Suppression of intrinsic B-cell function in Dengue-infected mice

Mitzi Nagarkatti and Prakash S. Nagarkatti[1]

Department of Microbiology, Defence Research and Development Establishment, Gwalior-474002 (India), 8 January 1979

Summary. Mice infected with Dengue virus show a depressed immune response to lipopolysaccharide (LPS), a helper T-cell-independent antigen, when LPS was administered on day 0, 6 and 12 post infection. Mice injected with inactivated virus failed to show immunosuppression.

Dengue virus is known to infect the lymphoid and reticuloendothelial system[2-4] and cause extensive damage to the lymphoid tissue[5-7]. These effects on the lymphoid system could lead to some alterations in the function of the immune system. To study the repercussion of the infection on the function of T and B lymphocytes, we have infected mice with Dengue virus and studied the immune response to helper T-cell dependent and independent antigens. Our earlier study using sheep red blood cells (SRBC), a helper T-cell dependent antigen, revealed that Dengue-infected mice show a depressed primary and secondary humoral immune response to SRBC as measured by antibody forming cells, haemagglutinin and haemolysin titres. The delayed hypersensitivity reaction (DH) to SRBC was also suppressed[8], indicating that the virus interferes with the function of helper T-cells and/or B cells as well as effector T-cells involved in the DH reaction. The present investigation is a continuation of our earlier work, carried out to study the response of Dengue-infected mice to helper T-cell independent antigen such as lipopolysaccharide (LPS).

Materials and methods. Swiss albino mice, 4 weeks old, were inoculated intracerebrally with 10^5 LD_{50} Dengue virus, type 3, while the control mice received normal brain suspension by the same route. With this dose of the virus, the mice became sick by day 10 but overcame the infection. This is also confirmed by the fact that the virus titres reached a peak around day 12, and thereafter gradually declined and by day 21 the virus was cleared from the brains[8]. The preparation of viral antigen has been described by us in detail elsewhere[9,10].

Formation of specific plaque forming cells (PFC) and antibody response to LPS was determined after the administration of 100 µg of LPS (Difco Laboratories, USA). PFC to LPS was determined by suitably modifying the method of Watson and Riblet[12]. In brief, to 0.5 ml of washed and packed SRBC was added 2.5 ml of LPS (1 mg/ml) and this mixture was incubated at 37 °C for 90 min. The LPS-coated cells were next washed thrice with phosphate-buffered saline (PBS) and used on agar plates as 20% cells. Controls were run with uncoated SRBC and the specific PFC response to LPS was determined by subtracting PFC to uncoated cells from PFC to LPS-coated cells. The LPS was heated for 2 h in boiling waterbath before being used for coating. The antibody titres were determined by coating SRBC with LPS as described above, but were adjusted to 0.5% cells before being used in the indirect haemagglutination (IHA) test, carried out using microtitre equipment as described in our earlier report[11].

Results and discussion. The kinetics of PFC response to LPS in Dengue-infected and control mice are shown in figure 1. On day 0, the infected group received both Dengue virus and LPS, while the controls received normal brain antigen and LPS. The PFC response to LPS was studied on days 6, 7 and 8 post inoculation. The peak PFC response was seen on day 7 in both control and Dengue-infected groups. However, in the Dengue-infected group there was a significantly lower PFC response on day 6 and 7 ($p < 0.001$).

Figure 2 shows the immune response to LPS, when LPS was injected at different stages of infection, on day 0, 6 and 12. The antibody and PFC response was studied 7 days after priming with LPS. In the Dengue-infected group responses to LPS were significantly depressed ($p < 0.05$), when LPS was administered on day 0, 6 and 12 post infection. Control animals which received normal brain antigen were also primed with LPS on day 0, 6 and 12 and the immune response studied. However, as there was no significant difference noticed in the response to LPS on these days, we have represented the control values by depicting only a single mean value in figure 2 corresponding to C on the graph.

The table shows the response to LPS of mice injected with live and UV-inactivated Dengue virus. Unlike the mice injected with live virus, those which received inactivated virus failed to exhibit signs of significant immunosuppression.

The present investigation therefore suggests that Dengue virus infection in mice suppresses the intrinsic B-cell function, as LPS response is known to bypass the T-cell helper

Antibody response to LPS in animals injected with inactivated Dengue virus

Number	Animals	Log_2 antibody titres
1	Control animals	3.87 ± 0.37
2	Inactivated Dengue virus injected	4.34 ± 0.66*

* $p > 0.1$.

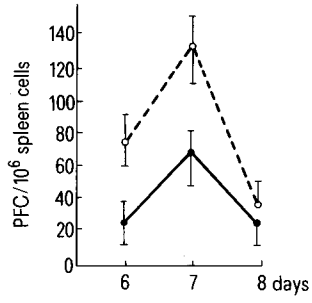

Fig. 1. Kinetics of PFC response to LPS in Dengue infected (●) and control (○) mice. Dengue virus and LPS were injected on day 0.

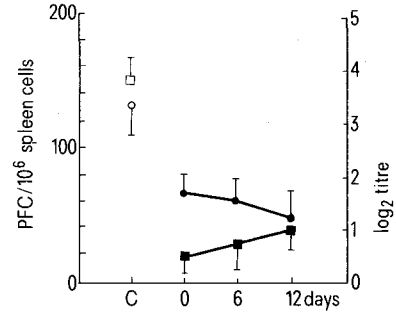

Fig. 2. Antibody (squares) and PFC (circles) response to LPS in Dengue-infected (■, ●) and control (□, ○) mice. Dengue virus was injected on day 0 and LPS on day 0, 6 and 12 and the response studied after 7 days. C represents control values.

function. However, in addition to the B-cell function, the possibility of the virus affecting helper T-cell function cannot be ruled out as, in our earlier study, we have found that the virus suppresses the response to helper T-cell dependent antigen[8]. The present investigation also suggests that the immunosuppression is caused by the active multiplication of the virus and not by the viral antigen alone, since the administration of UV-inactivated virus failed to cause immunosuppression. This finding rules out the possibility in Dengue virus infection of the immunosuppression as noticed in certain viral infections to result from the competition of viral antigen with another antigen in the production of antibody, thereby suppressing the response to the latter antigen[13].

1 We are grateful to Dr P.K. Ramachandran, Defence Research and Development Establishment, Gwalior, Dr B.P. Saxena, and Dr K.M. Rao for the encouragement and sustained interest. Thanks are also due to Mr A. Daniel for the help rendered in preparing the manuscript.

2 S.B. Halstead, H. Shotwell and J.J. Casals, J. infect. Dis. *128*, 15 (1973).
3 N. J. Marchette, S.B. Halstead and W.A. Falkler, Jr, J. infect. Dis. *128*, 23 (1973).
4 A.N. Theofilopoulos, W.E. Brandt, P.K. Russell and F.T. Dixon, J. Immun. *117*, 953 (1976).
5 N. Bhamarapravati, P. Toochinda and V. Boonyapaknavik, Ann. trop. Med. Parasit. *61*, 500 (1967).
6 M. Aung-Khin, Khin, Ma-Ma, Thant-Zin and M. Tinu, J. trop. Med. Hyg. *78*, 256 (1975).
7 M.B. D'Souza, P.S. Nagarkatti and K.M. Rao, J. Hyg. Epidem. Microbiol. Immun., in press (1979).
8 P.S. Nagarkatti, M. Nagarkatti and K.M. Rao, Int. Archs Allergy appl. Immun., in press (1979).
9 P.S. Nagarkatti, M.B. D'Souza and K.M. Rao, Indian J. exp. Biol. *16*, 10 (1978).
10 P.S. Nagarkatti, M.B. D'Souza and K.M. Rao, J. immun. Meth. *23*, 341 (1978).
11 M.B. D'Souza, P.S. Nagarkatti and K.M. Rao, J. trop. Med. Hyg. *81*, 142 (1978).
12 J. Watson and R. Riblet, J. exp. Med. *140*, 1147 (1974).
13 J.E. Osborn, A.A. Blazkovec and D.L. Walker, J. Immun. *100*, 835 (1968).

Alteration of early T lymphocyte count in patients with herpes genitalis[1]

T. Ishiguro, Y. Ozaki, M.M. Yokoyama, W. Chao and C.H. Tseng

Department of Obstetrics and Gynecology and Department of Microbiology, Shiga University of Medical Science, Shiga (Japan), and Department of Pathology and Microbiology, University of Illinois College of Medicine, Chicago (Illinois 60680, USA), 15 February 1979

Summary. Peripheral blood samples from 52 women, including 16 with herpes genitalis and 36 healthy persons, were studied to enumerate subpopulations of lymphocytes. It was found that the mean percentage of 'active' T lymphocytes was significantly less in the patients with herpes genitalis than in the controls.

Progenital herpes or cervical herpes is one of the most common infectious diseases in female genitalia and is caused by herpes simplex virus type 1 or type 2 (HSV-1 or HSV-2). Current studies suggest that cell-mediated immunity may play an important role in recovery from herpetic infection. The present study was designed to assess whether cellular immunity is depressed in patients with herpes genitalis, utilizing determination of T and B lymphocyte subpopulations in their peripheral blood.

Materials and methods. A total of 52 Japanese women, 16 with herpes genitalis and 36 healthy persons, were studied. Herpetic involvement was confirmed by viral isolation, exfoliative cytological examination or both. All patients received serological examination for HSV antibodies by the methods described previously[2]. 4 of the 16 sera had no antibodies against either HSV-1 or HSV-2 and were designated as primary infections. The remaining 12 sera had demonstrable antibodies against either virus and were considered to be secondary infections, although 11 of these patients had no previous history of herpes genitalis.

Lymphocytes were isolated from freshly drawn heparinized peripheral blood by Ficoll-Hypaque density gradient centrifugation[3], and the cells were adjusted to a final concentration of 2×10^6/ml in PBS containing 0.1% bovine serum albumine (PBS-BSA).

'Active' T lymphocyte rosette assay was done by the method described by Wybran and Fudenberg[4]. 'Total' T lymphocytes were determined by the method described by Jondal et al.[5].

'Total' B lymphocytes were assayed by immunobead rosette tests described previously[6,7] using polyacrylamide beads coated with anti-human immunoglobulin light chains ($\varkappa$ and λ). Immunobeads were obtained from Bio Rad · Laboratories (Richmond, California). Each bead population was suspended in PBS to give a concentration of 1×10^8 beads/ml.

Results. Normal percentages of total T lymphocytes were encountered in the patient group, whereas mean percentages of active T lymphocytes were less than those of the healthy controls, and the difference of active T lymphocyte counts between the 2 groups was statistically significant ($p < 0.001$) as shown in table 1. 7 of 16 (43.8%) with herpes genitalis had lower percentages of active T lymphocytes than the lowest limit of normal controls (normal range: 12.0–41.5%).

Mean percentages of total B lymphocytes are shown in table 2. The patient group showed no differences from controls in the percentages of total B lymphocytes.

Table 1. 'Active' and 'total' T lymphocytes in patients with herpes genitalis and in healthy women

	T lymphocytes (%)			
	Active	p	total	p
Healthy women	25.0±8.3		62.8±7.6	
Patients	13.9±6.8	<0.001	62.2±7.9	n.s.

Table 2. Total B lymphocytes in patients with herpes genitalis and in healthy women

	Total B lymphocyte (%)	p
Healthy women	14.0±4.2	
Patients	12.8±3.9	n.s.