ABROGATION OF HELPER T CELLS BY DENGUE VIRUS-INDUCED CYTOTOXIC FACTOR

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ABSTRACT

The mechanism of depletion of helper T cells (Th) in dengue-type 2 virus (DV)-infected mice is studied. The treatment of DV-induced Th *in vitro* with a DV-induced cytotoxic protein (CF) abrogates helper activity for the DV-specific direct antibody forming cell (PFC) response. Pretreatment of mice with CF abolishes generation of Th and post-treatment of mice removes Th activity from the spleen cells. Thus DV-induced CF removes helper T cell activity and may also contribute to immunosuppression in other viral infections.

INTRODUCTION

SELECTIVE destruction of the subpopulation of T cells is mainly responsible for helper/inducer functions ($OKT4^+$) and is a feature of infection by human immunodeficiency virus (HIV)[1,2]. Dengue is another viral infection in which depletion of OKT4 positive cells have been described[3]. Dengue virus replicates mainly in acrophages and to some extent in B lymphocytes or cell lines with B-cell characteristics but not in T lymphocytes[4,5]. However HIV infects and replicates in OKT4 positive helper cells producing cytopathic effects[1,2].

The production of a cytotoxic protein by T-cells, the cytotoxic factor (CF) is well-documented in dengue virus infection. CF kills about one third of the T lymphocytes and two thirds of the macrophages *in vitro* and *in vivo* severelly affecting their functions[6]. Dengue virus induces generation of helper T cells (Th) enhancing the virus-specific humoral immune response in mice[7,8]. The aim of the present study was to investigate the mechanism of depletion of Th in dengue virus-infected mice. The results show that virus-induced cytotoxic protein (CF) kills Th and may also contribute to immunosuppression in other viral infections.

MATERIALS AND METHODS

Experiments were carried out on 3 to 4-month-old inbred adult Swiss albino mice. Dengue type 2 virus strain P23085 was used in the form of infected adult mouse brain suspension as described elsewhere[9]. Putative helper cells were generated in mice by stimulation with DV as described earlier[7,8]. In brief, mice inoculated with 10^2 LD_{50} of DV iv were killed on day 4 and the spleens were collected aseptically. A single cell suspension of the spleens was used as putative helper cells. The DV-induced helper cells are Lyl^+ T lymphocytes and not the macrophages or B lymphocytes; therefore, these cells are considered[8] helper T cells. Cells obtained from normal mice, used as controls, had no helper activity; therefore, this data is not presented. The CF was prepared from the spleen homogenate of DV-infected mice[10,11].

Assay of helper activity

The helper activity was assayed by counting DV-specific direct antibody plaque forming cells (PFC) using haemolysis in gel technique[12] as described earlier[9,13]. Mice used to assay helper activity were treated with cyclophosphamide (CY) i.p. 200 mg/kg body weight (Endoxon-ASTA, Khandelwal Industries) followed (24 h later) with 10^3 LD_{50} DV ip and (72 h later) with 10^8 helper cells i.v. CY was used to remove the precursors of the endogenous helper T cells[14]. Mice inoculated with CY and DV were used as controls. DV-specific PFC were counted on 3rd day of DV ip as maximum help was generated on this day[9].

Mean values ± SD of the data obtained from repeated experiments are presented.

RESULTS

Effect of CF on Th

Putative helper cells (4×10^8) suspended in 1 ml MEM without serum were incubated with 0.2 ml CF at 4°C for 1 h. The cells were washed thrice, resuspended in MEM and their helper activity was assayed by transferring 1×10^8 cells in recipient mice. Figure 1 shows the helper activity of the untreated cells was 51% which was reduced to 5% on treatment with 10^{-1} dilution of CF. To study the dose response of CF the above experiment was repeated where Th were treated *in vitro* with different dilutions of CF. Figure 2 shows

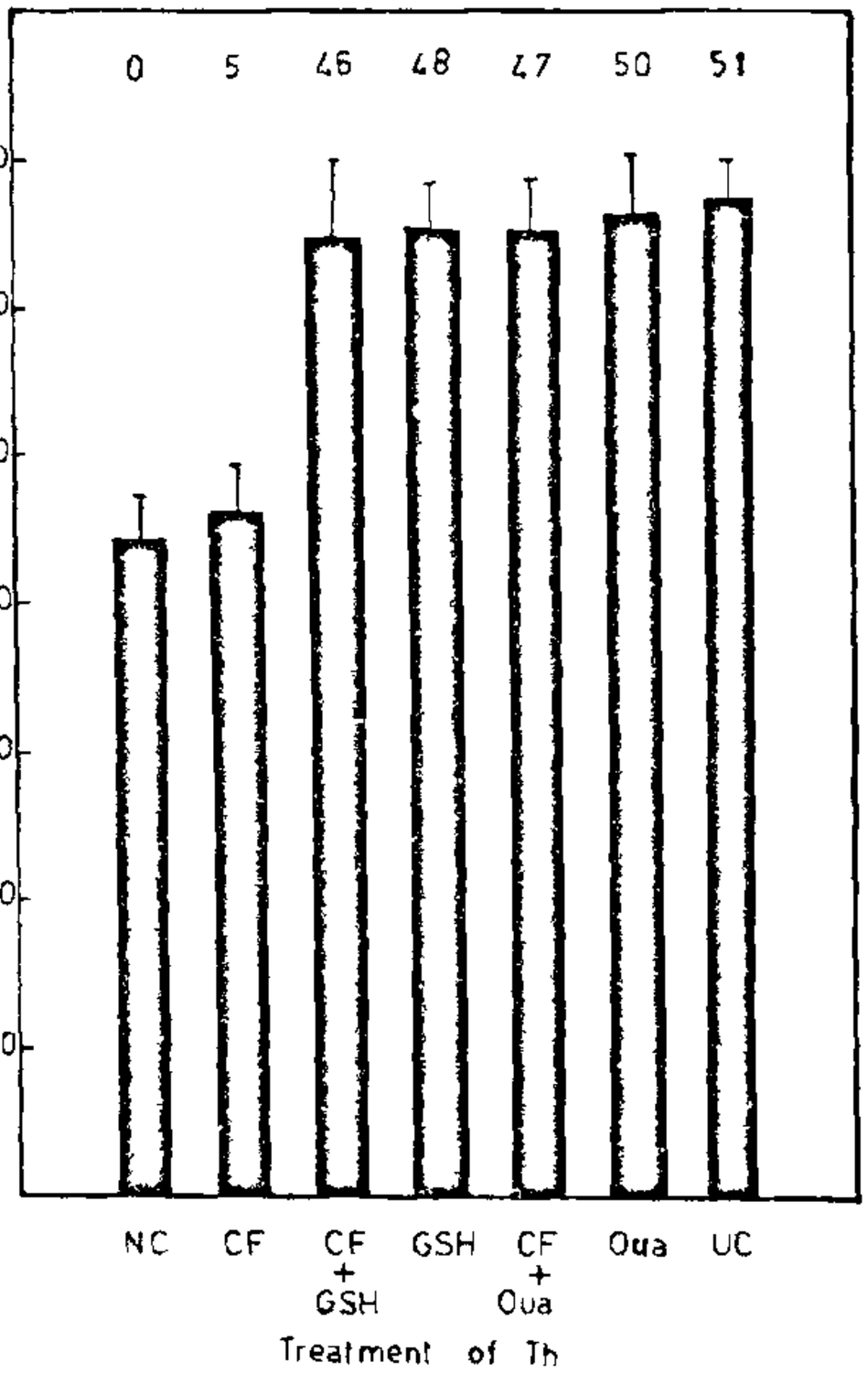

Figure 1. Abrogation of the activity of Th following *in vitro* treatment with CF. Pretreatment of Th with reduced glutathione (GSH) or ouabain (Oua) protected the cells against the effects of CF [NC, No cells; UC, Untreated Th].

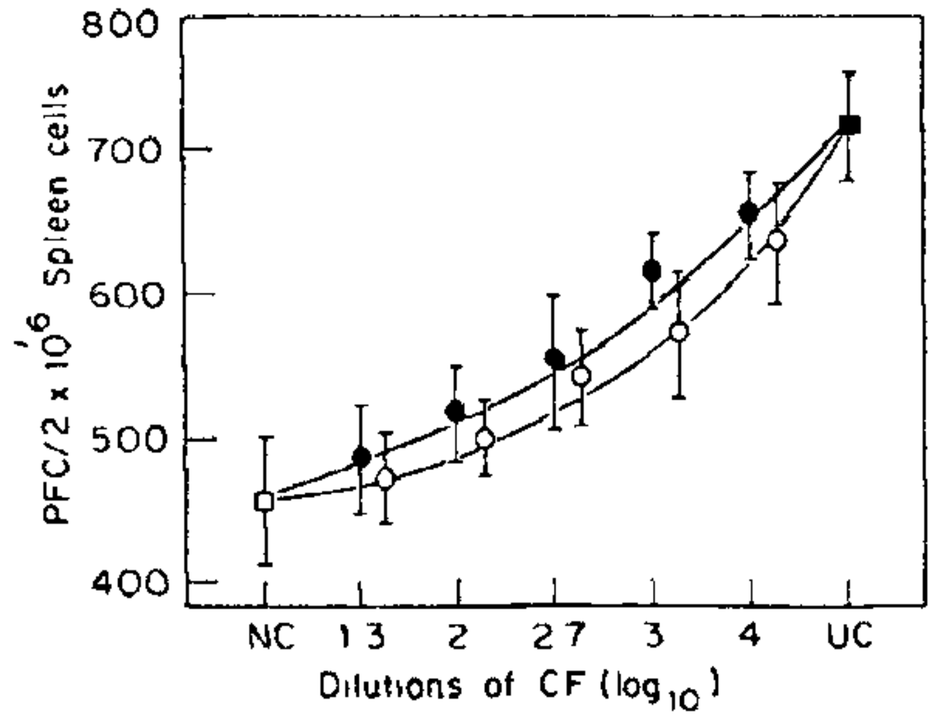

Figure 2. Dose-dependent effects of CF on Th by *in vitro* (○) or *in vivo* (●) treatment [NC, No cells; UC, Untreated Th].

the helper activity decreased with increasing concentration of CF.

Effect of drug treatment of Th on the cytotoxicity of CF

Pretreatment of target cells with plasma membrane stabilizers, such as 2,4-dinitrophenol, ouabain and reduced glutathione prevents the cytotoxicity of CF[15]. Therefore, an effort was made to examine if the adverse effects of CF on the helper cells can be abrogated by pretreatment with such drugs. The optimum dose of the drugs was established in preliminary experiments. For this experiment 4×10^8 putative helper cells were treated with 10^{-4} M reduced glutathione (Sarabhai M. Chemicals) or 10^{-4} M ouabain (E. Merck, Darmstadt) for 1 h at 4°C[15]. The cells were washed thrice, treated with CF and then assayed for the helper activity. The data presented in figure 1 show that CF had no significant ($P > 0.2$) effect on the helper activity of the cells pretreated with the drugs.

Effect of CF on the generation of Th

To study the effect of CF on the production of Th, groups of mice were inoculated with 0.2 ml (10^{-1}) of CF i.v. one day before or 1, 2 or 3 days after 100 LD_{50} of DV i.v. Mice were sacrificed on 4th day after DV inoculation and the spleen cells obtained from individual mice were assayed for the helper activity. Figure 3 shows the helper activity of the cells obtained

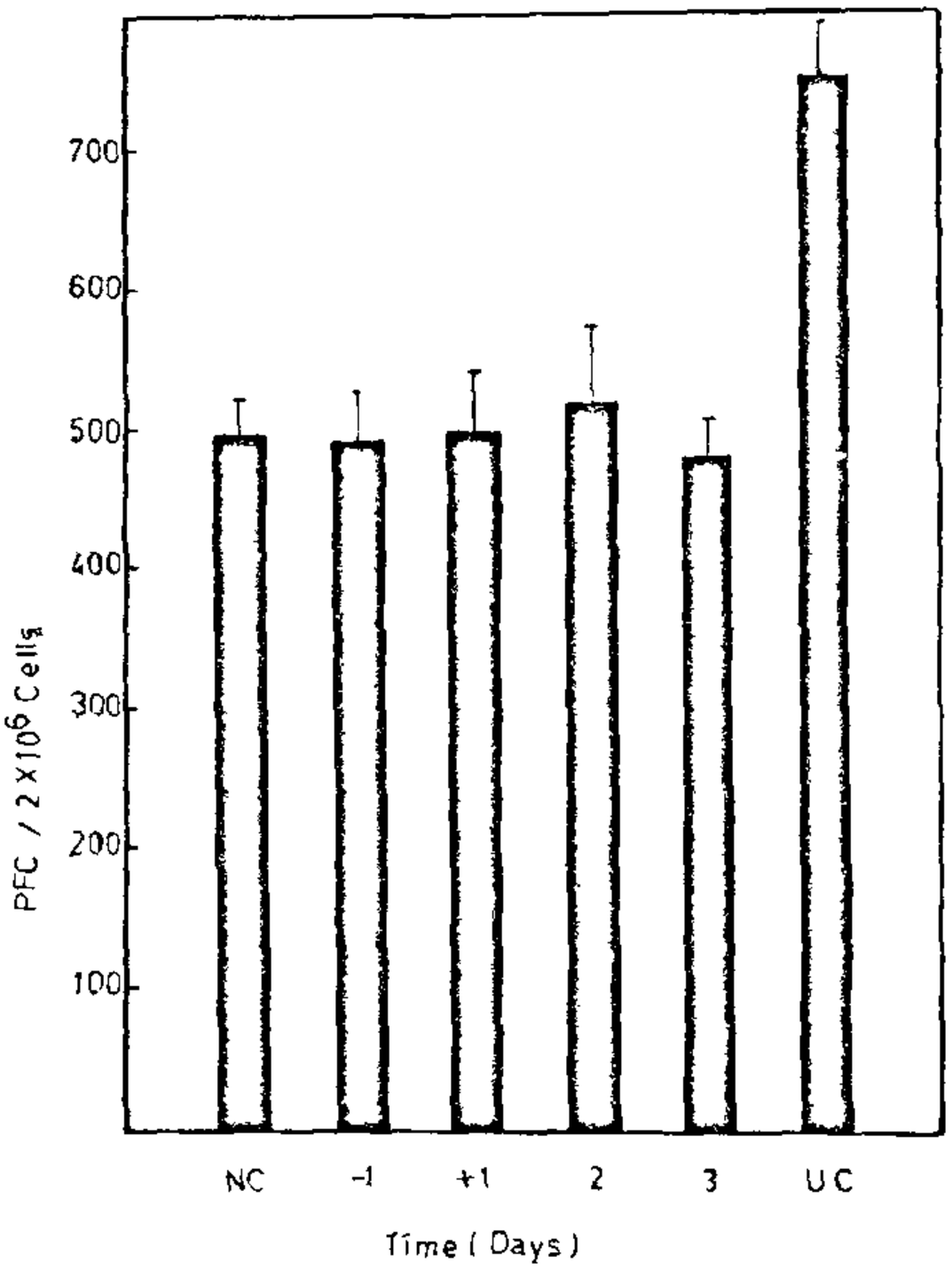

Figure 3. Abrogation of the activity of Th following treatment of donor mice with CF at different periods before or after induction of Th with DV [NC, No cells; UC, Untreated Th].

from untreated mice to be 54%. On the other hand, the cells obtained from mice treated with CF at different periods had no helper activity. To study the effect of the dose of CF the above experiment was repeated by giving different dilution of CF to donor mice on the 3rd day of priming. Figure 2 shows that the helper activity gradually decreased with increasing concentration of CF.

DISCUSSION

The present results demonstrate that DV-induced Th are killed by treatment *in vitro* with the DV-induced CF thus abrogating their activity. The killing effect of CF was dose-dependent. Similar results were obained when nylon wool column enriched T cells containing Th were treated *in vitro* with CF (data not presented here). Pretreatment of the donor mice with CF abrogated the generation of the Th. CF was equally effective in killing the Th *in vivo* in a dose-dependent manner. Pretreatment of the helper cells with plasma membrane stabilizing drugs viz. reduced glutathione or ouabain protected the cells from the killing effect of CF. This indicates that CF acts on the plasma membrane of the Th as discussed elsewhere[15].

Earlier studies have shown necrosis and haemorrhage in the thymus-dependent areas of lymphoid tissue[16] and depletion of OKT4 positive cells[3] in cases of dengue haemorrhagic fever; and selective depletion of helper and effector T lymphocytes with sparing of suppressor T cells in the spleen of DV-infected mice[17]. Lymphopoenia and depletion of T lymphocytes have been observed in a number of viral infections[18], in some it is due to selective depletion of a subpopulation of T lymphocytes[19]. Besides DV infection, selective depletion of helper T cells also occurs in HIV infection but the mechanism of such cell destruction is not known. DV does not replicate in T cells[4] but still causes T cell destruction. The present study provides direct evidence of the selective destruction of the helper T cell subpopulation by the virus-induced cytotoxic protein. This could be a mechanism contributing to T cell depletion and immunosuppression in other viral infections.

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ANNOUNCEMENTS

THE SECOND ASIAN FISHERIES FORUM

After the great success of the First Asian Fisheries Forum in Manila in 1986, the Second Asian Fisheries Forum will be held at the Central Administration Building of Nihon University in Tokyo from 18 to 21 April, 1989. The Fisheries Forum is open to all scientists, technicians and others interested in topics related to fisheries and aquaculture in the Asian region. The implications of this breakdown are that broader economic, social or institutional constraints are operating and that these constraints to effective fisheries management will not simply vanish by pointing to the good biological research conducted.

The scientific programme will include plenary lectures, verbal and poster presentations. The official language of the Forum is English. Several simultaneous sessions will be held on the following subjects: Aquaculture systems—monoculture integrated farming, polyculture, Capture fisheries—development, gear and method, management and resources assessment. Diseases, Environmental issues—oceanography, pollution, red tides, toxicology, Fisheries education, Genetics and reproduction, Life history, Nutrition, Physiology, Post-harvest and marketing, Socioeconomics.

Further particulars may be had from: The Secretariat, The Second Asian Fisheries Forum, Department of Fisheries, Faculty of Agriculture. The University of Tokyo, Yayoi 1–1–1 Bunkyoku, Tokyo 113, Japan.

INSTITUTION OF CHEMISTS (INDIA): ASSOCIATESHIP EXAMINATION, 1989

The 39th Associateship Examination of the Institution of Chemists (India) will be held in November. The last date for Registration is 30 November 1988. The Examination in applied analytical chemistry is divided into the following 11 sections and each candidate will be examined in two of them according to his choice as approved by the Council, in addition to general chemistry including organic, inorganic, physical and applied analytical chemistry:— (i) Analysis of minerals, silicates, ores and alloys; (ii) Analysis of drugs and pharmaceuticals; (iii) Analysis of foods; (iv) Analysis of water and sewage; (v) Biochemical analysis; (vi) Analysis of oils, fats and soaps; (vii) Fuel and gas analysis; (viii) Analysis of soils and fertilizers; (ix) Analysis connected with forensic chemistry; (x) Analysis connected with leather chemistry; (xi) Analysis connected with textile chemistry. The examination is recognized by the Government of India as equivalent to M.Sc. in chemistry for purposes of recruitment of Chemists. Further enquiries regarding this and for Membership may be made to the Honorary Secretary, Institution of Chemists (India), 11/4, Dr Biresh Guha Road, Calcutta 700 017.