Encephalopathy in mice following inapparent Semliki forest virus infection. *Br. J. exp. Path.* **53**, 125-129.