# Brain Viral Persistence and Myelin Damage in Nude Mice

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ABSTRACT

**Multiple foci of secondary demyelination were observed in the cerebellum of nude mice given two inoculations of avirulent Semliki Forest virus compared to single lesions seen in similarly treated Swiss $A_2G$ mice. The increased number of demyelinative plaques were attributed to brain viral persistence for 35 days in the nude mice with correspondingly low serum antibody titres. No neurological signs were observed in any of the mice.**

RÉSUMÉ

**L'auteur a décelé plusieurs foyers de démyélinisation secondaire dans le cervelet des souris atriches auxquelles il avait administré deux injections d'une souche avirulente du virus de la forêt Semliki. L'administration d'injections similaires à des souris suisses $A_2G$ provoqua cependant des lésions beaucoup moins marquées. Le nombre plus élevé de foyers de démyélinisation, chez les souris atriches, sembla attribuable à la persistance du virus dans leur cerveau, pour une période de 35 jours, ainsi qu'à une faible teneur de leur sérum en anticorps. Aucune des souris expérimentales ne développa de signes nerveux.**

## INTRODUCTION

Cerebellar lesions were induced in adult Swiss $A_2G$ mice by two or three intraperitoneal inoculations of avirulent neurotropic Semliki Forest virus (SFV) with apparent remyelination by seven and eight weeks (8). An ultrastructure study revealed the axonal damage to be secondary demyelination and no viral particles were identified in the lesions (10).

Adachi and co-workers reported that there was 100 per cent survival of nude mice given the E variant strain of the encephalomyocarditis virus while the mortality of litter mates and Swiss mice during the five to seven days after inoculation was more than 40 per cent (1). Other workers have shown that nude mice given an intracerebral high-egg-passage strain of rabies vaccine virus developed a lethal infection as compared to the clinically inapparent disease normally observed in Balb/c mice (17). Nude mice appear to respond differently to virus infections compared to the ordinary laboratory strains of mice.

The purpose of this paper is to investigate whether adult nude mice given two intraperitoneal injections of SFV would develop clinical signs of a lethal encephalitis or behave similarly to the Swiss $A_2G$ mice where the infection was asymptomatic in spite of single demyelinative plaques in the cerebellar white matter.

## MATERIALS AND METHODS

### Virology and Serology

The avirulent SFV A774 strain was supplied by Dr. C. J. Bradish (Microbiological Research Establishment, Porton, Wiltshire). Originally the virus was recovered from *Aedes* in Portuguese East Africa (21). It had undergone seven intracerebral passages in two to five day old mice and was a clonal selection from plaques of virus

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grown in monolayers of primary chicken embryo cells (7).

The virus used for the experiment was titrated in suckling mice and half brains of mice were sampled for assay of virus presence by the plaque count in agar suspensions of primary chicken embryo cells (7). Serum samples from exsanguinated mice were tested for humoral antibody by calculating the serum neutralization index (SNI). The SNI was the logarithm of antiserum dilution giving 50 per cent reduction of SFV plaques (6, 13). An SNI >2.5 protects the mice against challenge with the virulent strain of SFV (7).

### Experimental Animals

Six to eight week old nude mice used in the experiment were male and female CBA/H and Balb/C crosses from a randomized colony. Swiss $A_2G$ mice of the same age were used for a comparison. These strains of mice were the only ones available on our premises for research purposes.

### Experimental Design

The virus inoculated into the animals was in a 0.75% suspension of bovine serum albumen phosphate saline (BAPS) at pH 7.3. All mice in the experiment were given an intraperitoneal injection of $10^{5.5}$ $ICLD_{50}$/0.1 ml SFV in BAPS. Nine nude and ten Swiss $A_2G$ mice were each given one inoculation of SFV followed by a second dose 14 days after the initial injection. Two nude mice and two Swiss $A_2G$ mice were killed with anaesthetic ether on postinoculation days 7, 14, 21 and 28. On day 35 one nude mouse and two Swiss $A_2G$ mice were sampled. Single serum samples were assayed for the SNI and half brains of the corresponding mice were subjected to virus titration.

Routine autopsy was done on the mice for presence of macroscopic abnormalities but only brains were taken for histology.

### Histology

Brains were fixed in 5% formal saline for at least seven days. Whole brains were sectioned in the frontal coronal plane into three pieces. The first cut was in front of the hippocampus and the second cut was at the junction of the midbrain with the brain stem. Half brains were cut sagittally. Paraffin wax blocks of brains cut 5-6 $\mu$m thick were stained routinely with haematoxylin and eosin. Myelin damage was demonstrated by luxol-fast-blue counterstained with cresyl-echt-violet (18).

## RESULTS

During the course of the experiment none of the nude mice or the Swiss $A_2G$ mice showed any clinical signs such as paralysis of the limbs. Table I illustrates the brain virus titres (V) and the SNI in the nude mice and Swiss $A_2G$ mice.

Histology of the brain showed evidence of encephalitis in all the nude mice on postinoculation day 7 but this was much less compared to that observed in the Swiss $A_2G$ mice. By postinoculation day 14 the perivascular cuffing in the Swiss $A_2G$ mice reached maximal proportions consisting of several layers of lymphocytes with a few plasma cells. The inflammatory response in the nude mice on postinoculation day 14 was not increased and perivascular cuffs consisted of single layers of lymphocytes with rare plasma cells. Focal lesions in the cerebellar white matter were obvious in the nude mice on postinoculation day 21, whereas microcystic foci and single plaques of myelin damage were seen in the Swiss $A_2G$ mice. Multiple foci of myelin damage were apparent in the nude mice on day 28 and several foci could be identified in one microscopic field (Fig. 1) compared to the isolated foci in the Swiss $A_2G$ mice.

On postinoculation days 7 and 14 when the nude mice had positive SNI's of 3.0 and 3.3 respectively, attempts at virus recovery from the brains were negative at a dilution of $10^{0.3}$. On days 21 and 28 when SNI's were reduced, brain virus titres increased to $10^{2.0}$ and $10^{2.5}$, respectively, and at the end of the experiment on day 35 the nude mice had an SNI of <1.5 and brain virus titre of $10^{2.0}$. No virus was isolated from any of the brains of the Swiss $A_2G$ mice at a dilution of $10^{0.3}$ and SNI remained high and greater than 3.0 up to day 35. The magnitude of the immune response in the Swiss $A_2G$ mice seemed to correlate

TABLE I. Half Brain Virus Titres in Logs[10] p.f.u./ml with Corresponding SNI

| Postinoculation Day | Nude Mice | | Swiss $A_2G$ Mice | |
|---|---|---|---|---|
| | Given SFV on Day 0 and 14 | | Given SFV on Day 0 and 14 | |
| | Virus titre | SNI | Virus titre | SNI |
| 7 | <0.3[a] | 3.0 | <0.3 | 4.0 |
| 14 | <0.3 | 3.3 | <0.3 | 4.3 |
| 21 | 2.0 | 2.3 | <0.3 | 4.0 |
| 28 | 2.5 | 2.0 | <0.3 | 4.4 |
| 35 | 2.0 | <1.5 | <0.3 | 4.0 |

[a] <0.3 means that attempts at virus recovery were negative at a dilution of $10^{0.3}$

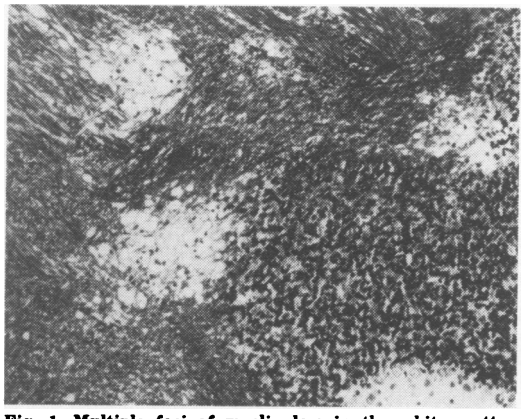

**Fig. 1. Multiple foci of myelin loss in the white matter of the cerebellum of the nude mice on postinoculation day 28. Luxol fast blue/cresyl echt violet. X112.**

with the degree of perivascular cuffing as quantified by layers of round cells surrounding the blood vessel.

## DISCUSSION

Cell mediated lysis of cells expressing viral antigens may be beneficial. For example, sensitized T cells may operate either alone to eliminate virus transformed tumour cells as in the rejection of allogenic tumours (16) or in collaboration with macrophages to limit growth of cytolytic viruses in tissues (3, 4). Several groups of research workers have reported that specifically sensitized thymus derived (T cells) may act directly to lyse virus infected target cells (5, 12, 19), otherwise the net effect of T cell depletion may be that the animal dies from extensive virus-induced cytopathology (2).

Nude mice have an abnormal development of the thymus and thymus-dependent systems and show a profound deficit of immune functions, especially of the thymus-dependent type (26).

Chew-Lim and co-workers have shown that two or three injections of avirulent SFV produced single foci of myelin lesions with occasional neuronophagic nodules in adult Swiss $A_2G$ mice when SNI was high and no virus was isolated from the brains (8). An electron microscopic study revealed that the myelin damage could be attributed to damage of whole groups of axons seen in areas where oligodendrocytes appeared to be normal (10). The demyelination and neuronophagia were likely to be secondary to axonal degeneration and is a picture very reminiscent of canine distemper demyelinating encephalitis (20, 24). However, with the SFV infection at the time when myelin loss and astrocytic hypertrophy were observed no virus was recovered from the brains and no virus particles were seen ultrastructurally in any of the cells, whereas in distemper the viral material identified accompanied both demyelination and cellular destruction and was located most frequently in astrocytes. The abnormal astrocytes of SFV secondary demyelination are not comparable to the distemper infected reactive astrocytes which have vesiculation of their nuclei and many nuclei contained viral inclusions with accumulations of paramyxovirus nucleocapsids in the cell cytoplasm and astroglial process (24).

Fleming using the same strain of SFV in random-bred Porton mice found that repeated inoculation of virus gave rise to a preponderance of $IgG_{2a}$ antibody titres in the serum samples (14). It can be deduced that when myelin damage is observed in the SFV infection that the high SNI index would also reflect a predominance of $IgG_{2a}$ antibodies. As such, the SFV cerebellar demyelination may have some similarity to the recent work on transmissible congenital demyelinating encephalopathy of lambs in

which a Togavirus could be implicated. Here affected lambs showed high IgG concentrations before they were given colostrum and the secondary demyelination was probably the result of a congenital viral infection causing degeneration of axons or neurones or both (11).

When the Swiss $A_2G$ mice were immunosuppressed with 500 rad total body irradiation and subsequently given three doses of SFV they developed multiple demyelinative plaques with brain viral persistence up to day 10 (9). The cerebellar lesions in the nude mice were similar to that seen in the irradiated mice with the exception that by days 14 and 21 the Swiss $A_2G$ mice had no virus recoverable from their brains whereas the nude mice had $10^{2.0}$ virus even on day 35.

Initially, in the nude mice on days 7 and 14 no virus was recovered from the brains at a dilution of $10^{0.3}$ and the SNI was 3.0 and 3.3 respectively. After a second virus infection on day 14 the SNI was reduced to 2.3 on day 21 and brain virus titre had increased to $10^{2.0}$. On days 28 and 35 the nude mice had brain virus titres of $10^{2.5}$ and $10^{2.0}$ and SNI's were 2.0 and less than 1.5 respectively. In contrast to this with the Swiss $A_2G$ mice all attempts to isolate virus from brains at dilutions of $10^{0.3}$ were negative and SNI remained high and more than 3.0 throughout the experiment. The SNI titres of the nude mice indicate that they were immuno-incompetent compared to the Swiss $A_2G$ mice. Whether SFV was able to persist in the brain or not was apparently dependent on the immune response of the mice as measured by the SNI. These results suggest the importance of the thymus-dependent system in the SFV infection for the elimination or inhibition of virus growth in the brain.

The multiple foci of myelin damage in the nude mice could be a result of viral presence or the action of persisting virus with antibody. This protracted action occurring in the immediate vicinity of myelinated fibres could precipitate the release of myelinolytic factors such as the soluble products from activated lymphocytes acting directly on myelin or through recruitment of accessory cells such as macrophages. This situation probably accounted for the more widespread demyelination. It is likely that the nude mice have some small proportion of T cells which were responsible for lysis of the first inoculation of virus but when given a second infection the virus cytolytic mechanism was less efficient and there was a corresponding reduction in circulating antibody such that by day 35 the SNI was much less than 2.5 and the mice probably would have succumbed to a challenge of virulent SFV. However, the mice appeared to be healthy throughout the experiment.

Zinkernagel and Althage demonstrated that in some virus infections the T cells can inhibit virus growth and spread and be an important antiviral effector mechanism (27). A competent immune system is essential for removal of SFV in a central nervous system infection and the development of antibody response probably requires T helper cells. An inadequate antibody response apparently resulted in a failure to restrict virus growth in the brains of the nude mice in our study.

This experiment illustrates that the nude mouse is a good laboratory animal for the study of SFV persistence in the brain with reduced antibody synthesis and how this relates to the severity of myelin lesions. The T cells in the SFV infection could be cytotoxic, causing lysis of virus-infected cells before infectious virus progeny are assembled and released. Alternatively, the T cells do not lyse the infected brain cells, but rather upon recognition of the relevant and self-cell surface antigens, the T cells release soluble mediators which then directly prevent virus spread. The role of T cells in the nude mice may have a direct association with the increased myelin damage. Using larger numbers of nude mice and combining this with daily sampling and an ultrastructure study it may be possible to elucidate how this avirulent neuroinvasive virus affects an adult immuno-incompetent animal and to give a clearer picture as to why a deficit of T cells exacerbates the secondary demyelination. Semliki Forest virus in the nude mouse could contribute to an understanding as to the pathogenesis of certain demyelinating human diseases such as subacute sclerosing panencephalitis (22, 23) and multiple sclerosis (15, 25).

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