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Immune Response to Japanese Encephalitis Virus in Mother Mice and their Congenitally Infected Offspring

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SUMMARY

The immune response to Japanese encephalitis virus (JEV) was assessed in JEV-infected mice (mothers) and their offspring. The congenitally infected baby mice responded poorly in all assays for cell-mediated immunity. The total number of their splenic cells remained unaltered but the percentage of T cells was significantly reduced; a depressed delayed hypersensitivity response was seen against both homologous (JEV) and heterologous (sheep erythrocytes) antigens. In addition, significantly higher leukocyte migration inhibition (LMI) of spleen cells in the presence of specific antigen was observed. Adult mice infected during pregnancy demonstrated an impaired delayed hypersensitivity response to JEV antigen only. LMI was positive in mothers at 2 weeks post-partum, but not at later periods.

During studies on the transplacental transmission of Japanese encephalitis virus (JEV) in mice, we have shown persistence of the virus in thymus and brown fat for up to 17 weeks after infection and its reactivation during a consecutive pregnancy in spite of the presence of maternal antibodies (Mathur *et al.*, 1982). Persistent infections can be produced by failure of the host's defence mechanism to eliminate invading viruses; on the other hand, viruses may develop strategies to bypass normal immune surveillance and clearance mechanisms (Stroop & Baringer, 1982). Recently, a transient cell-mediated immune response (CMI) during JEV infection of adult mice has been described. In its early stages, both a CMI and IgM antibodies aid in the clearance of virus; later, neutralizing IgG antibodies are present but they have no protective value (Mathur *et al.*, 1983*a*).

JEV infection of mice during pregnancy may be followed by *in utero* transmission of virus to the foetus, resulting in a variable degree of foetal and neonatal wastage (Mathur *et al.*, 1981, 1982). Congenitally JEV-infected mice which were apparently normal at birth manifested clinical (runting) or virological evidence of the virus infection in later life (A. Mathur, unpublished results). This suggests an underlying defect in the host immune response. In the present study, the cell-mediated immune response of such apparently normal baby mice at different ages, and of their mothers, was studied.

JEV was given intraperitoneally (i.p.) to pregnant Swiss albino mice (6 months old) on day 8 of pregnancy. About one-third of the newborn mice died during the first week after birth while the others remained asymptomatic. Congenital infection in these mice was demonstrated on day 1 post-partum by virus isolation from brain and spleen; also, IgM antibody activity (titres 16 to 32) could be shown by haemagglutination inhibition (HAI), using techniques described previously (Mathur *et al.*, 1981).

In the experiments reported here, we first determined the total number of cells in the spleens of (i) congenitally infected baby mice at 4 and 8 weeks of age and (ii) their mothers. The findings were compared with age-matched controls. Groups of mice were killed at these times and the spleens were aseptically removed. Spleen cells were teased out and single-cell suspensions were

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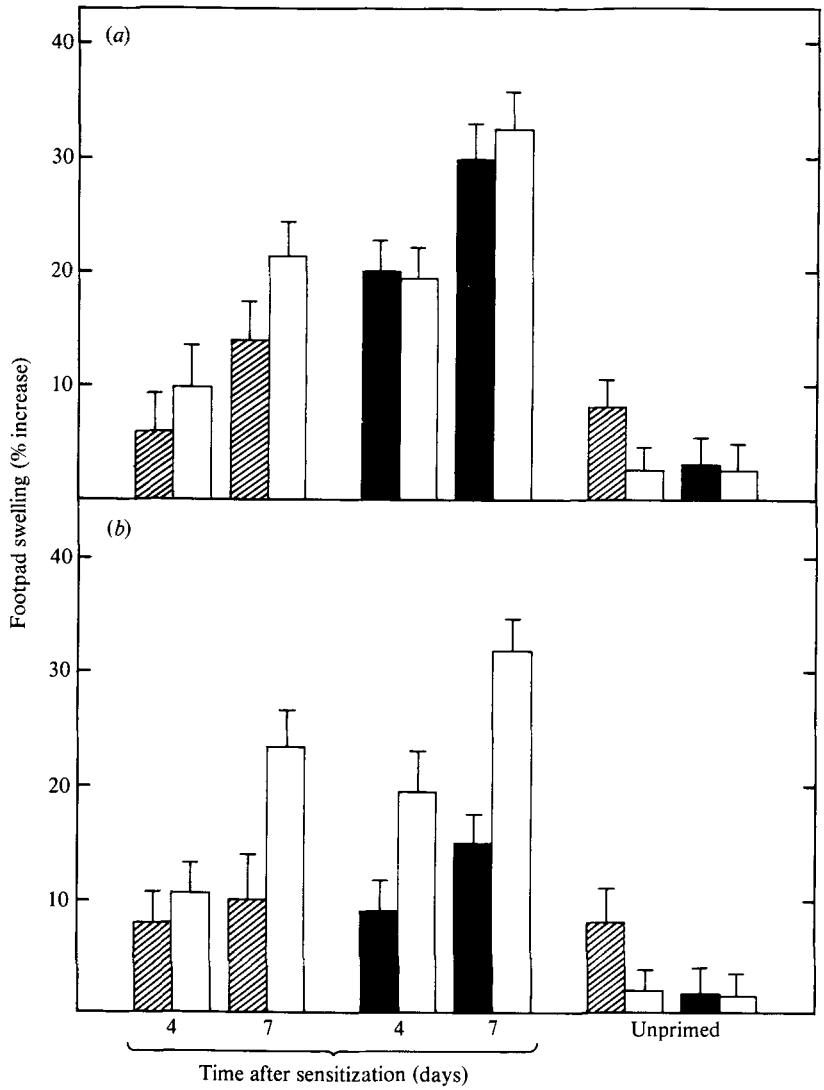

Fig. 1. Comparison of DTH responses between congenitally JEV-infected baby mice and their mothers with control mice. Groups of mice were primed with a 20% JEV-infected mouse brain suspension containing 10^2 LD_{50} units or SRBC (4×10^8) i.p., and another group of congenitally infected and control mice was left unprimed. The 24 h footpad swelling reaction to JEV or SRBC of mice sensitized with the respective antigens 4 and 7 days previously, or in unprimed mice was measured. Congenitally JEV-infected 8-week-old baby mice (*a*) and their mothers (*b*) were compared with age-matched control mice. ▨, JEV; ■, SRBC; □, uninfected control of each group. Each column represents the mean value and standard error for groups of five to seven mice.

obtained. The findings are expressed as the mean values of triplicate tests on six mice. The total number of spleen cells in congenitally infected baby mice was $3{\cdot}7(\pm 1{\cdot}3) \times 10^8$ and $4{\cdot}1(\pm 1{\cdot}7) \times 10^8$ cells while in the controls it was $4{\cdot}0(\pm 1{\cdot}5) \times 10^8$ and $4{\cdot}7(\pm 1{\cdot}5) \times 10^8$ cells at 4 and 8 weeks respectively. The difference was not significant ($P > 0{\cdot}05$), applying Student's *t*-test. Subsequently, the fraction of T lymphocytes was determined by treating the spleen cell suspension with monoclonal anti-Thy 1.2 antiserum (New England Nuclear) and complement (Golub, 1971) and then counting non-viable cells after trypan blue staining. Significant reductions ($P < 0{\cdot}001$) in the percentage of T cells [$24(\pm 3{\cdot}7)\%$ and $28(\pm 2{\cdot}1)\%$] in congenitally

Table 1. *Comparison of leukocyte migration inhibition responses to JEV in congenitally infected mice and their mothers*

Mice	Leukocyte migration inhibition (%)* 2 weeks†	4 weeks†	8 weeks†
Baby mice			
Congenitally infected	64 ± 5·6	52 ± 3·1	46 ± 4·5
Control (primed)	58 ± 7·3	56 ± 7·2	62 ± 9·8
Control‡	12 ± 4·1	14 ± 9	14 ± 8·3
Adult mice (mothers)			
Infected mothers	23 ± 4·5	15 ± 5·1	14 ± 7·2
Control (primed)	62 ± 5·1	60 ± 6·3	64 ± 5·7
Control‡	16 ± 7·2	15 ± 7·9	17 ± 4·3

* LMI of spleen cells was measured as described previously (Mathur *et al.*, 1983*a*); each value represents the mean value (±S.D.) of percentage migration inhibition in the presence of specific antigen, from triplicate tests on cells from five to seven mice.

† Age of congenitally infected mice or age-matched control (baby mice), or time post-partum and age-matched control (adult mice). Age-matched control (i.e. not congenitally infected) baby mice or adult mice were primed with JEV 8 days before the LMI test.

‡ Non-immune control mice.

infected baby mice as compared to the controls [40(±4·7)% and 41(±1)%] were observed at 4 and 8 weeks of age respectively. Total numbers of spleen cells ($5·0 \times 10^8$ to $5·5 \times 10^8$) in the mothers remained unaltered as were the percentages of T lymphocytes [43(±1·1)% to 46(±8·0)%].

The degree of delayed type hypersensitivity (DTH) was assessed by measuring the inflammatory response after footpad challenge of primed and unprimed mice with homologous (JEV) or heterologous (sheep red blood cells; SRBC) antigens. Congenitally infected 8-week-old baby mice and age-matched controls were primed i.p. either with JEV or SRBC. The mice were challenged 4 or 7 days later with the respective antigen by footpad inoculation (10^2 LD_{50} JEV or $2·5 \times 10^8$ SRBC in phosphate-buffered saline). Unprimed mice from congenitally infected and control groups were challenged similarly to measure the basal response. The footpad swelling at 24 h was measured using a dial gauge calliper (Oditest, H.C. Kroeplin GMBH, Messzeugfabrik, F.R.G.). The findings summarized in Fig. 1 (*a*) show that the DTH response against homologous (JEV) as well as heterologous (SRBC) antigens was significantly suppressed in primed, congenitally infected baby mice ($P < 0·001$) as compared to that in controls at both times. It was interesting to note that the DTH response in unprimed congenitally infected baby mice was similar to that of primed mice against JEV and was negligible with SRBC (Fig. 1*a*), indicating the presence of specific antigen-reactive T cells in unprimed congenitally infected mice.

The DTH response was determined simultaneously in mothers of all the above groups of baby mice with or without priming with JEV or SRBC. The data presented in Fig. 1 (*b*) show a significantly lower DTH to JEV but not to SRBC in primed mothers as compared to controls. In unprimed mothers the DTH was present against JEV only.

The cell-mediated immune response as studied by the leukocyte migration inhibition (LMI) test in congenitally infected baby mice of different age groups and in their mothers was compared with that in age-matched controls. The migration patterns of leukocytes were assayed using spleen cell suspensions and inhibition was calculated as described earlier (Mathur *et al.*, 1983*a*). Since peak LMI responses against JEV are present on day 8 after infection (Mathur *et al.*, 1983*a*), observations in controls were recorded on that day. The findings presented in Table 1 show that the LMI in 2-week-old congenitally infected mice was 64%, which is similar to the response observed in primed age-matched controls given JEV i.p. 8 days earlier. At 8 weeks, the level of LMI had declined to 46%. The kinetics of decline are slow compared to that in the primed adult mice studied earlier, where we observed a CMI lasting up to 2 weeks after priming (Mathur *et al.*, 1983*a*). This could be due to constant stimulation by persistent virus. In

the infected mothers, borderline positive values were seen at 2 weeks post-partum; after that the extent of inhibition was insignificant.

A normal LMI after 2 weeks in infected mothers with a depressed DTH response indicates that only certain subpopulations of T lymphocytes were depleted. Such selective depletions of T lymphocytes have been observed in many virus infections (e.g. mouse thymic virus, Cross *et al.*, 1976; dengue virus, Tandon *et al.*, 1979). In human cases of Japanese encephalitis (Chaturvedi *et al.*, 1979), the early response was a leukocytosis with lymphocytopenia which was due to a reduction in the number of T lymphocytes. A significantly higher than normal LMI was seen even in those cases where there was significant lymphocytopenia and reduced counts of T cells. All counts tended to return to normal levels by 4 weeks.

An impairment of the virus-specific cell-mediated immune response in humans has been observed in cytomegalovirus infection of mothers and their congenitally infected children (Gehrz *et al.*, 1977; Reynolds *et al.*, 1979; Starr *et al.*, 1979); CMI to other antigens remained intact. Chong & Mims (1982) reported a depressed DTH response of neonatally cytomegalovirus-infected mice during pregnancy and lactation. A reduction in T cells has been reported in a number of virus infections, e.g. in cytomegalovirus-infected infants (Schauf *et al.*, 1976).

The present study demonstrates that in congenitally infected baby mice (i) the number of T lymphocytes in the spleen is considerably reduced, (ii) the DTH response against viral antigen as well as against SRBC is significantly suppressed and (iii) a significant degree of leukocyte migration inhibition can be demonstrated. The number of suppressor T cells in JEV infection peaks sharply at 3 weeks after priming, as shown by suppression of IgM antibody plaque-forming cells against JEV (Mathur *et al.*, 1983*b*). We have also observed suppressor T cells in JEV-primed mice which suppress the DTH response (A. Mathur *et al.*, unpublished results). Similar observations have been made in influenza (Liew & Russell, 1980) and herpes simplex (Nash *et al.*, 1981) virus infections. Experiments are under way to study the relationship of suppressor cells and the depressed CMI in infected mothers and offspring in the establishment of persistent infection.

JEV-infected mothers show a virus-specific depressed DTH response. We have demonstrated previously that JEV persists in thymus and brown fat of pregnant mice for up to 17 weeks after infection (Mathur *et al.*, 1982). In adult mice, protection against JEV infection involves immune T lymphocytes and IgM antibody but both begin to decline 2 weeks post-infection (Mathur *et al.*, 1983*a*). Since even this transient immune response is not available in pregnant mice, virus persistence may be favoured.

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