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Transplacental Japanese Encephalitis Virus (JEV) Infection in Mice During Consecutive Pregnancies

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SUMMARY

Transplacental transmission of Japanese encephalitis virus (JEV) has been demonstrated in consecutive pregnancies of mice. Pregnant mice inoculated intraperitoneally with JEV transmit the virus to the foetus. When such female mice were mated again after 6 months, the virus could be isolated from the foetuses of the ensuing pregnancy. The incidence of abortion was increased significantly though the neonatal deaths were considerably less than during the first pregnancy. Intra-uterine infection occurred in spite of the presence of HAI antibodies against JEV in the preconceptional sera of the mice. The findings of the present study indicate the value of such a system for further investigations of the pathogenesis of JEV infection during pregnancy in humans.

A number of viruses have been incriminated as causing intra-uterine infections in humans (Hanshaw & Dudgeon, 1978) and animals (Kilham & Margolis, 1975). However, congenital virus infection in consecutive pregnancies is a rare phenomenon and has been described only in cytomegalovirus (CMV) infection (Embil *et al.*, 1970; Krench *et al.*, 1971; Stagno *et al.*, 1973, 1977).

Japanese encephalitis virus (JEV) has been implicated as causing intra-uterine infection in swine with stillbirths and the virus recovered from the brain of stillborn piglets (Morimoto *et al.*, 1972). Impaired spermatogenesis and virus discharge into swine semen have been demonstrated experimentally (Habu *et al.*, 1977). JEV transplacental transmission and foetal injury has also been shown in humans (Chaturvedi *et al.*, 1980) and experimentally in mice (Mathur *et al.*, 1981). Intra-uterine infection appears to be acquired by the foetus as a result of viraemia after primary maternal infection (Chaturvedi *et al.*, 1980; Mathur *et al.*, 1981). The immunity acquired as a result of primary infection usually provides protection during future pregnancies.

Persistent maternal infection or reactivation of a virus may cause foetal infection. Persistence of JEV infection has been shown in suckling mouse brain and mosquito cells (Gavrilov *et al.*, 1974; Rehacek, 1968). Shiraki (1970) has provided evidence that JEV persists in the human brain for 8 to 15 years after the onset of encephalitis. It is not known whether JEV, like CMV, reactivates during immunosuppression (Jordan *et al.*, 1977; Lang *et al.*, 1976). The immune response seems to be depressed during pregnancy (Purtilo *et al.*, 1972). Chantler *et al.* (1979) have suggested that altered immune responses may reactivate persistent CMV. In this report, using the murine model, we have shown that maternal JEV infection can be transmitted to the foetus during consecutive pregnancies.

Before studying the transplacental transmission of JEV in consecutive pregnancies in mice, experiments were done to demonstrate *in utero* transmission of virus to foetuses during the first pregnancy. The virus used was a 10% (w/v) homogenate of adult mouse brain-passaged JEV (strain 78668A) having an infectivity titre of $10^{4.5}$ LD_{50} per g of tissue (Mathur *et al.*, 1981). The initial infection was established in 23 Swiss albino pregnant mice, negative for JEV HAI antibodies (estimated by the method of Clarke & Casals, 1958), by intraperitoneal inoculation with 10^2 LD_{50} of JEV on day 8 of pregnancy. This time of gestation and route of

Table 1. (*a*) *Effects on consecutive pregnancy and foetal and neonatal wastage of maternal infection with JEV on day 8 of first pregnancy*

Group	No. inoculated	Maternal death	Number/total (%) of: Mice aborted or completely resorbed	Stillborn	Neonatal death	Apparently normal offspring
First pregnancy						
Infected	18	1	4/18 (22·2)	15/78 (19·2)	21/63 (33·3)	42/78 (53·8)
Control	7	–	–	0/63	4/63 (6·3)	59/63 (93·6)
Consecutive pregnancy						
Infected	10	–	3/10 (30·0)	2/35 (5·7)	3/33 (9·0)	30/35 (85·7)
Control	4	–	–	0/35	2/36 (5·5)	34/36 (94·4)

(*b*) *Virus isolation in consecutive pregnancy*

Organ of animal tested	No. of animals positive/total inoculated	Virus titre
First pregnancy		
Placenta	4/5	$10^{3\cdot5}$–$10^{4\cdot8}$
Foetal brain	4/5	$10^{2\cdot8}$–$10^{3\cdot2}$
Foetal spleen	1/5	$10^{4\cdot1}$
Newborn mouse brain	17/22	
Consecutive pregnancy		
Placenta	2/14	$10^{2\cdot8}$–$10^{3\cdot0}$
Foetal brain	2/14	$10^{1\cdot5}$–$10^{2\cdot3}$
Newborn mouse brain	3/30	$10^{2\cdot5}$–$10^{3\cdot1}$

inoculation gives high incidence of infection of the foetus (Mathur *et al.*, 1981). JEV produces no morbidity or mortality in the pregnant mice after intraperitoneal inoculation.

HAI antibodies against JEV were detected in all sera at day 12 after virus inoculation. Five virus-inoculated and three control pregnant mice were exsanguinated and examined approximately 1 day before the expected day of delivery. One mother showed total resorption (20%) while four mothers had 25 foetuses. Among these foetuses three showed partial resorption. In control mice all the 28 foetuses were normal. Virus titres in four out of seven placentas were $10^{3\cdot5}$ to $10^{4\cdot8}$ LD_{50} per g; in four of five foetal brains $10^{2\cdot8}$ to $10^{3\cdot2}$ LD_{50} per g, and in one of five foetal spleens $10^{4\cdot1}$ LD_{50} per g (Table 1*b*). The remaining 18 mice were allowed to deliver. Four mothers showed complete resorption or abortion (22·2%). A total of 78 mice were born of JEV-infected mothers. There was a significant increase in the incidence of stillbirths and neonatal deaths in infants of infected mothers as compared to controls (Table 1*a*). Brains were harvested from two or three normal infants from each mother and virus was detected in 17 out of 22 infants (76%). Mice that failed to transmit infection to the foetus were excluded from subsequent studies. These findings demonstrate that JEV can be transmitted from the infected mother to the foetus as shown earlier (Mathur *et al.*, 1981).

Presence of virus in different tissues of three to four JEV-infected mice was studied at weekly intervals by intracerebral inoculation of tissue suspensions in 1-day-old suckling mice. From 17 weeks after infection until prior to the next mating, the inoculated mice showed no detectable virus in the brain, thymus, ovary, uterus, brown fat, spleen, kidney or liver (A. Mathur *et al.*, unpublished results). Six months later 15 of these mice were mated with healthy male (Swiss albino) mice and day 1 of pregnancy was counted from the day of observing the vaginal plug. HAI antibody titres in serum specimens 6 months after the original infection

Table 2. *HAI antibody titres in those JEV-infected mice which transmitted the virus to the foetus during consecutive pregnancy*

Mouse no.	Virus isolation	JEV HAI antibody titre in mice: During first pregnancy	JEV HAI antibody titre in mice: Before second pregnancy
1	Foetal brain	40	40
2	Newborn infant (brain)	80	160
3	Newborn infant (brain)	80	80
4	Resorbed foetus	40	40
5	Resorbed foetus	160	160
6	Resorbed foetus	40	80
7	Resorbed foetus	20	20
8	Resorbed foetus	80	80

were roughly the same as that at delivery of the first litter (Table 2). Five of these mice pregnant for the second time, were sacrificed approximately 1 day before the day of delivery. Two of them showed complete resorption of the foetuses, while three had a total of 14 foetuses. Three control mice, in contrast, each had nine foetuses. When no foetuses were found, pregnancy was considered to have occurred when there was thickening and vascularization of the uterine wall and an increase in the size of the ovaries. The placenta and foetal organs were collected, washed thoroughly three times in cold serum-free minimal essential medium, homogenized and 10% (w/v) tissue suspensions titrated in 1-day-old infant mice by intracerebral inoculation. Two of the 14 placentas examined had detectable JEV (titres $10^{2\cdot8}$ and $10^{3\cdot0}$ LD_{50} per g). The virus titre in two out of 14 foetal brains ranged from $10^{1\cdot5}$ to $10^{2\cdot3}$ LD_{50}. The virus isolated was identified as JEV by a rapid complement fixation test and by a neutralization test in infant mice, using hyperimmune sera provided by the Director of the National Institute of Virology, Pune.

The remaining 10 mice were allowed to deliver (Table 1 *a*). Three of the inoculated mice showed evidence of abortion or resorption of foetuses (30%), which was a higher incidence to that during the first pregnancy. No virus isolations were made from the two stillborn mice. Out of the total 33 live-born mice, virus was isolated from three infant mice that died within 24 h after birth (Table 1 *b*). Five mothers did not transmit the virus to their offspring.

These preliminary results show that transplacental transmission of JEV occurs in consecutive pregnancies in mice. Primary maternal JEV infection accounts for a high rate of foetal wastage and smaller percentage of normal-looking infants, while during consecutive pregnancy the rate of abortion as well as the rate of normal offspring were higher. JE epidemics have long been wide-spread in South-East Asia and *in utero* transmission in swine population has been observed (Burns, 1950). JEV has caused many epidemics in India affecting large sections of the population (Indian Council of Medical Research, 1980) but human transplacental transmission was observed for the first time during an epidemic of JE in Uttar Pradesh during 1978 (Chaturvedi *et al.*, 1980; Mathur *et al.*, 1982). The virus strain (78668A) isolated during this epidemic has caused transplacental transmission in mice (Mathur *et al.*, 1981) and also infection during consecutive pregnancies as shown in this study. It is important to determine whether the same phenomenon occurs in humans in JE-infected areas. This particular strain has been shown to be markedly different from 15 other strains of JEV in forming plaques of larger size (Dr K. Banerjee, National Institute of Virology, Pune, personal communication). The present findings and our earlier ones are perhaps due to strain variation of the virus.

The intriguing questions arising from the present findings are how the virus persists, and what are the mechanisms by which it is reactivated? The work of Gavrilov *et al.* (1974) on

suckling mouse brain cell lines persistently infected with JEV showed that defective particles or infectious ribonucleoprotein are a major factor in establishment and maintenance of the chronically infected state. Pregnancy by itself is known to produce a state of immunosuppression (Purtilo *et al.*, 1972). Immune response of JEV-infected pregnant mice is suppressed. We have observed that in such mice footpad swelling reaction, leukocyte migration inhibition against JEV and sheep red blood cells after sensitization with respective antigens are markedly reduced as compared to normal mice. Furthermore, uptake of [^{3}H]thymidine by spleen cells following stimulation by PHA is significantly less in pregnant mice than in normal mice (A. Mathur *et al.*, unpublished results). How far persistence of the virus in our model can be attributed to immunosuppression remains to be investigated.

The possibility that there was infection by a heterologous strain of JEV during the second pregnancy is unlikely because of the steady maintenance of good antibody titres in these mice, and the rigorously controlled experimental conditions. Further confirmation of our observations needs direct demonstration of the virus antigen in the tissues by immunofluorescent technique. Therefore, it appears that the virus is reactivated during subsequent pregnancy. This is in agreement with the previous observations on CMV, referred to above.

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