J. gen. Virol. (1983), **64**, 2399–2407. *Printed in Great Britain*

Key words: *dengue virus/Fc-receptor/macrophage/phagocytosis*

Effect of Dengue Virus Infection on Fc-receptor Functions of Mouse Macrophages

By U. C. CHATURVEDI,* RACHNA NAGAR AND ASHA MATHUR

Virus Laboratory, Postgraduate Department of Pathology & Bacteriology, K.G. Medical College, Lucknow – 226 003, India

(*Accepted 30 June 1983*)

SUMMARY

Fc-receptor-mediated attachment and ingestion of opsonized sheep erythrocytes (EA) by the macrophages of spleen and peritoneal cavity were studied during dengue virus type 2 (DV) infection of Swiss albino mice. Following intracerebral inoculation, virus antigen could be demonstrated by immunofluorescence in the splenic macrophages from day 4 and in peritoneal macrophages from day 5 post-infection, with a higher number of positive cells discernible on the 7th and 8th days. The virus could be isolated from spleen tissue from day 5. The total number of cells was markedly reduced from day 4 onwards both in the spleen and peritoneal cavity. A loss in the capacity to attach and ingest EA was noticed, the lowest values of attachment index (AI) and phagocytic index (PI) being reached on day 4. At later periods the AI values increased markedly but continued to be significantly less than those in uninfected control mice. The PI values continued to be lower throughout. The dichotomy between the Fc-mediated attachment and ingestion may be a mechanism for prevention of virus infection of macrophages.

INTRODUCTION

Evidence accumulated during recent years indicates that the cells of the macrophage–monocyte phagocytic system are the cells that principally support replication of dengue virus in man, monkey and mouse. In man, studies have been carried out mainly on isolates from cases with haemorrhage/shock. Dengue virus has been recovered from the peripheral blood monocytes (Scott *et al.*, 1980); shown by electron microscopy in macrophages of glomeruli (Boonpucknavig *et al.*, 1976); and by a fluorescent antibody technique in the mononuclear cells of skin rash (Boonpucknavig *et al.*, 1979), Kupffer cells of liver, and macrophages of thymus, spleen and lungs of such cases (see Halstead, 1981*a*). The virus also replicates in blood phagocytic cells cultured *in vitro* (Halstead *et al.*, 1977). Similar observations have been made in rhesus monkeys with respect to virus isolation from blood monocytes, replication in cultures of spleen, thymus and lymph node macrophages and the distribution of virus antigen in macrophages of different organs (Marchette *et al.*, 1976; Halstead *et al.*, 1977; Halstead & O'Rourke, 1977; Halstead, 1981*a*). In the mouse, dengue virions have been demonstrated in macrophages of spleen (Nath *et al.*, 1983) and virus antigen has been demonstrated in Kupffer cells, and in macrophages of the spleen and peritoneal cavity (Hotta *et al.*, 1981 *a*, *b*). Replication of dengue virus in human (Brandt *et al.*, 1981) and mouse macrophage cell lines (see Halstead, 1982) has been reported. On the other hand, the phagocytic and migratory functions of macrophages are adversely affected in dengue virus-infected mice due to the production of a cytotoxic factor (CF) in the spleen (Gulati *et al.*, 1982) which also suppresses the macrophage functions of human blood leukocytes *in vitro* (Chaturvedi *et al.*, 1982*a*) and induces mouse macrophages to produce a cytotoxin (Gulati *et al.*, 1983*a*, *b*, *c*). The role of macrophages in various immune phenomena in dengue virus infection have been described (Chaturvedi *et al.*, 1981*b*, 1982*b*;

* Address for correspondence: Medical College Quarters, 50, Jagat Narain Road, Lucknow – 226 003, India.

0022-1317/83/0000-5753 $02.00

Shukla & Chaturvedi, 1982, 1983). Due to the extensive involvement of macrophages in dengue virus infection it has been termed 'Macrophagitis' (Halstead, 1981*b*).

Recently the mechanism of macrophage infection by dengue virus has been studied in some detail by Russell and co-workers. They have suggested that the virus can infect monocytes in one of two ways: through a trypsin-sensitive virus receptor or through a trypsin-insensitive Fc-receptor (Russell, 1981; Daughaday *et al.*, 1981). Immune enhancement of dengue virus infection by a non-cytophilic antibody, as shown by Halstead (1981*b*), occurs through Fc-receptors of monocytes. The present study was undertaken to investigate Fc-receptor-mediated functions of macrophages during dengue virus infection of mice.

METHODS

Mice. The inbred Swiss albino mice used were about 4 months old and were obtained from the colony maintained in this Department.

Virus. Dengue virus (DV) type 2 was in the form of adult mouse brain suspension. The virus was titrated in mice by intracerebral (i.c.) inoculation of tenfold dilutions of a 10% (wet w/v) suspension of the organ and the titres have been expressed as $\log_{10}$ LD_{50} per g wet weight (Chaturvedi *et al.*, 1978*a*). The details of the virus and the DV-infected mouse experimental model have been described (Chaturvedi *et al.*, 1977, 1978*a*). In all the experiments reported, a dose of 1000 LD_{50} i.c. of the virus was used.

Fluorescent antibody study of DV antigen. Smears prepared from the spleen and peritoneal cells were fixed in chilled acetone, then treated with virus-specific antisera and prepared for indirect fluorescent antibody study (Gardner & McQuillin, 1981). The smears were examined in transmitted light using a Leitz Dialux 20 fluorescence microscope.

Peritoneal macrophages. The peritoneal cavities of mice were lavaged with 5 ml heparinized medium containing antibiotics and the resident cells were collected. A portion of the cell suspension was used for total cell count and for preparation of smears which were stained with Leishman's stain for differential cell counts (Dacie & Lewis, 1977). The remaining cell suspension was washed once and cultured on 20 mm by 20 mm coverslips placed in Petri dishes. The medium consisted of Eagle's minimal essential medium (MEM) buffered with HEPES and containing 10% foetal calf serum. The cultures were incubated in a humidified incubator at 37 °C in the presence of 5% CO_2. After 2 h the coverslips were flushed with medium to remove non-adherent cells. The glass-adherent cells were considered to be macrophages on the basis of phagocytosis of neutral red dye and latex particles and the morphology of the cells. Monolayers of these cells were also tested for rosetting and phagocytosis of opsonized sheep erythrocytes, which were prepared by the technique of Stuart *et al.* (1978).

Splenic macrophages. A single-cell suspension of spleen was prepared (Chaturvedi *et al.*, 1978*b*). The cells were cultured on coverslips placed in Petri dishes as described for peritoneal macrophages and glass-adherent cell monolayers were obtained for further testing.

Tests for attachment and phagocytosis of sheep erythrocytes. Monolayers of macrophages cultured on coverslips for 2 h were washed and incubated with fresh haemolysin-coated or control sheep erythrocytes, at about 100 red blood cells per macrophage, for 1 h at 37 °C with frequent gentle shaking. The coverslips were then washed thoroughly with Hanks' balanced salt solution then air-dried, fixed in methanol and stained with Leishman's stain (Dacie & Lewis, 1977). The numbers of red blood cells (RBC) bound to each macrophage were counted in 100 or more macrophages on duplicate coverslips. The attachment index (AI) is expressed as the mean number of erythrocytes attached to 100 macrophages. Similarly, the red blood cells ingested by the macrophages were counted and the number of cells ingested by each macrophage was scored. The phagocytic index (PI) was calculated and is expressed as the mean number of red blood cells ingested per 100 macrophages (Bianco, 1976) as AI or PI = total no. of RBC attached or ingested × 100/total no. of macrophages − no. of negative macrophages.

Plan of study. The effect of DV infection on Fc-mediated sheep erythrocyte attachment and their phagocytosis by peritoneal and splenic macrophages was studied. Mice were inoculated with 1000 LD_{50} of DV i.c. and were sacrificed in groups of 6 to 12 at days 1 to 10 post-infection. At autopsy the peritoneal and splenic cells were collected from individual mice, macrophage monolayers were prepared, and the above tests were done in duplicate on cells from each mouse. The mean values ± standard deviation are presented after subtraction of background attachment or ingestion of non-opsonized RBC. The data were analysed using Student's *t*-test. A probability (*P*) value of less than 0·05 was considered significant.

RESULTS

Mice were inoculated with DV i.c. and various parameters were studied daily up to day 10 after infection. The mice remained apparently healthy up to the 5th day and then became seriously ill with development of hind limb paralysis from the 8th day, and all had died by day 10.

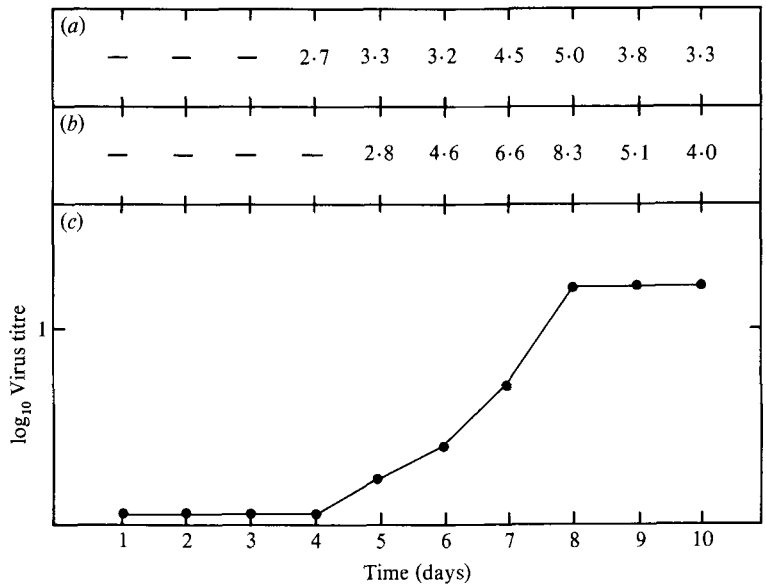

Fig. 1. Demonstration of the presence of virus at different days after inoculation of DV i.c. in mice. (*a*) Virus antigen-positive immunofluorescence cells per 10^3 cells in spleen macrophages; (*b*) virus antigen-positive immunofluorescence in peritoneal macrophages; (*c*) virus titre in the spleen.

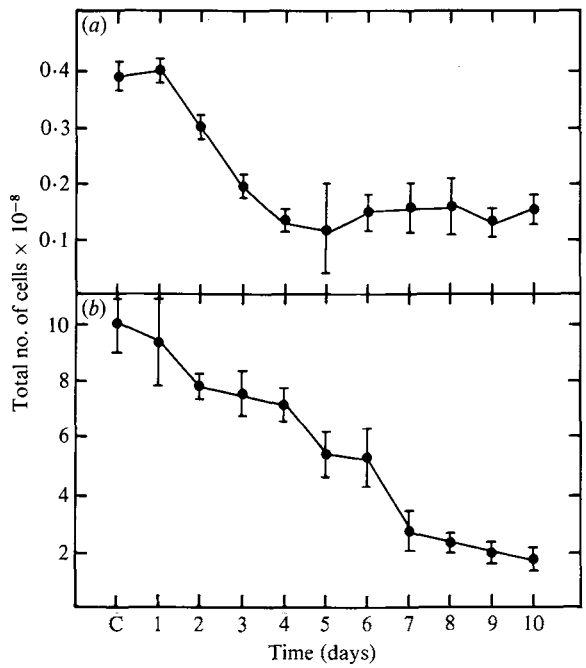

Fig. 2. Total number of cells in the peritoneal cavity (*a*) and spleen (*b*) of mice at different days after inoculation of DV i.c. C, Uninfected control.

Fluorescent antibody study of macrophages for DV antigen

Smears prepared daily were screened for the presence of dengue virus antigen in glass-adherent cells. The spleen cells showed DV antigen from day 4 (Fig. 1*a*) and the peritoneal macrophages from day 5 post-infection (Fig. 1*b*) with maximum numbers of cells showing antigen on day 8.

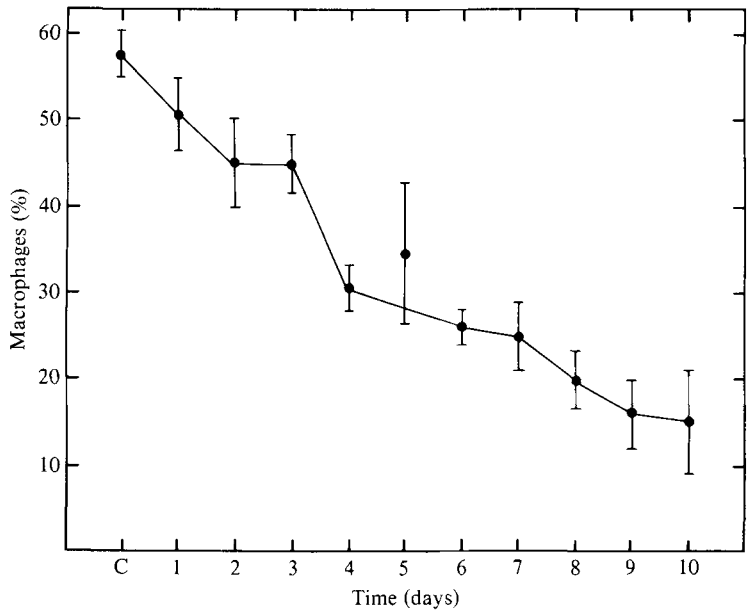

Fig. 3. Percentage of macrophages in the peritoneal cavity of the mice at different days after DV inoculation i.c. C, Uninfected control.

Table 1. *Attachment of antibody-coated sheep red blood cells (EA) to peritoneal and splenic macrophages**

	Number of EA per macrophage					
	Peritoneal macrophages			Splenic macrophages		
Day post-infection	0	1–3	>4	0	1–3	>4
1	44 ± 10	27 ± 14	28 ± 19	48 ± 9	36 ± 14	14 ± 6
2	29 ± 14	25 ± 10	46 ± 14	71 ± 15	19 ± 11	10 ± 5
3	29 ± 10	29 ± 6	42 ± 12	79 ± 6	13 ± 8	8 ± 1
4	53 ± 10	32 ± 11	15 ± 9	88 ± 8	6 ± 2	6 ± 0·4
5	41 ± 15	30 ± 12	29 ± 10	63 ± 11	25 ± 10	12 ± 5
6	30 ± 11	30 ± 7	40 ± 11	41 ± 11	30 ± 15	29 ± 15
7	43 ± 9	28 ± 9	29 ± 13	63 ± 14	25 ± 11	12 ± 6
8	29 ± 9	29 ± 13	42 ± 9	55 ± 27	25 ± 15	20 ± 16
9	28 ± 9	27 ± 8	44 ± 13	65 ± 13	20 ± 9	13 ± 5
10	27 ± 5	29 ± 11	44 ± 10	50 ± 4	31 ± 9	18 ± 8
Uninfected (control)	7 ± 2	13 ± 10	80 ± 8	39 ± 7	39 ± 10	22 ± 4

* Macrophages from spleen or peritoneum were cultured for 2 h and then assayed for binding capacity. Values represent the mean percentage of cells that bind the given number of EA ± S.D. from duplicate tests on 6 to 12 mice at each period.

Virus titre in spleen

Spleens collected from two mice daily were homogenized individually and titrated for DV. The data presented in Fig. 1(*c*) show that the maximum titre reached was 1·5 $\log_{10}$ LD_{50} and was found on 8 to 10 days post-infection.

Effect on total number of cells

The data summarized in Fig. 2(*a*) show a gradual decline in the total number of cells that could be lavaged from the peritoneal cavity, reaching the lowest value on the 5th day. The data

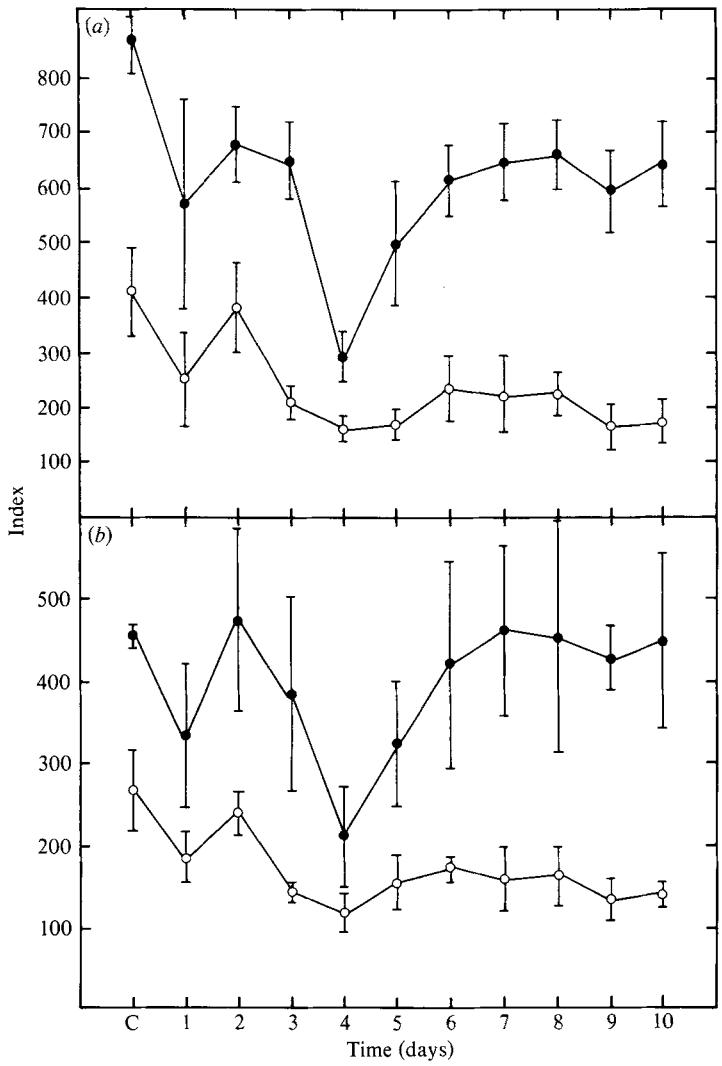

Fig. 4. Attachment index (●) and phagocytic index (○) of (*a*) the peritoneal and (*b*) splenic macrophages at different days after inoculation of DV i.c. C, Uninfected control.

presented in Fig. 3 show that in control mice 58 (±10)% of resident peritoneal cells were macrophages on the basis of morphological appearance and phagocytosis of latex particles. The proportion of macrophages gradually decreased as the infection progressed and in moribund mice on the 10th day 15 (±6)% of the cells were macrophages. A similar decline in the total number of spleen cells was observed, reaching the lowest value on day 10 (Fig. 2*b*). In control mice 12 (±4)% of spleen cells were macrophages; this proportion declined to 3 (±1·5)% on day 10 post-infection.

Effect on Fc-mediated attachment by macrophages

The pattern of Fc-mediated attachment of EA at different periods is presented in Table 1. In control mice the proportion of cells binding four or more erythrocytes was 80 (±8)%. As the infection progressed a significant proportion of the macrophages did not attach any EA, with consequent decrease in the number of cells binding > 4 EA, with the lowest values on day 4. No marked alterations were seen in the proportion of cells attaching one to three EA. A similar pattern was seen with splenic macrophages (Table 1).

Table 2. *Phagocytosis of antibody-coated sheep red blood cells (EA) by peritoneal and splenic macrophages**

Day post-infection	Number of EA per macrophage: Peritoneal macrophages 0	Peritoneal macrophages 1–3	Peritoneal macrophages >4	Splenic macrophages 0	Splenic macrophages 1–3	Splenic macrophages >4
1	61 ± 17	29 ± 8	9 ± 4	75 ± 14	27 ± 15	1 ± 1
2	63 ± 9	24 ± 8	11 ± 5	83 ± 7	16 ± 8	0
3	69 ± 12	27 ± 6	3 ± 2	83 ± 5	16 ± 6	1·2 ± 1
4	68 ± 11	32 ± 13	4 ± 3	83 ± 5	16 ± 8	0·5 ± 0·5
5	54 ± 17	40 ± 18	6 ± 5	76 ± 14	22 ± 11	2 ± 1
6	55 ± 22	38 ± 11	6 ± 3	76 ± 9	22 ± 9	0
7	63 ± 17	29 ± 13	7 ± 6	83 ± 7	15 ± 7	1·4 ± 1
8	78 ± 20	19 ± 8	3 ± 1	71 ± 17	25 ± 16	4 ± 3
9	66 ± 14	27 ± 9	5 ± 4	80 ± 4	17 ± 4	2·5 ± 2
10	62 ± 10	29 ± 11	11 ± 7	87 ± 17	12 ± 3	1 ± 1
Uninfected (control)	37 ± 2	41 ± 11	22 ± 9	58 ± 3	35 ± 8	7 ± 4

* Macrophages from spleen or peritoneum were cultured for 2 h and then assayed for ingestion ability. Values represent the mean percentage of cells that ingest the given number of EA ± S.D. from duplicate tests on 6 to 12 mice at each period.

Data for the attachment index of splenic and peritoneal macrophages have been summarized in Fig. 4. It was observed that after an initial fall in the value of the AI at day 1, the values increased with a sharp decline on day 4. At later periods the value of AI increased but remained well below the control values.

Effect on Fc-mediated phagocytosis by macrophages

The findings presented in Table 2 show that in DV-infected mice a large proportion of splenic and peritoneal macrophages did not ingest any EA and there was no improvement in the phagocytic activity of the cells as the infection progressed.

Fig. 4 presents the phagocytic index of peritoneal and splenic macrophages which show a pattern similar to that of Fc-mediated attachment at initial periods, with the lowest values seen on day 4.

DISCUSSION

The findings presented here show a sharp decline in the Fc-receptor-mediated attachment and phagocytosis of EA by peritoneal macrophages of mice given DV i.c., reaching lowest values 4 days after infection. Similar findings were observed with splenic cells. At later periods the ability of macrophages to attach EA recovered quickly but significantly less than the controls ($P < 0{\cdot}001$). The phagocytic activity continued to be suppressed throughout. A marked reduction of macrophage migration from capillary tubes and a reduction in phagocytosis of neutral red dye and latex particles have been observed from the 4th day in DV-infected mice (Gulati *et al.*, 1982). A marked reduction in total number of splenic and peritoneal cells was recorded as the virus infection progressed, reaching lowest values 9 or 10 days after infection. This can be compared with the reduction in weight of the spleen, and in the reduced percentage of splenic T lymphocytes earlier reported by Tandon *et al.* (1979). A significant reduction in the proportion of macrophages in peritoneal and splenic cells was also observed in the present study.

Increase in Fc-receptor-mediated activity can be induced by various mechanisms. Herpes simplex virus type 1 and cytomegalovirus induce formation of a virus glycoprotein which acts as Fc-receptor, even on non-phagocytic cells (Westmoreland & Watkins, 1974; Keller *et al.*, 1976; Westmoreland *et al.*, 1976; Para *et al.*, 1980). Another mechanism is mediated by interferon, as seen in lactate dehydrogenase-elevating virus and Newcastle disease virus infection of mice

(Hamburg *et al.*, 1978; Lussenhop *et al.*, 1982). There is no evidence that dengue virus is a good inducer of interferon. Besides this, the results of several experiments show that the migratory and phagocytic functions of DV-infected mice are significantly suppressed (Gulati *et al.*, 1982). Further, interferon enhances both rosette formation and phagocytosis of EA by macrophages but the data presented here show enhanced rosetting at later periods with suppressed phagocytosis of EA. Therefore, increased Fc-mediated attachment does not appear to be due to interferon production. It is also not due to increased stickiness of the surface of the cells, as they did not attach a greater number of non-opsonized control RBC.

Functional defects, including impaired phagocytosis by murine macrophages, have been observed in influenza, Sendai and cytomegalovirus infections (Sawyer, 1969; Warr & Jakab, 1979; Jakab *et al.*, 1980; Shanley & Pesanti, 1982). In Sendai virus-infected mice maximum suppression of Fc-mediated ingestion of EA by alveolar macrophages is seen 7 days after virus infection (Warr *et al.*, 1979), whereas in DV-infected mice it is seen on the 4th day. The initial drop in AI and PI at 24 h after DV inoculation i.c. is similar to that seen at 18 h in mice given Newcastle disease virus intravenously (Donahoe & Huang, 1976). In the later case, however, phagocytic activity recovered in the next 16 h and remained at higher levels.

Oliver & Berlin (1976) have suggested that the functional assay for Fc-receptor activity is not simply a measure of the existence of receptors, but also of their ability to move within the cell surface. Consequently the apparent loss of Fc-receptors could be due to their effective immobilization and the increase could be the result of movement of Fc-receptors in clusters. Findings similar to those observed in the present study have been observed in mice given dengue virus-induced cytotoxic factor intravenously (Nagar *et al.*, 1983). We have observed that the cytotoxic factor (CF) kills all susceptible splenic cells *in vitro* (Chaturvedi *et al.*, 1980*a*, *b*, 1981*a*) and the cells that are not killed are adversely affected functionally (Chaturvedi *et al.*, 1981*b*, 1982*a*; Gulati *et al.*, 1982). The defective functioning of macrophages from the 4th day after DV infection in the present study is associated with the presence of CF in the spleens and sera of mice (Chaturvedi *et al.*, 1980*a*). Therefore, the sharp drop in AI and PI values may be due to killing or functional impairment of susceptible cells.

The mechanism by which DV infects macrophages includes the ingestion of virus–antibody complexes through Fc-receptors. DV-immune complexes become irreversibly attached to the monocyte surface within 5 min and the attached virus can be neutralized up to 30 min (Halstead & O'Rourke, 1977; Halstead, 1982). In the absence of phagocytosis, DV may continue to remain attached to the cell surface and thus exposed to the action of neutralizing antibody. Further studies are needed to elucidate this point.

The study has been carried out with the financial assistance of the Indian Council of Medical Research, New Delhi. We sincerely thank the Alexander von Humboldt Foundation, Bonn, F.R.G. for the gift of the Leitz Fluorescent Microscope. R.N. is grateful to Dr K. C. Pandey for guidance. We are grateful to Mrs H. Dalakoti for the initiation of the study and for preparation of the figures.

REFERENCES

BIANCO, C. (1976). Methods for the study of macrophage Fc and C_3 receptors. In *In Vitro Methods in Cell-mediated and Tumor Immunity*, p. 407. Edited by B. R. Bloom & J. R. David. New York: Academic Press.

BOONPUCKNAVIG, V., BHAMARAPRAVATI, N., BOONPUCKNAVIG, S., FUTRAKUL, P. & TANPAICHITR, P. (1976). Glomerular changes in dengue hemorrhagic fever. *Archives of Pathology* **100**, 206–212.

BOONPUCKNAVIG, S., BOONPUCKNAVIG, V., BHAMARAPRAVATI, N. & NIMMANNITYA, S. (1979). Immunofluorescence study of skin rash in patients with dengue hemorrhagic fever. *Archives of Pathology* **103**, 463–466.

BRANDT, W. E., MCCOWN, J. M., GENTRY, M. K. & RUSSELL, P. K. (1981). Immune enhancement of dengue 2 virus replication in the U-937 human monocyte line by cross-reactive monoclonal antibodies (Abstract 4735). *Federation Proceedings* **40**, 1065.

CHATURVEDI, U. C., TANDON, P. & MATHUR, A. (1977). Effect of immunosuppression on dengue virus infection in mice. *Journal of General Virology* **36**, 449–458.

CHATURVEDI, U. C., TANDON, P., MATHUR, A. & KUMAR, A. (1978*a*). Host defence mechanisms against dengue virus infection of mice. *Journal of General Virology* **39**, 293–302.

CHATURVEDI, U. C., TANDON, H. O. & MATHUR, A. (1978*b*). Control of *in vitro* spread of Coxsackie virus B4 infection by sensitized spleen cells and antibody. *Journal of Infectious Diseases* **138**, 181–190.

CHATURVEDI, U. C., BHARGAVA, A. & MATHUR, A. (1980*a*). Production of cytotoxic factor in the spleen of dengue virus-infected mice. *Immunology* **40**, 665–671.

CHATURVEDI, U. C., DALAKOTI, H. & MATHUR, A. (1980*b*). Characterization of the cytotoxic factor produced in the spleen of dengue virus-infected mice. *Immunology* **41**, 387–392.

CHATURVEDI, U. C., MATHUR, K. R., GULATI, L. & MATHUR, A. (1981*a*). Target lymphoid cells for the cytotoxic factor produced in the spleen of dengue virus-infected mice. *Immunology Letters* **3**, 13–16.

CHATURVEDI, U. C., SHUKLA, M. I., MATHUR, K. R. & MATHUR, A. (1981*b*). Dengue virus-induced cytotoxic factor suppresses immune response of mice to sheep erythrocytes. *Immunology* **43**, 311–316.

CHATURVEDI, U. C., GULATI, L. & MATHUR, A. (1982*a*). Inhibition of E-rosette formation and phagocytosis by human blood leucocytes after treatment with the dengue virus-induced cytotoxic factor. *Immunology* **45**, 679–685.

CHATURVEDI, U. C., SHUKLA, M. I. & MATHUR, A. (1982*b*). Role of macrophages in the transmission of dengue virus-induced suppressor signal to a subpopulation of T-lymphocytes. *Annales d'Immunologie* **133C**, 83–96.

DACIE, J. V. & LEWIS, S. M. (1977). In *Practical Haematology*, 5th edn., pp. 68–83. Edinburgh: E.L.B.S. and Churchill Livingstone.

DAUGHADAY, C., BRANDT, W. E., MCCOWN, J. M. & RUSSELL, P. K. (1981). Evidence for two mechanisms of dengue virus infection of adherent human monocytes: trypsin-sensitive virus receptors and trypsin-resistant immune complex receptors. *Infection and Immunity* **32**, 469–473.

DONAHOE, R. M. & HUANG, K.-Y. (1976). Interferon preparations enhance phagocytosis *in vivo*. *Infection and Immunity* **13**, 1250–1257.

GARDNER, P. S. & MCQUILLIN, J. (1981). Rapid virus diagnosis. In *Applications of Immunofluorescence*, 2nd edn., pp. 56–91. London: Butterworths.

GULATI, L., CHATURVEDI, U. C. & MATHUR, A. (1982). Depressed macrophage functions in dengue virus-infected mice: role of the cytotoxic factor. *British Journal of Experimental Pathology* **63**, 194–202.

GULATI, L., CHATURVEDI, U. C. & MATHUR, A. (1983*a*). Dengue virus-induced cytotoxic factor induces macrophages to produce a cytotoxin. *Immunology* **49**, 121–130.

GULATI, L., CHATURVEDI, U. C. & MATHUR, A. (1983*b*). Characterization of the cytotoxin produced by macrophages in response to dengue virus-induced cytotoxic factor. *British Journal of Experimental Pathology* **64**, 192–197.

GULATI, L., CHATURVEDI, U. C. & MATHUR, A. (1983*c*). Plasma membrane-acting drugs inhibit the effect of dengue virus-induced cytotoxic factor on target cells. *Annales d'Immunologie* **134C**, 227–234.

HALSTEAD, S. B. (1981*a*). The pathogenesis of dengue. Molecular epidemiology in infectious disease. *American Journal of Epidemiology* **114**, 632–648.

HALSTEAD, S. B. (1981*b*). Immune enhancement of dengue infection: research questions. In *Proceedings of the First ICMR Seminar on Dengue Haemorrhagic Fever* (1980, Kobe, Japan), pp. 115–127. Edited by S. Hotta. Kobe: International Centre for Medical Research, Kobe University School of Medicine.

HALSTEAD, S. B. (1982). Immune enhancement of viral infection. *Progress in Allergy* **31**, 301–364.

HALSTEAD, S. B. & O'ROURKE, E. J. (1977). Dengue viruses and mononuclear phagocytes: infection enhancement by non-neutralizing antibody. *Journal of Experimental Medicine* **146**, 201–217.

HALSTEAD, S. B., O'ROURKE, E. J. & ALLISON, A. C. (1977). Dengue viruses and mononuclear phagocytes. II. Identity of blood and tissue leucocytes supporting *in vitro* infection. *Journal of Experimental Medicine* **146**, 218–229.

HAMBURG, S. I., MANEJIAS, R. E. & RABINOVITCH, M. (1978). Macrophage activation: increased ingestion of IgG-coated erythrocytes after administration of interferon inducers to mice. *Journal of Experimental Medicine* **147**, 593–598.

HOTTA, H., MURAKAMI, I., MIYASAKI, K., TAKEDA, Y., SHIRANE, H. & HOTTA, S. (1981*a*). Inoculation of dengue virus into nude mice. *Journal of General Virology* **52**, 71–76.

HOTTA, H., MURAKAMI, I., MIYASAKI, K., TAKEDA, Y., SHIRANE, H. & HOTTA, S. (1981*b*). Experimental dengue virus infection using athymic nude mice. In *Proceedings of the First ICMR Seminar on Dengue Haemorrhagic Fever* (1980, Kobe, Japan), pp. 151–158. Edited by S. Hotta. Kobe: International Centre for Medical Research, Kobe University School of Medicine.

JAKAB, G. J., WARR, G. A. & SANNES, P. L. (1980). Alveolar macrophage ingestion and phagosome–lysosome fusion defect associated with virus pneumonia. *Infection and Immunity* **27**, 960–968.

KELLER, R., PEITCHEL, R., GOLDMAN, J. N. & GOLDMAN, N. (1976). An IgG-Fc-receptor induced in cytomegalovirus-infected human fibroblasts. *Journal of Immunology* **116**, 772–777.

LUSSENHOP, N., HOLMES, B., CAFRUNY, W. A. & PLAGEMANN, P. G. W. (1982). Acute infection of mice with lactate dehydrogenase-elevating virus enhances Fc and complement receptor activity of peritoneal macrophages. *Journal of General Virology* **61**, 25–32.

MARCHETTE, N. J., HALSTEAD, S. B. & CHOW, J. S. (1976). Replication of dengue viruses in cultures of peripheral blood leucocytes from dengue-immune rhesus monkeys. *Journal of Infectious Diseases* **133**, 274–282.

NAGAR, R., CHATURVEDI, U. C. & MATHUR, A. (1983). Effect of dengue virus-induced cytotoxic factor on Fc-receptor functions of mouse macrophages. *British Journal of Experimental Pathology* **64** (in press).

NATH, P., TANDON, P., GULATI, L. & CHATURVEDI, U. C. (1983). Histological and ultrastructural study of spleen during dengue virus infection of mice. *Indian Journal of Medical Research* **78**, 83–90.

OLIVER, J. M. & BERLIN, R. D. (1976). Macrophage membranes. In *Immunobiology of the Macrophage*, pp. 259–273. Edited by D. S. Nelson. London: Academic Press.

PARA, M. F., BAUMKE, R. B. & SPEAR, P. G. (1980). Immunoglobulin G (Fc) binding receptors on virions of herpes simplex type 1 and transfer of these receptors to the cell surface by infection. *Journal of Virology* **34**, 512–520.

RUSSELL, P. K. (1981). Specificity and biological activity of monoclonal anti-dengue antibodies. In *Proceedings of the First ICMR Seminar on Dengue Haemorrhagic Fever* (1980, Kobe, Japan), pp. 81–93. Edited by S. Hotta. Kobe: International Centre for Medical Research, Kobe University School of Medicine.

SAWYER, W. D. (1969). Interaction of influenza virus with leucocytes and its effects on phagocytosis. *Journal of Infectious Diseases* **119**, 541–556.

SCOTT, R. M., NISALAK, A., CHEAMUDON, U., SERIDHORANAKUL, S. & NIMMANNITYA, S. (1980). Isolation of dengue viruses from peripheral blood leucocytes of patients with hemorrhagic fever. *American Journal of Tropical Medicine and Hygiene* **29**, 638–648.

SHANLEY, J. D. & PESANTI, E. L. (1982). Effect of antiviral agents on murine cytomegalovirus-induced macrophage disfunction. *Infection and Immunity* **36**, 918–923.

SHUKLA, M. I. & CHATURVEDI, U. C. (1982). *In vivo* role of macrophage in transmission of dengue virus-induced suppressor signal to T lymphocytes. *British Journal of Experimental Pathology* **63**, 522–530.

SHUKLA, M. I. & CHATURVEDI, U. C. (1983). Transmission of dengue virus-induced suppressor signal from macrophage to lymphocyte occurs by cell contact. *British Journal of Experimental Pathology* **64**, 87–92.

STUART, A. E., HABESHAW, J. A. & DAVIDSON, A. E. (1978). Phagocytes *in vitro*. In *Handbook of Experimental Immunology, vol. 2: Cellular Immunology*, pp. 31.1–31.30. Edited by D. M. Weir. London: Blackwell Scientific Publications.

TANDON, P., CHATURVEDI, U. C. & MATHUR, A. (1979). Differential depletion of T lymphocytes in the spleen of dengue virus-infected mice. *Immunology* **37**, 1–6.

WARR, G. A. & JAKAB, G. J. (1979). Alteration in lung macrophage antimicrobial activity associated with viral pneumonia. *Infection and Immunity* **26**, 492–497.

WARR, G. A., JAKAB, G. J. & HEARST, J. E. (1979). Alterations in lung macrophage immune receptor(s) activity associated with viral pneumonia. *Journal of the Reticuloendothelial Society* **26**, 357–366.

WESTMORELAND, D. & WATKINS, J. F. (1974). The IgG receptor induced by herpes simplex viruses: studies using radioiodinated IgG. *Journal of General Virology* **24**, 167–178.

WESTMORELAND, D., ST JEOR, S. & RAPP, F. (1976). The development by cytomegalovirus-infected cells of binding affinity for normal human immunoglobulin. *Journal of Immunology* **116**, 1566–1570.

(*Received 13 May 1983*)