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Differential depletion of T lymphocytes in the spleen of dengue virus-infected mice

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Summary. Following the i.c. inoculation of dengue type 2 virus (DV) the spleen weight of infected mice was reduced, as was the proportion of cells killed by ATS and complement (T lymphocytes) in spleen-cell suspensions. In DV-infected mice the mean haemolysin titre, 16 days after i.p. inoculation of 4×10^8 SRBC, was 47 compared with 406 in normal mice and spleen cells from DV-infected mice produced significantly reduced direct GVH reactivity in Parker strain (PS) infant mice. Adoptive transfer of spleen cells obtained from mice given three weekly i.p. doses of DV or a single i.c. dose, suppressed antigen-specific antibody secretion as detected by Jerne plaque technique. This suppression was abrogated by pretreating the transferred cells with ATS and complement. Thus DV selectively depletes T-lymphocyte subpopulations responsible for helper and effector functions and spares suppressor T cells in the spleen of infected mice.

INTRODUCTION

Cell-mediated immune response (CMI) plays an important role in host resistance against a number of viral infections (Allison, 1972). On the other hand some viral infections, both natural and vaccine-induced, are associated with depressed CMI (Notkins, Mergenhagen & Howard 1970; Woodruff & Woodruff, 1975). During studies of host defence mechanisms in dengue type 2 virus (DV)-infected mice, we observed that CMI against the virus does not develop. The reasons to believe this were (i) the failure of adoptively transferred sensitized spleen cells to protect mice against challenge with a small dose of DV; (ii) the failure of anti-thymocyte serum treatment of mice to potentiate DV infection; (iii) the failure of adoptive transfer or reconstitution of immunosuppressed mice by sensitized spleen cells to protect against DV; and (iv) the absence of significant leucocyte migration inhibition in tests using spleen cells from DV infected mice (Chaturvedi, Tandon & Mathur, 1977; Chaturvedi, Tandon, Mathur & Kumar, 1978a). At the same time we observed evidence of suppressor cell activity as shown by the suppression of antigen-specific antibody secretion after transfer of sensitized spleen cells (Chaturvedi *et al.* 1978a). These findings prompted us to investigate various functions of thymus-dependent lymphocytes in the spleen of DV-infected mice. Our findings show differential depletion of the helper and effector T cells with sparing of suppressor T cells in the spleen of DV infected mice.

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MATERIALS AND METHODS

Mice

The mice were 4–6 months old Swiss albino male mice, obtained from the colony of this Department. For graft *v.* host reaction (GVH), 1 day old mice of Parker

strain (PS) were kindly provided by Dr O. P. Babbar, of the Central Drug Research Institute, Lucknow.

Virus

Dengue type 2 virus (DV) was in the form of infected adult mouse brain suspension (Chaturvedi *et al.* 1977; Agrawal, Tandon, Chaturvedi & Kumar, 1978). Mice were inoculated with 10^3 LD_{50} of DV i.c. in doses of 0·03 ml, and were killed in batches of four to five from day 1 to 10. In some of the experiments a small dose of 1–5 LD_{50} was used i.c.

Preparation of antithymocyte serum (ATS)

Antiserum to thymocytes of Swiss mice was prepared in albino rabbits by the method of Levey & Medawar (1966) as described earlier (Chaturvedi *et al.* 1978a).

Weight of spleen

Spleen weights were recorded and expressed in mg/100g body weight.

Preparation of spleen cells

The spleen cells were teased out in MEM and a single cell suspension was obtained. The cells were washed and viable count done by the trypan blue dye exclusion test. In different preparations about 95% cells were viable.

T-cell counts in spleen

The proportion of T cells in the spleen cell suspension was determined by counting the cells killed by incubation with ATS and complement by the technique of Golub (1971) as described elsewhere (Chaturvedi, Tandon & Mathur, 1978b). Sufficient ATS was used to kill all T cells, the amounts being calculated by extrapolation from the cytotoxicity titre curves of the ATS. A further check was made by treating the cells with additional ATS and complement which did not kill additional cells.

Study of T-cell functions

Various functions of the T cells in the spleen of DV infected mice were studied as follows.

Suppressor function. In previous experiments, there was evidence of suppression of antigen-specific antibody secretion as detected by Jerne's haemolytic plaque technique after transfer of sensitized spleen cells obtained after three i.p. doses of DV (Chaturvedi *et al.*, 1978a). These experiments were repeated using one set of sensitized cells after treatment with ATS and complement to deplete T cells. Briefly, groups of mice were immunized by three weekly i.p. injections of 0·5 ml of 20% DV-infected mouse brain suspension. Spleen cells were collected 1 week after the last dose and were divided into two batches. One batch was treated with ATS and complement (Golub, 1971) while the other was treated with saline in place of ATS. The cells were then washed twice. As a control, cells from normal mouse spleen were used. Recipient mice were given 0·5 ml DV 10^3 LD_{50} i.p. followed 48 h later by the adoptive transfer of 10^8 spleen cells i.v. from one of the three groups of donor mice, a fourth group of control mice received virus only. Mice from each group were sacrificed in batches of four to five on day 6 and 7 after DV inoculation. The spleens were collected and direct antibody forming cells against DV were counted by the technique of Jerne & Nordin (1963).

In a second experiment the donor mice were given about 1000 LD_{50} of DV i.c. on day 8, the spleen cells were collected for adoptive transfer and the above experiment was repeated.

Helper function. Mice were given 1–5 LD_{50} of the virus i.c. 10 days later three mice were killed and the weight of spleens were recorded and the cells were tested to confirm that T cells were depleted. The remaining mice of the batch given DV, were challenged with 4×10^8 sheep red blood cells (SRBC) i.p. Normal control mice received similar amounts of SRBC. Mice from both groups were sacrificed on day 16 and the sera were assayed for their haemolysin titre (Cruickshank, 1962).

Graft v. host (GVH) reaction

Single cell suspension of spleen cells was prepared as described above. One day old Parker strain mice were injected i.p. with 10^5, 10^6 or 10^7 donor cells in a volume of 0·05 ml. One set of mice were given spleen cells obtained from DV-infected mice, 8 days after i.c. inoculation of the virus, on which the cytotoxicity test with ATS showed depletion of T cells. Spleen cells from uninoculated normal mice were given to second set of mice and a third set of littermate mice was used as such for control. The mice were killed 9 days later, their spleen indices (SI) were recorded (Eikman & Bowser 1972). Each group consisted of seven to fourteen mice. Spleen indices greater than 1·3 were considered to represent significant GVH reactivity (Simonsen, 1962).

RESULTS

Changes in spleen weight

In control mice, the mean weight of the spleen was 720 ± 65 mg/100g body weight. In DV-inoculated mice the mean weight of the spleen was 637 ± 80 mg/100g body weight on day 1 after i.c. inoculation of the virus. Thereafter spleen weight decreased, with a sharp drop on day 5 and then more gradually, reaching its lowest weight on day 10 when it was 216 ± 52 mg (Fig. 1).

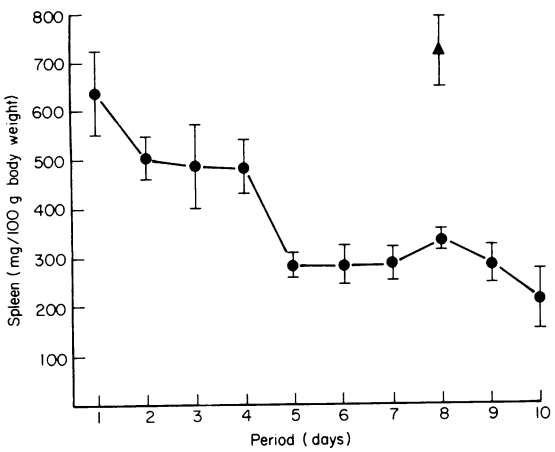

Figure 1. Reduced spleen weight of infected mice after intracerebral inoculation of DV. Each point represents mean value with standard error of the mean, of five to seven DV infected (●) or normal (▲) mice.

Depletion of T cells

Findings summarized in Fig. 2 show the proportion of T cells present in the spleen of mice on various days after i.c. inoculation of DV. In normal mice, the spleen contained 33–42% T cells. In the DV-infected mice, the number of T cells started declining from day 4 (28 ± 4%) but the diminution was more severe from day 8 onwards when the spleen cells contained only 12–15% T cells.

Haemolysin titre

Figure 3 summarizes the haemolysin titre after inoculation of SRBC. The mean haemolysin titre in the controls was 406 while in DV infected it was 47.

Suppressor function

In earlier studies, the peak antibody forming cell response against DV was observed on day 6 or 7 after i.p. inoculation (Tandon & Chaturvedi, 1977; Chaturvedi *et al.*, 1978a), therefore, observations were obtained on these days only. The data presented in Table 1 show that the adoptive transfer of sensitized spleen cells

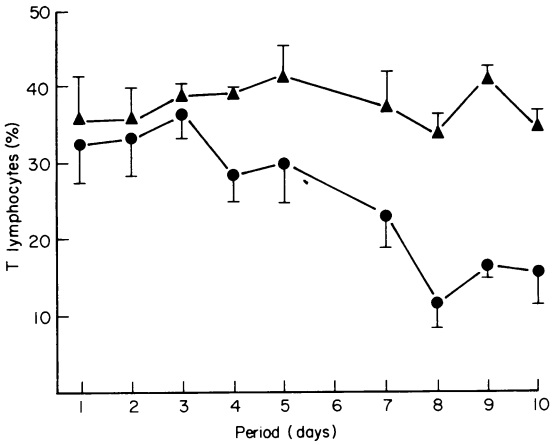

Figure 2. Depletion of T lymphocytes in the spleen of infected mice after intracerebral inoculation of DV. Those spleen cells killed by treatment with ATS and complement were considered T cells. Each point represents mean value, with standard error of the mean of four to seven DV infected (●) or normal (▲) mice.

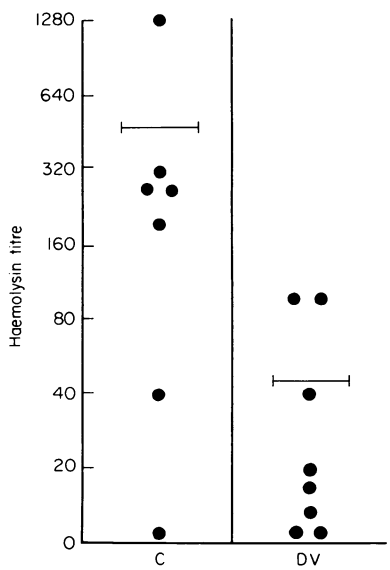

Figure 3. Suppression of haemolysin titre in DV infected mice. 4×10^8 SRBC were injected i.p. in normal control (C) mice, and mice which had received 1–5 LD_{50} of DV i.c. 10 days earlier (DV). The haemolysin titre was assayed 16 days after SRBC inoculation.

Table 1. Suppression of PFC against DV in the spleen by adoptive transfer of sensitized spleen cells.

	PFC (after DV challenge)			
	Day 6		Day 7	
Donor spleen cells*	Number§	Suppression (%)	Number	Suppression (%)
Exp. I: DV i.p.†				
Sensitized cells	296 ± 50	57	259 ± 49	67
ATS-treated sensitized cells	407 ± 24	41	391 ± 43	51
Exp. II: DV i.c.‡				
Sensitized cells	272 ± 50	60	334 ± 29	58
Controls				
Normal cells	530 ± 85	22	638 ± 88	20
No cells	680 ± 98	0	792 ± 7	0

* 10^8 spleen cells were transferred i.v.
† Spleen cells obtained 7 days after three weekly i.p. doses of DV.
‡ Spleen cells obtained 8 days after DV i.c.
§ Number of PFC/2 × 10^6 spleen cells with ± standard error of the mean.

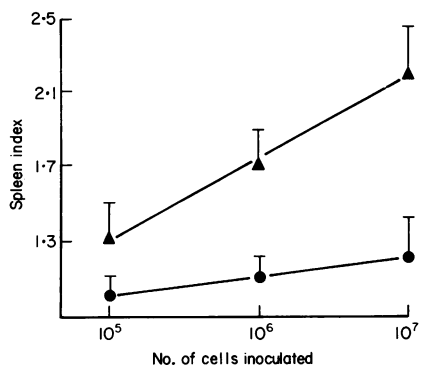

Figure 4. GVH reaction of Parker mice injected with varying numbers of spleen cells from normal or DV-infected Swiss mice which received the virus intracerebrally 8 days earlier. Each point represents mean value of Simonsen spleen index, with standard error of the mean, from seven to eight mice, which received cells from DV infected (●) or normal mice (▲).

from mice given three i.p. doses of DV, suppressed specific antibody forming cells in the spleen of recipient mice as the PFC were 259–296/2 × 10^6 spleen cells compared with the very high count seen in controls. Pretreating the sensitized spleen cells with ATS and complement abrogated their suppressor activity (Table 1); the PFC count was 391–407/2 × 10^6 spleen cells which was significantly higher ($P < 0{\cdot}001$) than that with untreated sensitized cells.

In a second experiment, suppressor activity was also demonstrated in the spleen cells of mice infected with DV i.c., 8 days earlier. The suppression of PFC in the recipient mice was 53–61% (Table 1).

GVH reaction

The reactivity of the spleen cells from normal and DV-infected adult Swiss mice in the infant mice of Parker strain is presented in Fig. 4. Significant splenomegaly with a spleen index exceeding 1·3 was induced by all three doses of normal spleen cells. The capacity of spleen cells from DV-infected mice to produce direct GVH reactivity was significantly reduced, and at all cell doses the spleen index was 1·2 or less.

DISCUSSION

The present study shows that DV infection of mice altered the functions of T cells in the spleen. As the infection progressed, the proportion of T cells diminished in the spleen, with a comparable reduction in spleen weight. This was associated with suppressed T-cell helper function as shown by poor response to a thymus-dependent antigen (SRBC), and impaired capacity of spleen cells from DV-infected mice to produce direct GVH reactivity (Fig. 4). Since lymphoid tissues normally contain both precursor and amplifier T cells participating in GVH reaction, the

later experiment reflects the function of both populations and indeed has been shown to be a good measure of precursor-cell function (Morse & Asofsky, 1974; Cross, Morse & Asofsky, 1976). Absence of leucocyte migration inhibition of the spleen cells of DV-infected mice by specific antigen as reported in an earlier study (Chaturvedi *et al.*, 1978a), shows the lack of yet another T-cell function. At the same time, it is interesting to note that the suppressor T-cell function was not affected during DV infection as shown by the suppression of antigen-specific antibody secretion detected by Jerne's plaque technique after transfer of sensitized spleen cells (Table 1).

Lymphocytopenia and depletion of T cells have been observed in a number of viral infections (reviewed by Woodruff & Woodruff, 1975), but the present study shows that only some parameters of T-cell function are affected, sparing suppressor cells, thus indicating selective destruction of subpopulations of T cells by DV. Another virus infection where selective destruction with sparing of suppressor cells occurs is the mouse thymic virus (Cross *et al.*, 1976). The subpopulations of T cells, *viz.* suppressor and helper cells differ from each other in a number of aspects including sensitivity to irradiation (Taylor & Basten, 1976). These differences may be one of the factors responsible for differential depletion of T cells in DV infection.

In different virus infections, T-cell depletion could be due to transient lymphocytopenia, depletion of lymphocytes in thymus-dependent areas of lymphoid tissue (Woodruff & Woodruff, 1970); changes in T-lymphocyte traffic (Woodruff & Woodruff, 1974; 1975); or destruction of T lymphocytes directly by the virus or through a cytotoxic factor (Snodgrass, Lowrey & Hanna, 1972; Huang, Lattos, Nelson, Reeb & Hong, 1973). After i.c. inoculation, DV replicates in the spleen of mice and is detectable from day 5 onwards (Chaturvedi *et al.*, 1978a). Further, DV replicates in B lymphocytes and cell lines with B-cell characteristics but not in the T lymphocytes or cell lines with T-cell character (Theofilopoulos, Brandt, Russell & Dixon, 1976). These authors did not note any cell destruction but necrosis and haemorrhage in the thymus-dependent areas of lymphoid tissues have been reported in cases of dengue haemorrhagic fever (Aung-Khin, Ma-Ma, Thant-Zin & Tin-U, 1975). Thus the exact mechanism of T-cell depletion in DV infected mice is not known.

Our data show that the lack of CMI, observed in earlier studies on DV-infected mice (Chaturvedi *et al.*, 1977; 1978a) may be due to selective depletion of subpopulations of T cells.

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