voltage was found to change from -2.4 mV/K to -3.5 mV/K in case of 1N4007 Si diode when the current was changed from 200 μA to 10 nA. The current dependence of the sensitivity and $[dV/(nE_g/2)\,dT]$ as $E_g/2$ is independent of current, are shown in Figure 5. As for 1N4007 Si diode, the change of $nE_g/2$ is little with respect to current, both the quantities seem to vary linearly (Figure 5 *a*). Of course, the match is excellent in the latter case. Similarly, in case of the Cryo diode (Figure 5 *b*), it is noticed from the (dV_f/dT) vs $\ln I$ that the data points are quite away from a straight line, whereas when plotted as $[dV_f/(nE_g/2)dT]$, the data points fit reasonably to a straight line as expected from eq. (4).

In order to test the T^* dependence of the current, we define V_f^* as the voltage that corresponds to T^*. At $T = T^*$, eq. (2) becomes

$$\frac{nE_g/2 - V_f^*}{nT^*} = \frac{k_B}{e}\left[\log Q + \tfrac{5}{2}\log T^* - \log I\right]. \qquad (5)$$

Eq. (5) implies that the quantity in the left hand side is logarithmic in I. The procedure to obtain T^* is described in ref. 7. In order to verify experimentally, we have plotted $(nE_g/2 - V_f^*/(nE_g/2)T^*)$ vs $\ln I$ for 1N4007 Si diode and cryo diode (Figure 6). Interestingly, the variation is found to be linear for both the diodes. Similar investigations on different diodes of the same batches confirm the result qualitatively.

The most interesting feature associated with the forward characteristics that the band gap can be determined by extrapolating V_f–T curve to 0 K (ref. 10). In the case of 1N4007 Si diode, though $nE_g/2$ varies logarithmically with current, the slope is too small, i.e. all the V_f–T curves meet almost at a point to give the value of $E_g = 1.17$ eV. But, in case of cryo diode, the slope is different in different current regime. It is noted from Figure 4 that in regime II, the slope is of the same order of magnitude as in the case of 1N4007 Si diode and can be used for the determination of the band gap. Following this procedure, the value of E_g for silicon is found to be 1.189 eV, which is very close to the earlier reported value[10] and within 2% that obtained for 1N4007 diode.

In conclusion, the current dependence of ideality factor (n) is found to be different for different silicon diodes. The current dependence of sensitivity (dV_f/dT) and that of crossover temperature (T^*) are understood by taking the current dependence of n into account. The procedure for the determination of the band gap is also presented.

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CD4 positive T-cells produce cytotoxic factor in cases of dengue haemorrhagic fever

Renu Agarwal, U. C. Chaturvedi*, A. Misra, S. Kapoor, R. Nagar and R. Tandon

Postgraduate Department of Microbiology, K.G. Medical College, Lucknow 226 003, India
*Present address: Department of Microbiology, Faculty of Medicine, Kuwait University, PO Box 24923, Safat 13110, Kuwait

During dengue virus infection of mice and man, an unique cytokine, cytotoxic factor (CF), is produced which reproduces various pathological lesions in mice that are seen in human cases of dengue fever (DF) and dengue haemorrhagic fever (DHF). The present study was undertaken on 15 cases of DF/DHF and 5 normal healthy control individuals to find out the cell responsible for the production of the human cytotoxic factor (hCF). Peripheral blood mononuclear cells (PBMC) or their enriched subpopulations of monocytes, B- or T-cells were cultured for 24 h and the culture supernatants were assayed for the presence of hCF by ELISA, dot blot and cytotoxicity assay. The findings showed that hCF was produced by T-cells and not by the monocytes or B-cells. Pretreatment of the T-cells with anti-CD4 antibody abrogated the production of hCF while anti-CD8 antibody had no effect. Presence of hCF-specific mRNA was found by Northern hybridization in the CD4 positive T-cells only of the cases and not in the CD8 positive T-cells, B-cells or monocytes, thus confirming, for the first time, production of hCF by CD4 positive T-cells.

The cytotoxic factor is a unique cytokine that is produced in mouse (mCF) and man (hCF) during dengue virus infection only[1–3]. The amino terminal sequence of mCF has no homology with any known protein or cytokine as shown by matching at the data base till 1996. mCF

is a pathogenesis-associated protein, capable of reproducing all the pathological lesions in mice that are seen in cases of human dengue[4-6]. hCF purified from the sera of the cases of dengue fever (DF) or dengue haemorrhagic fever (DHF) is similar to mCF in all the properties, including the pathogenic effects on mice[3,7]. In an experimental study, the cultures of human peripheral blood mononuclear cells (PBMC) were stimulated with dengue type 2 virus to investigate *in vitro* production of hCF. These experiments showed that hCF is generated *in vitro* by the CD4 positive T-cells[8]. Such *in vitro* experiments are useful but may not truly represent the actual response *in vivo*. Therefore, it was considered necessary to confirm the finding in actual patients. The epidemic of DHF at Lucknow during 1996 provided an opportunity to investigate this aspect in a more realistic manner by *ex vivo* culture of PBMC from such cases. The findings presented here show that hCF is produced by CD4 positive T-cells in cases of DF/DHF.

The study was carried out on 15 cases admitted to the Gandhi Memorial and associated hospitals, Lucknow during the epidemic of DHF in November 1996. The diagnosis of dengue virus infection was made by isolation of dengue type 2 virus from two of the cases, or by demonstration of specific IgM (data to be published). On the basis of thorough clinical and laboratory examination, the cases were classified as DF and DHF grades I to IV according to the criteria of the World Health Organization[9]. For the present study, three cases of each category were taken (total 15 cases) and 5 normal healthy controls who had not suffered any illness in last three months were also included. From each individual; peripheral venous blood was collected in a heparinized tube and leucocyte-rich plasma was removed. The peripheral blood mononuclear cells (PBMC) consisting of monocytes and lymphocytes were separated by layering the leucocyte-rich plasma over Lymphoprep, density 1.077 *g*/ml (Nyegaard & Co., Oslo, Norway) and centrifuged. The interface layer was collected and the cells were washed thrice with Hank's basal salt solution as described earlier[8]. The PBMC were passed through glass wool column to remove monocytes and the dead cells and then filtered through nylon wool column to prepare enriched T- and B-cell subpopulations[10] and their purity was checked as described earlier[8,11]. To prepare monocytes-enriched cells the PBMC were suspended in Eagle's minimum essential medium (MEM) containing 5% foetal calf serum and layered in 4-cm petri dishes (Nunclon, Nunc, Denmark) and incubated at 37°C in presence of 5% CO_2 in air. After 2 h, the adherent cells were separated and their purity tested[12]. The T-cells were further enriched into CD4 or CD8 positive cells by treatment with mouse anti-human CD4 or CD8 monoclonal antibodies (mAB; Dako A/S, Glostrup, Denmark) and rabbit complement as described earlier[8]. The cells obtained by different procedures described above were washed and the count was adjusted to 5×10^6 cells/ml. The cells were cultured at 37°C for 24 h in the presence of 5% CO_2 in air. Then the cell-free culture supernatant (CS) was collected after centrifugation at 3000 *g* for 10 min. The CS obtained from the cases and the controls were screened for the presence of hCF using cytotoxicity assay, enzyme linked immunosorbent assay (ELISA) and the dot blot tests with appropriate controls as described elsewhere[8,13]. For ELISA and the cytotoxicity tests the mean value ± 2 SD obtained from the CS of the cells from normal healthy controls was considered as 'cut-off' value. hCF-specific mRNA was studied in the cells by Northern blot hybridization. The RNA was purified from the cells and 5 µg of it from each individual or one mouse spleen (for controls) was blotted on nitrocellulose membrane and hybridized using oligonucleotide probe as described earlier[8]. Statistical analysis of the data was done using Student's *t* test and a *p* value of less than 0.05 as considered significant.

Table 1 shows the presence of hCF in the CS obtained from the cells of the cases of dengue virus infection by all the three tests. A significant cytotoxic activity was found in CS of the total PBMC and the T-cells but not in those of monocytes or B-cells, indicating that hCF was produced by the T-cells. The findings in cases of different grades of illness, viz. DF or DHF grades I to IV were similar ($p > 0.05$) and their number in each group was small, therefore, they have not been

Table 1. *Ex-vivo* production of hCF by peripheral blood T-cells

	Cytotoxicity#		ELISA*		Dot blot@	
Cells	Cases	Controls	Cases	Controls	Cases	Controls
PBMC	20 ± 2	6 ± 2	78 ± 5	22 ± 3	+	–
Monocytes	3 ± 1	3 ± 1	15 ± 3	16 ± 4	–	–
B cells	5 ± 2	7 ± 2	12 ± 6	18 ± 4	–	–
T cells	25 ± 3	4 ± 2	80 ± 7	15 ± 5	+	–

Peripheral blood mononuclear cells (PBMC) or their enriched subpopulations of monocytes, B- or T-cells obtained from 15 cases of DF/DHF (cases) and 5 normal healthy control individuals (controls) were cultured in triplicate (5×10^6 cells/ml) at 37°C in presence of 5% CO_2 for 48 h. The culture supernatants were collected and assayed for the presence of hCF by ELISA, dot blot and cytotoxicity tests. The data has been presented as mean values ± SD.
#Per cent target cells killed; *, Per cent inhibition; @, + Positive, – Negative.

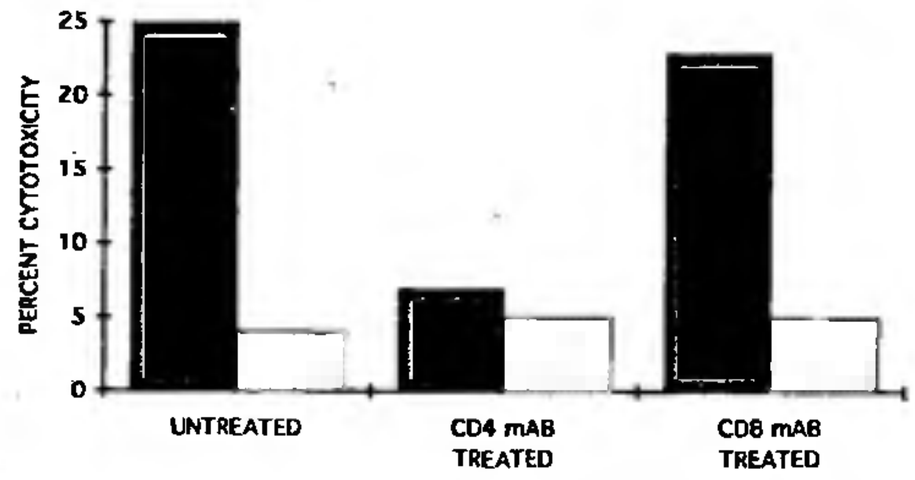

Figure 1. Cytotoxicity of culture supernatants (CS) from T-cell-enriched subpopulation pretreated with anti-CD4 or anti-CD8 antibody and complement. The cells were obtained from peripheral venous blood of normal healthy controls (□) and the cases of DF/DHF (■).

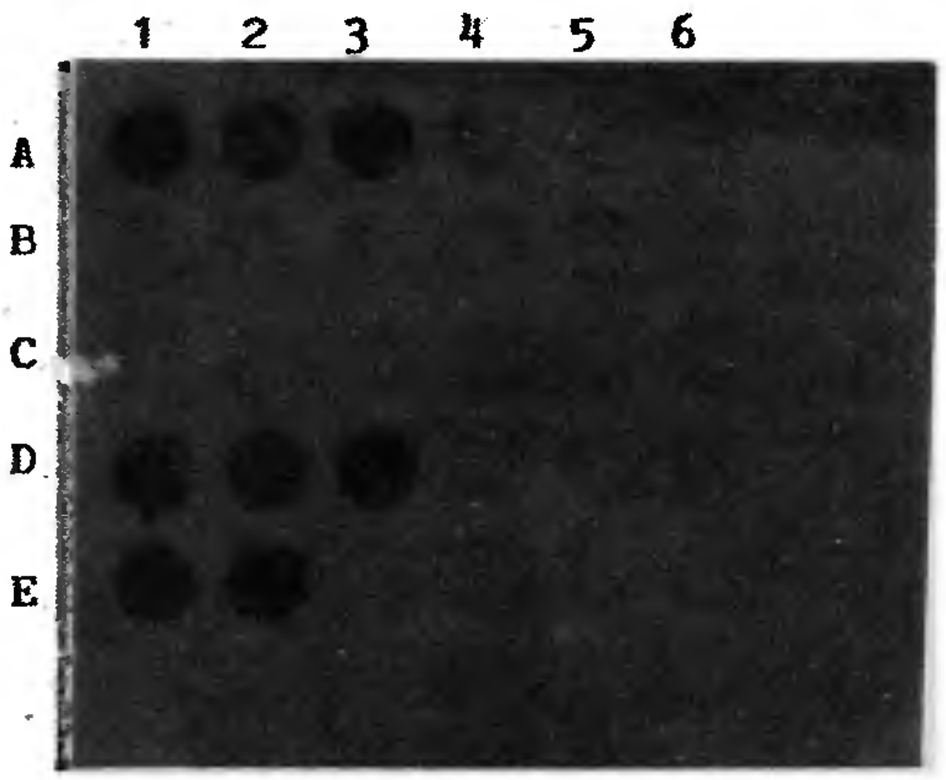

Figure 2. Northern blot hybridization analysis or total RNA from the PBMC of the cases of DF/DHF and the normal healthy controls. T-cells from the cases (lane A 1–3) or controls (lane A 4–6); B-cells from the cases (lane B 1–3) or controls (lane B 4–6); Monocytes from the cases (lane C 1–3) or controls (lane C 4–6); Total PBMC from the cases (lane D 1–3) or controls (lane D 4–6); Positive control, RNA from the dengue virus-infected mouse spleen (lane E 1–2) or negative control mice (lane E 3–6).

presented separately. Further experiments showed that the production of hCF was abrogated by pretreatment of the T-cells by anti-CD4 antibodies but not by the anti-CD8 antibodies (Figure 1). Pretreatment of the T-cells with a heterologous antibody, normal sera or complement alone for controls, had no effect. This was further confirmed by demonstration of hCF-specific mRNA in the T-cell-enriched subpopulation of the cases by Northern hybridization. The monocytes and B-cells from the cases of dengue and the cells from the normal healthy controls did not show the presence of mRNA for hCF (Figure 2).

The results show that hCF was produced by CD4 positive T-cells in the cases of DF/DHF. This conclusion was drawn from the findings of ELISA, dot blot and cytotoxicity assay of CS obtained from the cases of DF/DHF, for example, hCF activity was similar in CS from total PBMC and T-cell-enriched (depleted of monocytes and B-cells) subpopulations ($p > 0.005$) while the CS from B-cells or monocytes-enriched subpopulations had no hCF activity ($p > 0.001$). Abrogation of the production of hCF by pretreatment of T-cells with anti-CD4 antibody and not with anti-CD8 antibody confirmed this conclusion. This is in conformity with the production of mCF in mice by L_3T_4 (= human CD4) cells[1,14] and by *in vitro* stimulation of human CD4 positive T-cells with dengue type 2 virus[8]. A much more sensitive and specific technique is detection of cytokine by the expression of mRNA by *insitu* hybridization[15]. Expression of mRNA for hCF in PBMC and T-cells of the present series of cases of DF/DHF has further strengthened this conclusion. During the present epidemic we have observed presence of hCF in over 90% of the 333 sera tested (R. Agarwal *et al.*, unpublished data), which indicates that CD4 positive T-cells produce hCF in detectable amounts in majority of the cases of DF/DHF. hCF has been shown to increase the capillary permeability and damages the blood–brain barrier in mice[3]. Studies are underway to determine the precise role of hCF in the pathogenesis of DHF in man.

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