Clin. exp. Immunol. (1983) **52,** 115–120.

Reconstitution of Semliki forest virus infected mice, induces immune mediated pathological changes in the CNS

J. K. FAZAKERLEY, SANDRA AMOR & H. E. WEBB *Neurovirology Unit, Department of Neurology, The Rayne Institute, St Thomas' Hospital, London, UK*

(Accepted for publication 19 November 1982)

SUMMARY

Reconstitution of Semliki forest virus infected nude mice with spleen cells from their immunocompetent nu/+ litter mates resulted in an abolition of the otherwise persistent brain virus, production of anti-SFV IgG, and development of normally absent brain pathology. The brain pathological changes, including demyelination, seem to be mediated by T cells, and are maximum 14 days after sensitization of the reconstituting spleen cells. Sensitization of the spleen cells 7 days before transfer to the nude mice results in pathological changes advanced by about 8 days, compared to reconstitution with unsensitized cells. The involvement of T cells in the virus-induced pathology is discussed.

INTRODUCTION

Semliki forest virus (SFV), an alphavirus of the Togaviridae, has been shown to produce an encephalitis with demyelinating lesions in the central nervous system (CNS) of Swiss/A_2G mice, (Webb *et al.*, 1979). These lesions were found to be absent in SFV infected BALB/c-CBA mice homozygous for the nude (*nu*) gene, but present in their immunocompetent heterozygous *nu*/+ litter mates (Jagelman, *et al.*, 1978). Chew-Lim (1979) found the SFV-induced inflammatory reaction in the *nu/nu* mice to be less severe than in immunocompetent Swiss/A_2G mice. Bradish *et al.* (1979), using a different strain of *nu/nu* mouse, found that infection with SFV produced an encephalitis of the same severity and extent in *nu/nu* mice as in *nu*/+ or A_2G mice. Berger (1980) using BALB/c-CBA *nu/nu* mice found that pathology after SFV infection was reduced but not absent. Results on the outcome of an avirulent SFV infection in the *nu/nu* mouse are thus conflicting.

Nude mice are severely impaired in their T cell responses and in the production of thymus-dependent antibodies. Previous work in this laboratory (Suckling, Jagelman & Webb, 1982), on *nu/nu* mice has shown normal levels of neutralizing IgM, but low or undetectable levels of virus specific IgG. IgG1 and IgG2a seem to be the subclasses most notably affected. Reconstitution of these mice with spleen cells from their *nu*/+ litter mates, resulted in levels of total IgG similar to those of the *nu*/+ mice and an increase in specific anti-SFV IgG to *nu*/+ levels. This paper enlarges on these results, and comments on the brain virus titres and pathology of the reconstituted *nu/nu* mice.

MATERIALS AND METHODS

Mice. A breeding nucleus of BALB/c-CBA mice carrying the *nu* gene was obtained from a colony originating from the Laboratory Animals Centre, Carshalton, Surrey. Homozygous *nu/nu*

Correspondence: John K. Fazakerley, Neurovirology Unit, Department of Neurology, The Rayne Institute, St Thomas' Hospital, London SE1 7EH, UK.

0009-9104/83/0400-0115$02.00

mice and their heterozygous *nu*/+ litter mates were bred in the animal house at St Thomas' Hospital Medical School. The mice were maintained under barrier conditions throughout. Mice of either sex used for these experiments were 4–6 weeks of age.

Virus. The SFV used in these experiments was the avirulent strain A7(74) originally isolated by Bradish, Allner & Maber (1971), and obtained from Dr C. J. Bradish of the Microbiological Research Establishment, Porton Down, Wiltshire, England. The virus was made up in sterile phosphate-buffered saline with 0·75% bovine serum albumin added (BAPS) and stored at −70°C until use. The virus had a titre of $10^{8.5}$ $ICLD_{50}$/0·02 ml in 2–4 day old suckling mice.

Virus titres. 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 an appropriate range of dilutions intracerebrally (i.c.) into groups of 4–6 suckling mice, 2–4 days old. The i.c. 50% lethal dose/0·02 ml was calculated by the method of Reed & Muench (1938).

Antibody titres. An enzyme linked immunosorbent assay (ELISA) based on the method of Voller, Bidwell & Bartlett (1976), was used to measure anti-SFV IgG levels. This has been described in detail previously (Suckling *et al.*, 1982). Results were obtained as the highest log dilution giving a reading significantly above that of serum from an uninoculated mouse and are expressed as the mean value from five mice, with the standard error of the mean.

Histology. Half brains were fixed in 5% formol saline and processed using standard histological techniques. The half brains were sectioned sagitally, taking 5μm sections of different levels to ensure representative examination. Sections were stained using the haematoxylin & eosin method, for routine study, and the luxol fast blue and cresyl fast violet method to study demyelination. At least 10 sections were studied from each brain.

Reconstitution of the nu/nu *mice.* Spleen cells from uninfected *nu*/+ mice were used to reconstitute 35 *nu*/*nu* mice. Sensitized spleen cells from *nu*/+ mice infected with SFV 7 days previously were used to reconstitute a second group of 30 *nu*/*nu* mice.

The *nu*/+ mice were killed under ether anaesthesia and their spleens removed aseptically into RPMI 1640 media with HEPES buffer (Flow Laboratories) and 0·2% crystamycin was added. A single cell suspension was prepared by passing the spleens through a steel sieve, aggregated cells were removed and the suspension washed three times. Each mouse received approximately 8×10^7 nucleated splenocytes in 0·5 ml of media intraperitoneally (i.p.). The cell viability was 90% as assessed by trypan blue exclusion. Reconstituted *nu*/*nu* mice were injected 24 h previously with $10^{4.5}$ i.c. LD_{50}SFV i.p.

Five mice from each of the two reconstituted groups were sampled on each of post-inoculation days (PIDs) 4,7,9,12,15 and 18 (an additional five mice were sampled on PID 21 in the group receiving unsensitized cells).

RESULTS

Fig. 1 shows, for comparison, results from our laboratory on the brain virus titres and serum antibody levels in the *nu*/+ and the normal *nu*/*nu* mice, adapted from Jagelman *et al.* (1978) and Suckling *et al.* (1982).

Brain virus

Reconstitution with the unsensitized spleen cells clears virus from the brain by PID 21 (Fig. 2), only one of five mice were positive on PID 18. Over the time examined the virus did not produce a second stage of high brain titres, as found in the normal *nu*/*nu* mice (Fig. 1). The mice reconstituted with the SFV sensitized cells clear virus from the brain by PID 9, as is the case with the *nu*/+ mice.

Serum IgG

Reconstitution with the sensitized spleen cells produces a rapid rise to high levels of anti-SFV IgG in the sera reaching a maximum on PID 7 as seen in Fig. 2. Levels fall on PID 12 but rise to high levels again by PID 18. The rise to 5 logs by PID 8 is more rapid than in the *nu*/+ mice which do not reach these levels until PID 15.

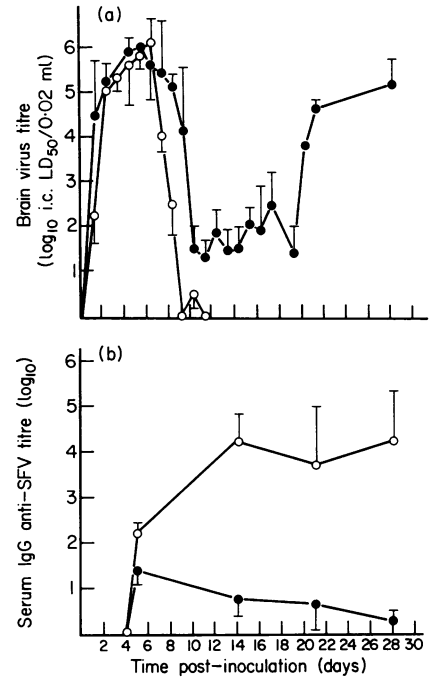

Fig. 1. (a) Brain virus titres and (b) serum IgG anti-SFV levels in *nu*/+ and normal (non-reconstituted) *nu*/*nu* mice, after infection with SFV A7(74). *nu*/+ mice (○), *nu*/*nu* (●) mice. Vertical bars represent 1 s.e. (mean) ($n = 5$).

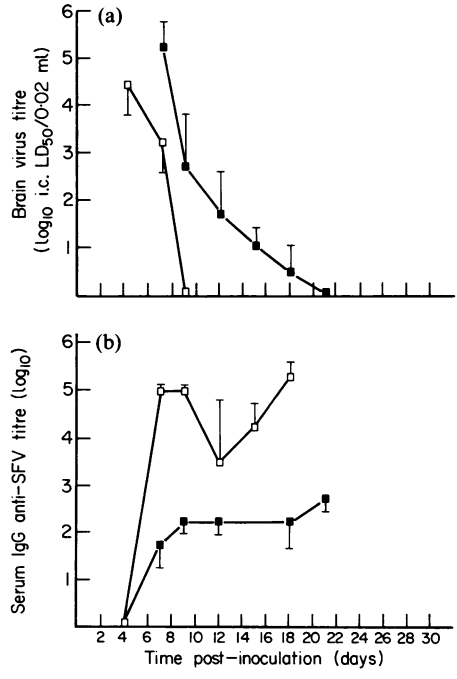

Fig. 2. Effect of reconstitution with *nu*/+ cells previously sensitized, (□) and not sensitized (■) to SFV A7(74), on (a) the brain virus titres and (b) the serum anti-SFV IgG levels in the *nu*/*nu* mice.

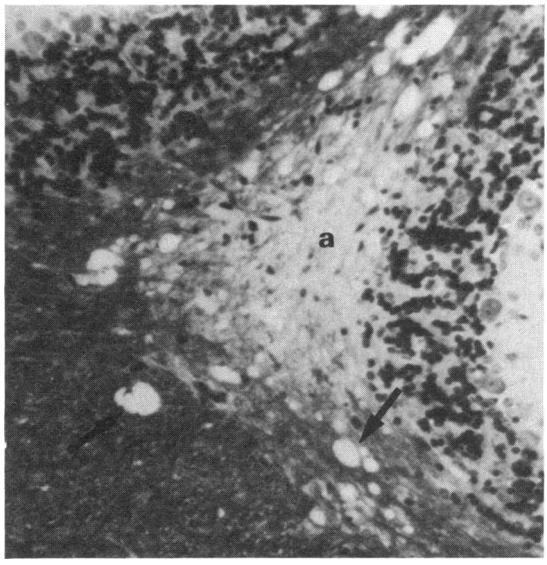

Fig. 3. Lesion in white matter of cerebellum showing demyelination (a) and microcysts (arrows) following reconstitution of infected mice with sensitized cells. PID 7. Magnification × 500. Stain: luxol fast blue and cresyl fast violet.

Reconstitution with the unsensitized spleen cells results in production of anti-SFV IgG at levels significantly above those in the normal *nu/nu* mice. No early peak in levels is present, the rise is later and the levels attained are lower than in the sensitized cell reconstitution.

Pathology

The CNS pathology in Swiss/A_2G mice (Webb *et al.*, 1979; Kelly *et al.*, 1982) and in *nu/+* and *nu/nu* mice (Jagelman *et al.*, 1978) have been described. On each sampling day, from each of the reconstituted groups, at least 10 sections from the brains of each of five mice, were examined. On day 4, in the *nu/nu* mice reconstituted with unsensitized cells no pathology was present, whereas in the group given sensitized cells perivascular cuffing (normally the earliest pathological change) was apparent. By day 7 the difference between the two groups was marked in that perivascular cuffing was now present, but minimal in the group given unsensitized cells, while in the group given sensitized cells, the cuffing was extensive, as were microcystic changes and demyelination (Fig. 3). Changes in this group were maximal on this day. It was not until day 15 that the changes in the group given unsensitized cells became maximal and included both microcystic change and demyelination. The pathological changes in the brains of the mice given unsensitized cells were never as extensive as in the mice given sensitized cells and were later by about 8 days.

DISCUSSION

Reconstitution of SFV infected *nu/nu* mice with SFV sensitized and unsensitized spleen cells from their *nu/+* litter mates results, in both cases, in abolition of infective brain virus, production of anti-SFV IgG and development of brain pathological changes, normally absent in these *nu/nu* mice. The dynamics of the response however differs between the two reconstituted groups.

The mice given sensitized spleen cells reduce brain virus titres, as do the unreconstituted *nu/nu* mice, to less than 3 logs by PID 10. This fall may be due to either IgM antibody which both groups of mice produce at high titres from PID 5 to PID 20 (Suckling *et al.*, 1982) or to IgG3 which has been found in the extravascular region of brain tissue from PID 5 in immunocompetent mice infected with SFV (Fleming, 1977). The brain virus is incompletely cleared in the normal *nu/nu* mice and a

second rise in infectivity occurs (Fig. 1). The *nu/nu* mice given unsensitized spleen cells do, however, produce anti-SFV IgG and this is probably responsible for the final slow clearance of brain virus by PID 21 in these mice. The mice given sensitized spleen cells clear infective brain virus by PID 9 as do the *nu/+* mice and this complete clearance is probably explained by the high levels of anti-SFV IgG, which reach a peak on PID 7 (Fig. 2).

The biphasic nature of the anti-SFV IgG antibody production in the mice reconstituted with sensitized cells is probably best explained by an early production of antibody by the primed donor *nu/+* cells, which reaches a peak on PID 7 and a second later rise in levels on PID 12–18 due to production of antibody by the hosts own B cells, now helped to produce this thymus-dependent antibody by the T lymphocytes of the donor spleen cells.

That both reconstitutions produce pathology in *nu/nu* mice is clear. Unsensitized cells produced a time course of pathogenesis essentially the same as in the *nu/+* mice, but the sensitized cells produced a time course advanced by about 8 days similar in type but more severe in nature. Since the microcystic and demyelinating changes are brought foward by reconstitution with sensitized cells, as compared to mice given unsensitized cells, these changes are clearly not a result of direct viral cytolysis as was previously suggested by Chew-Lim, Webb & Jagelman (1977) using the A7(74) strain of SFV in irradiated mice, and as has been suggested for the M136 mutant strain of SFV by Sheahan, Barrett & Atkins (1981).

In both reconstitution groups spleen cells are required for the production of pathology and it is the spleen cells, 14 days after priming with SFV, that are associated with maximum damage. Since the *nu/nu* mice lack T cells it would seem that it is the T cells in the reconstituting spleen cell population that are responsible for the pathogenesis. Lymphoblastic cells similar to T cells have been described in the vicinity of SFV induced demyelinating lesions in Swiss/A_2G mice (Pathak, Illavia & Webb, 1982). The production of pathology on reconstitution of our nude mice indicate that they do not contain other than an immunological deficit preventing SFV induced brain pathogenesis. The pathological changes found by other workers using avirulent SFV in nude mice conflict with our finding that the pathology produced is dependent upon T cells. It is possible that these results reflect strain differences, incomplete absence of T lymphocytes, which is known to occur in some nude mice (Raff, 1973; Loor & Roelants, 1974) or induction of functional T cells on infection (Scheid, Goldstein & Boyse, 1975). Two mechanisms of T cell-mediated pathological damage are possible. Firstly, that cytotoxic T cells are involved directly in recognition of viral induced cell surface antigens leading to cell damage and demyelination and secondly that helper T cells are required to induce production, perhaps locally of damaging antibodies. Further experiments using the above reconstitution system are underway to investigate these possibilities.

We thank the Multiple Sclerosis Society, the Philip Fleming Trust and the Charitable Funds of St Thomas' Hospital for the financial support of this work.

REFERENCES

BERGER, M.L. (1980) Humoral and cell-mediated immune mechanisms in the production of pathology in avirulent *Semliki Forest* virus encephalitis. *Infect. Immun.* **30,** 244.

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. *J. gen. Virol.* **12,** 141.

BRADISH, C.J., FITZGEORGE, R., TITMUSS, D. & BASKERVILLE, A. (1979) The response of nude-athymic mice to nominally avirulent togavirus infections. *J. gen. Virol.* **42,** 555.

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.

CHEW-LIM, M. (1979) Brain viral persistence and myelin damage in nude mice. *Can. J. Comp. Med.* **43,** 39.

FLEMING, P. (1977) Age dependent and strain related differences of virulence of *Semliki Forest* virus in mice. *J. gen. Virol.* **37,** 93.

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.

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. *Neuropathol. Appl. Neurobiol.* **8,** 43.

LOOR, F. & RORLANTS, G.E. (1974) High frequency of

T lineage lymphocytes in nude mouse spleen. *Nature*, **251**, 229.

PATHAK, S., ILLAVIA, S. & WEBB, H.E. (1982) The identification and role of cells involved in CNS demyelination in mice after *Semliki Forest* virus infection: an ultrastructural study. In *Progress in Brain Research*. Elsevier/North-Holland biomedical press. (In press.)

RAFF, M.C. (1973) θ-bearing lymphocytes in nude mice. *Nature*, **246**, 350.

REED, C.J. & MUENCH, H. (1938) A simple method of estimating fifty per cent end points. *Am. J. Hyg.* **27**, 493.

SCHEID, M.P., GOLDSTEIN, G. & BOYSE, E.A. (1975) Differentiation of T-cells in nude mice. *Science*, **190**, 1211.

SHEAHAN, B.J., BARRETT, P.N. & ATKINS, G.J. (1981) Demyelination in mice resulting from infection with a mutant of *Semliki Forest* virus. *Acta Neuropathol.* **53**, 129.

SUCKLING, A.J., JAGELMAN, S., & WEBB, H.E. (1982) Immunoglobulin synthesis in nude (*nu/nu*), *nu/+* and reconstituted *nu/nu* mice infected with a demyelinating strain of Semliki forest virus. *Clin. exp. Immunol.* **47**, 283.

VOLLER, A., BIDWELL, D. & BARTLETT, A. (1976) Microplate enzyme immunoassays for the immunodiagnosis of virus infections. In *Manual of Clinical Immunology* (ed. by N.R. Rose & H. Friedman) pp. 506. American Society for Microbiology, Washington, D.C.

WEBB, H.E., CHEW-LIM, M., JAGELMAN, S., OATEN, S.W., PATHAK, S., SUCKLING, A.J., & MACKENZIE, A. (1979) *Semliki Forest* virus infections in mice as a model for studying acute and chronic central nervous system virus infections in man. In *Clinical Neuroimmunology* (ed. by F.C. Rose) pp. 369. Blackwell Scientific Publications, Oxford.