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DEPRESSED MACROPHAGE FUNCTIONS IN DENGUE VIRUS-INFECTED MICE: ROLE OF THE CYTOTOXIC FACTOR

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Summary.—Dengue virus Type 2 (DV) infection causes immunosuppression in mice. Since macrophages are crucial for immune response, we have studied their functions in this condition and report our findings here. It was observed that in DV-infected mice the phagocytosis of neutral-red and latex particles by splenic and peritoneal-cavity macrophages was significantly reduced ($P<0.001$) from Days 3 to 10 after inoculation. Similarly the migration of splenic and peritoneal macrophages on a glass surface was reduced significantly ($P<0.001$) from Days 4 to 10 after inoculation. Pre-treatment of normal mouse spleen cells with DV-induced cytotoxic factor (CF) inhibited the phagocytic and migratory functions in the same way as observed in DV-infected mice. Higher dilutions of CF (10^{-3} and $10^{-3.7}$) did not kill the cells but affected their functions. It was concluded that macrophage functions are affected by killing and metabolic changes in these cells by DV-induced CF, thus producing immunosuppression.

ADULT SWISS ALBINO MICE inoculated intracerebrally (i.c.) with dengue Type 2 virus (DV) fail to develop cell-mediated immune response (CMI) to homologous antigen and have poor immune response to heterologous antigens. There is an associated reduction in the size of the spleen and the number of cells in it (Chaturvedi, Tandon and Mathur, 1977; Chaturvedi *et al.*, 1978*a*; Tandon, Chaturvedi and Mathur, 1979*a*, *b*). The T lymphocytes of spleens of DV-primed mice produce a suppressor factor (SF) and a cytotoxic factor (CF) which mediate antigen-specific and nonspecific immunosuppression respectively (Chaturvedi, Bhargava and Mathur, 1980*a*; Chaturvedi, Dalakoti and Mathur, 1980*b*; Chaturvedi, Shukla and Mathur, 1981*c*; Chaturvedi, Shukla and Mathur 1982*b*; Chaturvedi, 1981; Chaturvedi and Shukla, 1981; Shukla and Chaturvedi, 1981*a*, *b* and unpublished; Dalakoti, Chaturvedi and Mathur, 1982). The CF kills most of the mouse macrophages and one third of the T lymphocytes *in vitro* and *in vivo*, and affects the functions of these cells (Chaturvedi *et al.*, 1981*b*, *c*; Chaturvedi, Gulati and Mathur, 1982*a*).

Macrophages play a vital role in protection against a number of viral infections nonspecifically (reviewed by Mogensen, 1980). The fate of viruses in macrophages varies; for example, some of them are inactivated, some replicate in macrophages while others have no effect (reviewed by Denman & Pinder, 1974). Macrophages are known to play an important role in immune response; *viz.*, phagocytosis, processing and presentation of antigens; secretion of immuno-regulatory substances and control of immune response; and as effector cells in CMI (reviewed by Unanue, 1978; Friedman, 1978; Bloom and Rager-Zisman, 1975). Suppression of macrophage functions is known to affect immune responses *in vitro* as well as *in vivo* (reviewed by

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Friedman, 1978). Virelizier (1975) has suggested that infected macrophages, through failure to provide proliferative signals, or through release of toxic factors, may play an important role in virus-induced immunosuppression. We have made therefore, an attempt to study macrophage functions in DV-infected mice. The findings presented here indicate depression of macrophage functions which may be due to the effect of the cytotoxic factor.

MATERIALS AND METHODS

The details of dengue Type 2 Virus (DV), mice, and the DV experimental model have been described earlier (Chaturvedi, Tandon and Mathur, 1977; Chaturvedi *et al.*, 1978*a*; Agrawal *et al.*, 1978).

Preparation of spleen cells.—Spleen tissue was collected aseptically from mice. The spleen cells were teased out into cold minimum essential medium (MEM) supplemented with 5% foetal calf serum (Armour Pharmaceutical Co., U.K.) and a single-cell suspension was prepared. Viable nucleated cells were counted using trypan blue dye as described elsewhere (Chaturvedi, Tandon and Mathur, 1978*b*).

Preparation of peritoneal macrophages.—Five ml heparinized MEM was inoculated into the peritoneal cavity of normal mice. Peritoneal lavage washings were aspirated under aseptic conditions. The cells were washed twice with MEM and cultured in Leighton tubes with coverslips for 2 h at 37°. The cell sheet was washed with MEM to remove glass-nonadherent cells and phagocytic function was assayed. For the migration test total peritoneal cells were used.

Neutral red dye test.—This test was performed using the technique of Cohn and Weiner (1963) to assess the phagocytic activity. To 0·5 ml cell suspension ($5–10 \times 10^6$ cells (or cell sheet on coverslip 50 μl of 0·1% neutral red dye was added and incubated at 37° for 20 min with intermittent shaking. After incubation the cells were washed twice and resuspended in MEM and 200 or more cells were counted to calculate the percentage of cells containing neutral-red granules.

Latex particle test.—The phagocytic activity of macrophages was assessed using latex particles (Cline and Lehrer, 1968). 0·1 ml of latex-particle suspension was added to 1 ml of cell suspension or cell sheet on the coverslip and incubated at 37° for 1 h. Cells were washed to remove free latex particles and resuspended in MEM. The percentage of positive cells containing latex particles was calculated after 200 or more cells had been counted.

Latex-particle phagocytosis was also assessed by another technique. In this the spleen was cut longitudinally and an imprint smear of the cut surface was obtained on a sterile chilled microscope slide. The imprint smear was immediately covered with MEM containing the requisite amount of latex particles. The slide was incubated at 37° for 1 h in an atmosphere of 5% CO_2. The slide was then washed gently to remove free latex particles and the imprint smear was stained by the Giemsa technique. The slide was then examined under an oil-immersion lens for cells containing intracellular latex particles. The advantage of this technique was that the macrophages could be visualized in relation to other spleen cells as the splenic architecture was projected in the imprint smear.

Leucocyte migration test.—This test was performed by the technique of David *et al.* (1964) as described elsewhere (Chaturvedi *et al.*, 1978*a*, b). The area of cell migration was projected by the Camera Lucida on Whatman No. 1 paper. The paper was cut out and weighed on a precision balance. The area of migration was represented as the weight of the paper cut out.

Preparation of CF.—The cytotoxic factor (CF) was prepared from the spleen of DV-infected moribund mice as described in detail elsewhere (Chaturvedi *et al.*, 1980*a*, *b*).

Plan of study.—To study the effect of the virus infection on macrophage functions, groups of mice were inoculated i.c. with a dose of 1000 LD_{50} of DV. Four mice were harvested daily from Day 1 to Day 10 after inoculation in repeated experiments. Peritoneal fluid and spleen were collected and the above tests were performed.

To examine the effect of CF on macrophage functions, normal mouse spleen cells were treated with various dilutions of CF at 4° for 1 h. The cells were washed three times and the above tests were repeated. For control, homogenate similarly prepared from normal mouse spleen was used for treating the cells. It had no effect on the functions of the cells as described before (Chaturvedi *et al.*, 1980*a*, b, 1982*a*); these findings have therefore not been described here.

All the tests were set in triplicate or more. The mean values ± s.d. of the findings obtained from different mice are presented. The data have been subjected to Student's t test for P values. P values less than 0·05 were considered significant.

RESULTS

Cells from DV-infected mice

The phagocytic and migratory functions

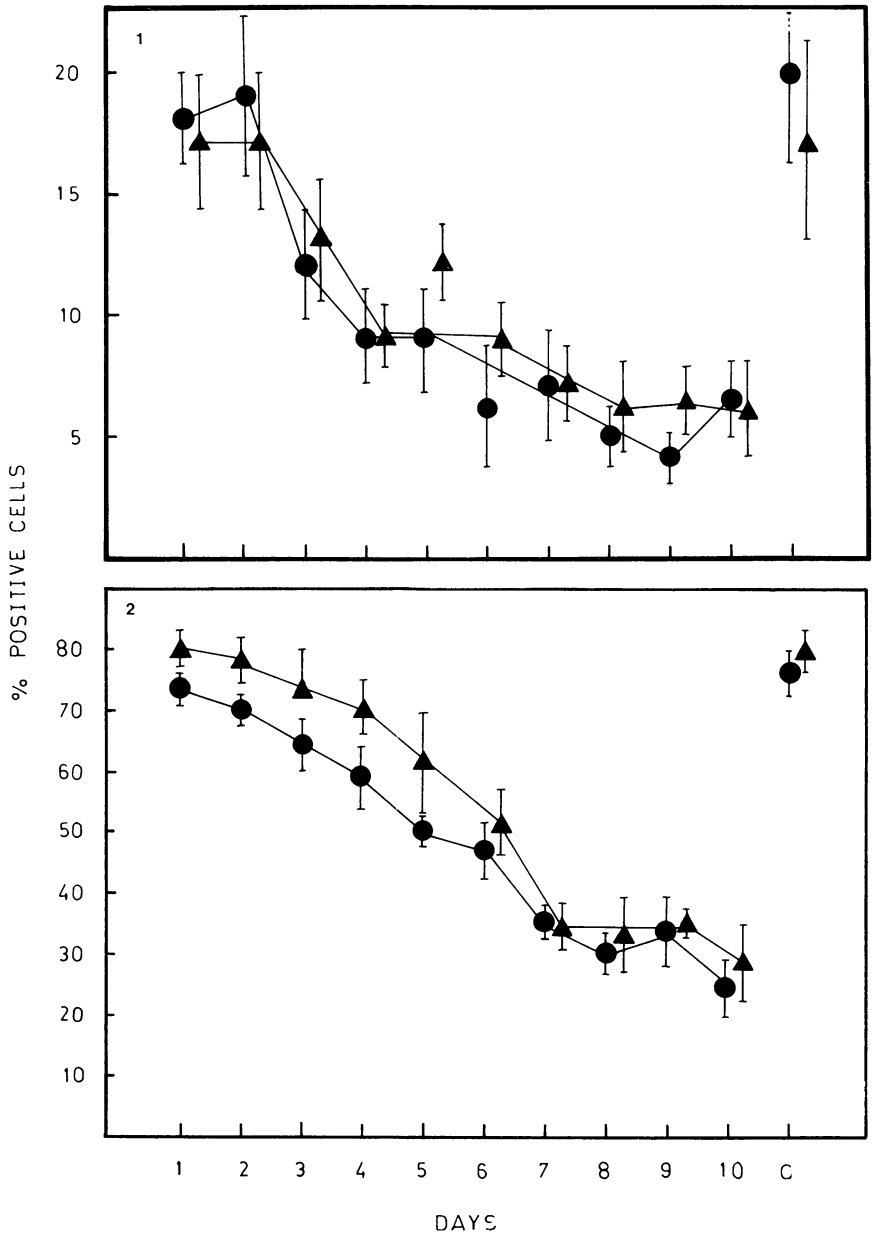

FIG. 1.—Depressed phagocytic activity of spleen cells of DV-infected mice. DV was inoculated i.c. in doses of 10^3 LD_{50} in mice. The phagocytic activity of the spleen cells was screened by uptake of neutral red dye (●) or latex particles (▲) at different periods. C, cells from normal control mice.

FIG. 2.—Depressed phagocytic activity of peritoneal lavage cells of DV-infected mice. The peritoneal lavage cells were collected from mice at different periods after 10^3 LD_{50} DV i.c. Phagocytic activity of the cells was screened by uptake of neutral red dye (●) or latex particles (▲). C, cells from normal control mice.

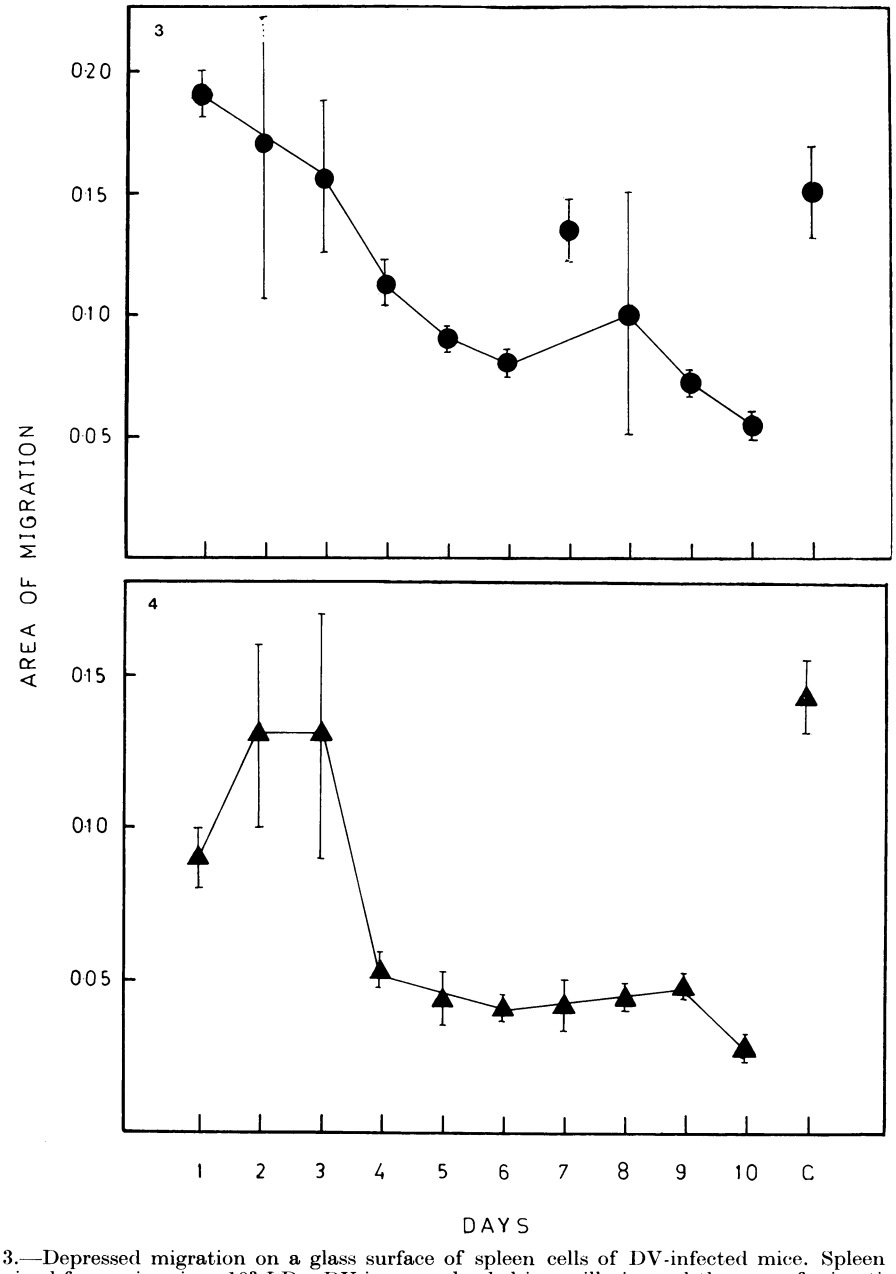

FIG. 3.—Depressed migration on a glass surface of spleen cells of DV-infected mice. Spleen cells obtained from mice given 10^3 LD_{50} DV i.c. were loaded in capillaries and the area of migration on a glass surface was recorded on Whatman No. 1 paper. The paper was cut and weighed. The area of migration has been represented as weight of the paper in g. C, cells from normal control mice.

FIG. 4.—Depressed migration on a glass surface of peritoneal lavage cells of DV-infected mice. For details see legend to Fig. 3.

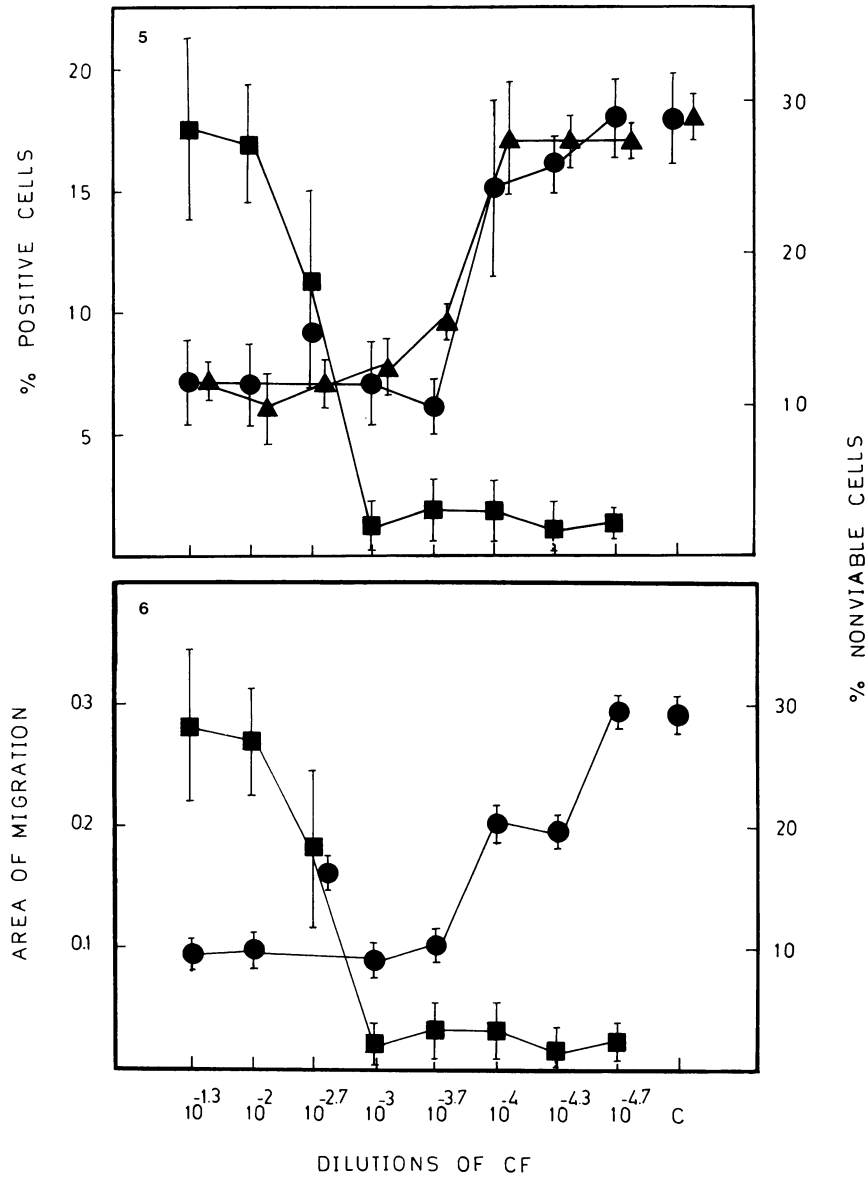

FIG. 5.—Inhibition of phagocytic activity of normal mouse spleen cells on treatment with DV-induced cytotoxic factor (CF). The spleen cells were treated with various dilutions of CF at 4° for 1h and then tested for killing of cells (■) and phagocytic activity by uptake of neutral red dye (●) and latex particles (▲). C, cells treated with normal mouse spleen homogenate.

FIG. 6.—Inhibition of migration on a glass surface of normal mouse spleen cells pretreated with DV-induced cytotoxic factor. For details see legends to Fig. 5.

of spleen and peritoneal cells obtained at different periods after i.c. inoculation of mice with 10^3 LD_{50} of DV were studied.

Effect on phagocytosis by spleen cells.—The phagocytic function of spleen cells was studied by the uptake of neutral-red dye and latex particles. The findings presented in Fig. 1 show a significant reduction in phagocytic activity from the 3rd day after inoculation onwards. Reduction in uptake

of neutral red and latex particles was 40% and 24% ($P < 0{\cdot}001$) respectively on Day 3, while that on Day 10 was 68 and 65% respectively.

Effect on phagocytosis by peritoneal macrophages.—Data summarized in Fig. 2 show that among normal mice peritoneal macrophages $76 \pm 1{\cdot}5\%$ cells had taken up neutral red and $79 \pm 1{\cdot}5\%$ latex particles. A highly significant ($P < 0{\cdot}001$) reduction in phagocytosis was observed in DV-infected mice starting from the 5th day after inoculation. $50 \pm 1{\cdot}5\%$ cells positive for neutral red and $61 \pm 6{\cdot}6\%$ cells had taken up latex particles at Day 5. Most peritoneal macrophages of infected mice lost their phagocytic function at the 7th to the 10th days after inoculation.

Effect on migration of spleen cell.—The areas of migration of the cells at different periods after the virus infection are presented in Figure 3. A progressive reduction in the area of migration was observed from Day 4 after inoculation. Reduction in the area of migration of cells of DV-infected mice was 24–65% at 4–10 Days after inoculation as compared to that in controls.

Effect on migration of peritoneal macrophages.—A highly significant reduction in the area of migration of peritoneal macrophages occurred from Day 4, the reduction being 64 to 83% as compared to that in controls (Fig. 4)

Cells treated with DV-induced cytotoxic factor.—To find out if the alterations observed in the functions of DV-infected mouse cells can be reproduced by DV-induced cytotoxic factor (CF), the following experiments were done. The normal mouse spleen cells were treated with various dilutions of CF at 4° for 1 h. The cells were then washed and their viable cells counted, and their phagocytic and migratory functions studied.

Effect of CF treatment on viability of spleen cells.—CF diluted $10^{-1{\cdot}3}$–$10^{-2{\cdot}7}$-fold killed 18 ± 6–$28 \pm 6\%$ of cells and among sets treated with higher dilutions of CF the percentage of non-viable cells was $1{\cdot}5 \pm 0{\cdot}5$ to $2{\cdot}2 \pm 0{\cdot}9$. A correlation of the findings of nonviable cells with the functions of CF-treated cells is presented in Figs 5 and 6.

Effect of CF treatment on phagocytosis by spleen cells.—In the untreated control sets $18 \pm 1{\cdot}7\%$ cells were positive for neutral red and $18 \pm 0{\cdot}6\%$ for latex particles. Among the cells treated with CF diluted $10^{-1{\cdot}3}$–$10^{-3{\cdot}7}$-fold, $7 \pm 1{\cdot}5$–$6 \pm 1{\cdot}1\%$ of cells had taken up the dye and $7{\cdot}25 \pm 0{\cdot}5$ to $9{\cdot}5 \pm 0{\cdot}6\%$ of cells had engulfed latex particles. The inhibition of phagocytosis by treatment with CF diluted 10^{-3}- and $10^{-3{\cdot}7}$-fold was highly significant ($P < 0{\cdot}001$), though the percentage of non-viable cells with these dilutions of CF was negligible (fig. 5). The percentage of cells phagocytosing after treatment with higher dilutions of CF was similar to that in controls.

Effect of CF treatment on migration of spleen cells.—Data summarized in Fig. 6 show that, on treatment with CF diluted $10^{-1{\cdot}3}$–$10^{-3{\cdot}7}$, a highly significant reduction ($P < 0{\cdot}001$) in the area of migration of the cells occurred. CF diluted up to 10^{-3} and $10^{-3{\cdot}7}$ had no cytotoxic activity but reduction in area of migration continued. Among the sets treated with higher dilutions of CF the area of migration was similar to that in controls.

DISCUSSION

The findings presented here demonstrate that the phagocytic and migratory functions of macrophages are depressed in DV-infected mice. The extent of inhibition of the functions increased with the period of infection and was maximum in moribund mice. Macrophage functions are adversely affected in a number of viral infections. The phagocytic functions of macrophages are depressed in mouse leukaemia virus (Old *et al.*, 1960) and influenza virus infections (Warshauer *et al.*, 1977), while intracellular killing is impaired in Sendai (Jakab and Green, 1976) and influenza virus (Warshauer *et al.*, 1977) infections. The migratory function of macrophages is suppressed in influenza virus infection (Kleinerman *et al.*, 1976), while the secretion of immuno-

regulatory substances is inhibited in influenza (Shayegani, Lief and Mudd, 1974), herpes simplex (Kirchner *et al.*, 1977) and poliovirus infections (Van Loon *et al.*, 1979). In some viral infections the macrophages are killed and depleted, *viz.* ectromelia (Mims, 1964), mouse hepatitis Type 3 viruses (Mallucci, 1965) and lymphocytic choriomeningitis (Löhler Ehlerding and Lehmann-Grube, 1974.

The mechanisms which influence macrophage function in viral infections are not clearly understood. Function can be affected either by killing of the macrophages or by disturbances of their metabolism. These can be mediated by replication of the virus within them or through production of a mediator which affects the macrophages. DV is known to replicate in different organs of mice including the spleen (Chaturvedi, Mathur and Mehrotra 1974; Chaturvedi *et al.*, 1978*a*; Agrawal *et al*, 1978; Hotta *et al.*, 1981) but information regarding its replication in mouse macrophages is scanty. Hotta *et al.*, (1981) have suggested replication of DV in mouse macrophages on the basis of their demonstration of DV-specific antigen in liver Kupffer cells by a fluorescent antibody technique. We have observed a mechanism in DV-infected mice which results in macrophage killing. The T lymphocytes of DV-infected mouse spleen produce a factor, the CF, which kills most of the mouse macrophages and one third of the splenic T lymphocytes *in vitro* and kills spleen cells of mice *in vivo* when injected i.v. or i.p. (Chaturvedi *et al.*, 1980*a*, *b*, 1981*b*, *c*). The findings presented in this paper show that treatment of macrophages with CF alters their phagocytic and migratory functions as is observed in DV-infected mice. Further, a correlation between the cell killing and functional alterations observed indicate that the latter can be produced with higher dilutions of CF without killing the cells. Lymphokines are released into blood and body fluids of man and animals including mouse (reviewed by Neta and Salvin, 1981). We have observed the presence of CF in the sera of some of the DV-infected mice.

From the present and earlier studies it appears likely that the alterations observed in macrophage function in DV-infected mice are mediated by CF. The reasons for believing this are: (i) the period of peak production of CF corresponds with the period of maximum reduction in spleen weight and number of spleen cells (Chaturvedi *et al.*, 1980*a*; Tandon *et al.*, 1979*a*); (ii) CF kills most of the macrophages *in vitro* and kills spleen cells *in vivo* (Chaturvedi *et al.*, 1981*b*, *c*); (iii) the suppressed immune functions observed in DV-infected mice can be reproduced by administration of CF (Tandon *et al.*, 1979*a*; Chaturvedi *et al.*, 1981*c*); and (iv) phagocytosis by monocytes and SRBC-rosette formation by T lymphocytes from human blood can be inhibited by pretreatment of the cells with CF (Chaturvedi *et al.*, 1982*a*), as confirmed in the present study with higher dilutions (10^{-3} and $10^{-3\cdot7}$) of CF.

We have observed that the numbers of macrophages in the spleen and peritoneal cavity of DV-infected moribund mice are significantly reduced (data to be published). Suppressed macrophage function is, thus brought about by the killing of these cells as well as by disturbance of their metabolism. Virelizier (1975) has suggested that virus-induced immunosuppression is caused by failure of infected macrophages to provide proliferative signals, or by release of toxic factors by infected macrophages. Poliovirus has been shown to produce immunosuppression by impairing macrophage metabolic functions required for the production of proliferative signals (Diamantstein and Ulmer, 1976) or by alterations in membrane structure needed for lymphocyte–macrophage action (Albrecht *et al.*, 1978). In DV-infected mouse macrophages the metabolic function appears to be disturbed but the membrane function of transfer of suppressor signal to T lymphocytes remains intact (Shukla and Chaturvedi, 1982). Strong arguments have been put forward by Woodruff and Woodruff

(1975) that virus-induced immunosuppression is caused by direct interaction of virus and lymphocytes, but the findings of our present and earlier studies do not subscribe to this view. Our preliminary studies have indicated that CF induces changes in the functions of cell membranes which may cause ionic imbalance resulting in cell death (Chaturvedi *et al.*, 1982*a*). However, the precise mechanism of cell killing and disturbances in their metabolic functions in DV-infected mice remains to be investigated.

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REFERENCES

AGRAWAL, D. K., TANDON, P., CHATURVEDI, U. C. & KUMAR, A. (1978) Biochemical Study of Certain Enzymes and Metabolites of Carbohydrate Metabolism in the Skeletal Muscle of Dengue Virus-infected Mice. *J. gen. Virol.*, **40**, 399.

ALBRECHT, R. M., HINSDILL, R. D., SANDOK, P. L. & HOROWITZ, S. D. (1978) Murine Macrophage–Lymphocyte Interactions: Scanning Electron Microscopic Study. *Inf. Immun.*, **21**, 254.

BLOOM, B. R. & RAGER-ZISMAN, B. (1975) Cell-mediated Immunity in Viral Infections. In *Viral Immunology and Immunopathology*, Ch. 7. Ed. by A. L. Notkins. London: Academic Press. p. 113.

CHATURVEDI, U. C., MATHUR, A. & MEHROTRA, R. M. L. (1974) Experimentally Produced Cardiac Injury following Dengue Virus Infection. *Indian J. Path. & Bact.*, **17**, 218.

CHATURVEDI, U. C., TANDON, P. & MATHUR, A. (1977) Effect of Immunosuppression on Dengue Virus Infection in Mice. *J. gen. Virol.*, **36**, 449.

CHATURVEDI, U. C., TANDON, P., MATHUR, A. & KUMAR, A. (1978*a*) Host Defence Mechanism against Dengue Virus Infection of Mice. *J. gen. Virol.*, **39**, 293.

CHATURVEDI, U. C., TANDON, H. O. & MATHUR, A. (1978*b*) Control of *in Vitro* Spread of Coxsackie Virus B_4 Infection by Sensitized Spleen Cells and Antibody. *J. inf. Dis.*, **138**, 181.

CHATURVEDI, U. C., BHARGAVA, A. & MATHUR, A. (1980*a*) Production of Cytotoxic Factor in the Spleen of Dengue Virus-infected Mice. *Immunol.*, **40**, 665.

CHATURVEDI, U. C., DALAKOTI, H. & MATHUR, A. (1980*b*) Characterization of the Cytotoxic Factor Produced in the Spleen of Dengue Virus-infected mice. *Immunol.*, **41**, 387.

CHATURVEDI, U. C. (1981) Study of Immune Mechanism in Dengue Virus-infected Mice. In Dengue Haemorrhagic Fever 1981. Ed. S. Hotta. Kobe, Japan: Yukosha Printing House, p. 159.

CHATURVEDI, U. C. & SHUKLA, M. I. (1981) Characterization of the Suppressor Factor Produced in the Spleen of Dengue Virus-infected Mice. *Ann. Immunol. (Pasteur Institute)*, **132c**, 245.

CHATURVEDI, U. C., SHUKLA, M. I. & MATHUR, A. (1981*a*) Thymus dependent Lymphocytes of the Dengue Virus-infected Mouse Spleen Mediate Suppression through Prostaglandin. *Immunol.*, **42**, 1.

CHATURVEDI, U. C., MATHUR, K. R., GULATI, L. & MATHUR, A. (1981*b*) Target Lymphoid Cells for the Cytotoxic Factor Produced in the Spleen of Dengue Virus-infected Mice. *Immunol. Letters*, **3**, 13.

CHATURVEDI, U. C., SHUKLA, M. I., MATHUR, K. R. & MATHUR, A. (1981*c*) Dengue Virus-induced Cytotoxic Factor Suppresses Immune Response of Mice to Sheep RBC. *Immunol.*, **43**, 311.

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. *Immunol.*, **45**, (in Press).

CHATURVEDI, U. C., SHUKLA, M. I. & MATHUR, A. (1982*b*) Role of Macrophages in Transmission of Dengue Virus-induced Suppressor Signal to a Subpopulation of T Lymphocytes. *Ann. Immunol.* (Pasteur Institute), **133c**, (in Press).

CLINE, M. J. & LEHRER, R. I. (1968) Phagocytosis by Human Monocytes. *Blood*, **32**, 423.

COHN, Z. A. & WEINER, E. (1963) The Particulate Hydrolases of Macrophages. I. Comparative Enzymology, Isolation and Properties. *J. exp Med.*, **118**, 991.

DALAKOTI, H., CHATURVEDI, U. C. & MATHUR, A. (1982) Production of Dengue Virus-induced Cytotoxic Factor is Inhibited by Treatment with Cycloheximide and Mitomycin C. *Ind. J. exp. Biol.*, **20**, 1.

DAVID, J. R., AL-ASKARI, S., LAWRENCE, H. S., THOMAS, L. (1964) Delayed Hypersensitivity *in Vitro*. I. The Specificity of Inhibition of Cell Migration by Antigens. *J. Immunol.*, **93**, 264.

DENMAN, A. M. & PINDER, M. (1974) Assessment of immunological function in man. *Proc. R. Soc. Med.*, **67**, 1219.

DIAMANTSTEIN, T. & ULMER, A. (1976) Two Distinct Lymphocyte-stimulating Soluble Factors (LAF) Released from Murine Peritoneal Cells. I. The Cellular Source and the Effect of cGMP on their Release. *Immunol.*, **30**, 741.

FRIEDMAN, H. (1978) Macrophages in Immunity—Concluding Remarks and General Summary. *Fed. Proc.*, **37**, 102.

HOTTA, H., MURAKAMI, I., MIYASAKI, K., TAKEDA., Y., SHIRANE, H. & HOTTA, S. (1981) Inoculation of Dengue virus into nude mice. *J. gen. Virol.*, **52**, 71.

JAKAB, G. J. & GREEN, G. M. (1976) Defect in Intracellular Killing of *Staphylococcus aureus* within Alveolar Macrophages in Sendai Virus-infected Murine Lungs. *J. clin. Invest.*, **57**, 1533.

KIRCHNER, H., DARAI, G., KEYSSNER, K., MUNK, K. & MERGENHAGEN, S. E. (1977) Virus–Macrophage–Lymphocyte Interactions. In *Regulatory Mechanisms in Lymphocyte Activation*–Proc. 11th Leucocyte Culture Conference, 1977. New York: Academic Press. p. 617.

KLEINERMAN, E. S., DANIELS, C. A., POLSSON, R. P. & SNYDERMAN, R. (1976) Depression of Macrophage Accumulation in Influenza infected Mice. *Am. J. Path.*, **85**, 373.

LÖHLER, J., EHLERDING, I. & LEHMANN-GRUBE, F. (1974) Pathologie des lymphatischen Gemebes bei der lymphozytären Choriomeningitis der

Maus. *Zt. Bakt. u. Hyge.* I. Abteilung Originale A, **227,** 458.

MALLUCCI, L. (1965) Observation on the Growth of Mouse Hepatitis Virus (MHV-3) in Mouse Macrophages. *Virology,* **25,** 20.

MIMS, C. A. (1964) The Peritoneal Macrophages of Mice. *Br. J. exp. Path.,* **45,** 37.

MOGENSEN, S. C. (1980) Role of Macrophages in Natural Resistance to Virus Infections. *Microbiol. Rev.,* **43,** 1.

NETA, R. & SALVIN, S. B. (1981) Production of Lymphokines *in Vivo.* In *Lymphokines,* Vol. 2. New York: Academic Press. p. 295.

OLD, L., BENACERRAF, B., CLARK, D. A. & GOLDSMITH, M. (1960) The Reticuloendothelial System and the Neoplastic Process. *Ann. N.Y. Acad. Sci.,* **88,** 264.

SHAYEGANI, M. F., LIEF, F. S. & MUDD, S. (1974) Specific and Non-specific Cell mediated Resistance to Influenza in Mice. *Inf. Immun.,* **9,** 991.

SHUKLA, M. I. & CHATURVEDI, U. C. (1981*a*) Cycloheximide and Mitomycin C Treatment Inhibits Production of Dengue Virus-induced Suppressor Factor. *Indian J. exp. Biol.,* **19,** 826.

SHUKLA, M. I. & CHATURVEDI, U. C. (1981*b*) Dengue Virus-induced Suppressor Factor Stimulates Production of Prostaglandin to Mediate Suppression. *J. gen. Virol.,* **56,** 241.

TANDON, P., CHATURVEDI, U. C. & MATHUR, A. (1979*a*) Differential Depletion of T Lymphocytes in the Spleen of Dengue Virus-infected Mice. *Immunol.,* **37,** 1.

TANDON, P., CHATURVEDI, U. C. & MATHUR, A. (1979*b*) Dengue Virus-induced Thymus-derived Suppressor Cells in the Spleen of Mice. *Immunol.* **38,** 653.

UNANUE, E. R. (1978) Macrophage Functions in Immunity. *Fed. Proc.,* **37,** 77.

VAN LOON, A. M., VAN DER LOGT, J. TH. M. & VAN DER VEEN, J. (1979) Poliovirus-induced Suppression of Lymphocyte Stimulation: A Macrophage-mediated Effect. *Immunol.,* **37,** 135.

VIRELIZIER, J. L. (1975) Mechanisms of Immunodepression Induced by Viruses: Possible Role of Infected Macrophages. *Biomedicine,* **22,** 255.

WARSHAUER, D., GOLDSTEIN, E., AKERS, T., LIPPERT, W. & KIM, M. (1977) Effect of Influenza Viral Infection on the Ingestion and Killing of Bacteria by Alveolar Macrophages. *Ann. Rev. res. Dis.,* **115,** 269.

WOODRUFF, J. F. & WOODRUFF, J. J. (1975) The Effect of Viral Infections on the Function of the Immune System. In *Viral Immunology and Immunopathology.* Ed. by A. L. Notkins. Academic Press. p. 393.