J. gen. Virol. (1986), **67**, 945–947. *Printed in Great Britain*

Key words: *JEV/congenital infection/latency/reactivation*

Japanese Encephalitis Virus Latency Following Congenital Infection in Mice

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(*Accepted 20 February 1986*)

SUMMARY

Latent Japanese encephalitis virus (JEV) infection was shown in inapparently congenitally infected Swiss albino mice after their mothers had been given JEV intraperitoneally during pregnancy. Only one of 37 (2·7%) of the baby mice showed persistence of infectious virus at 5 weeks of age. Reactivation of JEV in Swiss albino mice was demonstrated by stimulation with allogeneic spleen cells from Parks strain mice at 21 weeks of age; reactivation was demonstrated in 41% of the inapparently infected mice. The spleen cells of congenitally infected mice had depressed [^{3}H]thymidine uptake following stimulation with concanavalin A, and depressed ability to induce a graft-versus-host response.

A persistent infection followed by a latent infection with Japanese encephalitis virus (JEV) has been observed in Swiss albino mice (Mathur *et al.*, 1982). The latent virus could be activated by giving cyclophosphamide even 1 year after infection (Mathur *et al.*, 1986). Abortion or congenital infection can occur when mice are infected with JEV during pregnancy (Mathur *et al.*, 1981; Sugamata & Miura, 1982) and similar effects of JEV infection have been observed at different periods of gestation in human cases (Chaturvedi *et al.*, 1980; Mathur *et al.*, 1985). The affected mice may be small, show runting, have hydrocephalus or die (A. Mathur, unpublished data). Long-term congenital JEV infection leading to persistent or latent infection has not been studied before. This paper reports latent JEV infection in congenitally infected mice.

The Japanese encephalitis virus strain used, 78668A, was isolated from the brain of a case of Japanese encephalitis and propagated by intracerebral (i.c.) inoculation in adult mice (Mathur *et al.*, 1981). Infected mouse brain pools were stored at −70 °C. Groups of mice which had been pregnant for 8 days (inbred Swiss albino mice obtained from this Department) were injected intraperitoneally (i.p.) with 10^2 LD_{50} of JEV. Of the newborn mice, 37% died within the first 15 days and the others remained unaffected. From the brain tissue of some of the dead mice, JEV was isolated, indicating congenital infection. The unaffected mice were tested at the age of 5 weeks for JEV persistence. The thymus, spleen and brain tissue homogenates were inoculated i.c. into suckling mice. Out of 37 mice examined, only one (2·7%) yielded the virus. For the present study, unaffected mice whose mothers had been infected during pregnancy were selected when they were 21 weeks old (group A). Controls consisted of age-matched normal mice (group B) and age-matched normal mice which had been given JEV i.c. 6 days earlier (group C).

Virus-specific IgM antibody plaque-forming cells (PFC) were counted in the spleens of congenitally infected and control mice by the haemolysis in gel technique of Jerne & Nordin (1963) as described earlier (Mathur *et al.*, 1983*a*). The findings presented in Table 1 show that

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Table 1. *Antibody plaque-forming cells in the spleen of mice**

Group of mice	No. of mice	PFC/2 × 10^6 spleen cells	*P* value
A	13	151 ± 11	<0·001
B	9	48 ± 5	
C	9	619 ± 16	

* JEV-specific IgM PFC were counted in individual spleens from the various groups of mice. Multiple slides were prepared from each spleen and the mean value ± S.D. is presented for each group. The data were analysed by Student's *t*-test.

Table 2. *Comparison of spleen proliferative response of inapparently congenitally infected and control mice*

Spleen cells* from mouse group	Con A-induced [^{3}H]thymidine incorporation†
A	21175 ± 903
B	32231 ± 921
C	11247 ± 327

* Spleen cells from different groups of mice were cultured with Con A for 48 h and labelled with [^{3}H]thymidine for the last 4 h.

† Results are the means from five Con A-stimulated cultures minus mean values from unstimulated spleen cell cultures, ±S.D.

the number of PFC in the spleen cells of group A mice was significantly greater than in uninfected mice (group B). Group A mice were therefore assumed to have been congenitally infected. When the thymus and spleen of congenitally infected 21-week-old mice were assayed for the presence of virus, JEV was found in the spleen of one out of 41 (2·4%) mice, a rate which probably represents spontaneous reactivation. A similar phenomenon has been observed with cytomegalovirus and herpes simplex virus latent infections (Jordan *et al.*, 1977; McLennan & Darby, 1980). These animals were considered to be latently infected.

An effort was made to reactivate the virus in congenitally infected 21-week-old mice. Congenitally infected and control uninfected mice were injected i.p. with 1 × 10^8 spleen cells of Parks strain mice (purchased from the Central Drug Research Institute, Lucknow). On day 9 the recipient mice were killed and the brain, spleen and thymus were assayed for the presence of JEV. Out of 31 congenitally infected mice examined, 13 (41·9%) yielded the virus from thymus and 12 (38·7%) from the spleen. No virus was isolated from any organ in control mice. This shows that allogeneic cells induce reactivation of the infectious virus from the resting state.

The lymphoproliferative response of mice of groups A, B and C was compared by the uptake of [^{3}H]thymidine into spleen cells following stimulation with concanavalin A (Con A). Spleen cells were cultured in MEM containing 5% foetal calf serum and antibiotics (2 × 10^6 cells/ml) and were either left untreated or stimulated with 2 μg/ml Con A for 48 h. The cells were pulse-labelled with [^{3}H]thymidine (Amersham) for the last 4 h of culture and uptake was measured in a Beckman LS 9000 liquid scintillation counter. A decreased [^{3}H]thymidine uptake by spleen cells of congenitally infected mice was observed as compared to that in uninfected controls (Table 2).

The immunosuppressive effect of JEV in congenitally infected mice was further demonstrated by a decreased graft-versus-host (GVH) reaction. Spleen single-cell suspensions (10^6 and 10^7 cells) from inapparently congenitally infected (group A) or normal control (group B) mice were injected i.p. into infant mice of the Parks strain. The recipient mice were killed 9 days later and the weight of spleens was recorded. The relative spleen weight of each mouse was defined as the ratio of the spleen weight to total body weight. The spleen index (SI) was calculated for each mouse by dividing its relative spleen weight by mean relative spleen weight of non-infected control mice. An SI value greater than 1·3 was considered to represent significant GVH reactivity (Simonsen, 1962). A significant splenomegaly with SI values 1·6 (10^6

cells given) and 2·3 (10^7 cells given) was induced by normal mouse spleen cells, whereas when congenitally infected mouse spleen cells were given, the SI was 1·2 or less.

The decreased [^{3}H]thymidine uptake by the Con A-stimulated spleen cells from congenitally infected mice, and the significantly reduced direct GVH reaction produced in Parks strain mice confirms our previous observations of depressed cell-mediated immune response in congenitally infected mice (Mathur *et al.*, 1983*b*). Activation of the virus in inapparently infected animals by co-cultivation of spleen cells with allogeneic, syngeneic or both types of cells has been observed also in mouse cytomegalovirus infection (Olding *et al.*, 1975; Mayo *et al.*, 1978; Jordan *et al.*, 1982). The transmission of JEV has been described in piglets (Morimoto *et al.*, 1972), infant mice (Fujisaki *et al.*, 1976) and hamsters (Tamura *et al.*, 1977) after infection of pregnant animals, but the presence of latent JEV in congenitally infected animals has not previously been demonstrated.

This study was carried out with the financial assistance of the Indian Council of Medical Research, New Delhi.

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(Received 23 October 1985)