DENGUE VIRUS INFECTION OF HUMAN T LYMPHOCYTES

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Summary. – We have previously reported (Kurane *et al., Arch. Virol.* ***110,*** 91–101, 1990) that human T cell leukemia and lymphoma cell lines could be infected with dengue virus. In the present study we examined whether human T lymphocytes which are not leukemia or lymphoma cells can be infected with dengue virus. Dengue virus-specific human $CD4^+$ T cell clones, JK44 and JK49, and $CD8^+$ T cell clones, CB2.8 and CB6.17, were infected with dengue-2 virus. Dengue virus antigen-positive cells were detected in each of the four T cell lines by the immunofluorescence staining 20 – 60 hrs post infection (p.i.). Infectious dengue virus was detected in the culture fluids 40 hrs p.i. These results demonstrate that activated $CD4^+$ and $CD8^+$ human T lymphocytes can be infected with dengue-2 virus.

Key words: dengue virus; human T cell lines

Dengue virus infections are a serious health problem in Southeast Asia, Central and South America (Monath, 1994). Dengue viruses belong to the family *Flaviviridae*, genus *Flavivirus*, and are transmitted to humans by mosquitoes. Dengue virus infection causes two forms of syndromes, dengue fever (DF) and dengue haemorrhagic fever (DHF). DF is a self-limited febrile disease, while DHF is a life threatening illness with plasma leakage, haemorrhagic manifestations and shock (Halstead, 1988).

The pathogenesis of DHF has not been elucidated. It is believed that following feeding by mosquitoes, virus enters the blood stream via lymphatics (Monath, 1985) and that monocytes in peripheral blood mononuclear cells (PBMC) mainly support the virus infection (Halstead, 1980). However, it is possible that other types of cells in PBMC also support dengue virus infection. In the present study we examined whether human T cells can be infected with dengue virus, using non-leukemic or non-lymphoma T lymphocytes.

Dengue virus-specific human $CD4^+$ clones, JK44 and JK49, and $CD8^+$ T cell clones, CB2.8 and CB6.17, were used in the experiments. The establishment and characterization of these T cell clones were previously reported (Kurane *et al.,* 1991a; Livingston *et al.,* 1995). The T cell clones were maintained by periodic stimulation with dengue antigen in the presence of gamma-irradiated autologous PBMC in medium AIM (Gibco Laboratories) containing 10% foetal calf serum (FCS).

Dengue-2 virus, New Guinea C strain, was used for infection. The virus was propagated in *Aedes albopictus* (mosquito) cells (C6/36) as previously described (Kurane *et al.,* 1984). The titer of the virus stock used in the experiments was 1×10^8 PFU/ml in CV-1 cells (Mady *et al.,* 1991).

The cells (1×10^6) were infected with dengue-2 virus at a multiplicity of infection (MOI) of 20 PFU/cell at 37°C for 2 hours. The cells were cultured in AIM medium containing 10% FCS. The cells were stained for the presence of viral antigens by indirect immunofluorescence using hyperimmune mouse ascitis fluid to dengue-2 virus 20, 40 and 60 hrs p.i. as previously reported (Kurane and Ennis, 1987). The culture supernatants were collected 40 hrs p.i. and virus titers were determined. The amount of infectious virus produced by each T cell line was determined by the plaque assay in CV-1 cells (Mady *et al.,* 1991).

$CD4^+$ and $CD8^+$ human T cell clones were infected with dengue-2 virus as described above, and the percentage of

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Abbreviations: DF = dengue fever; DHF = dengue haemorrhagic fever; FCS = foetal calf serum; MOI = multiplicity of infection; PBMC = peripheral blood mononuclear cells; p.i. = post infection

Table 1. Percentage of dengue virus antigen-positive cells in CD4⁺ and CD8⁺ human T cell clones after infection with dengue-2 virus

T cell clones	Dengue virus antigen-positive cells (%)		
	20 hrs	40 hrs	60 hrs
JK44 (CD4⁺)	0.5	0.8	2.9
JK49 (CD4⁺)	ND	6.8	ND
CB.2 (CD8⁺)	0.4	0.5	1.7
CB6.17 (CD8⁺)	8.8	12.5	ND

Cells were infected with dengue-2 virus at MOI of 20 PFU/cell. ND = not done.

dengue virus antigen-positive cells was determined by the immunofluorescence staining. Dengue virus antigen-positive cells were detected in the CD4⁺ human T cell clones (JK44 and JK49) and CD8⁺ human T cell clones (CB2.8 and CB6.17) 40 hrs p.i. (Table 1). The percentage of dengue virus antigen-positive cells varied depending on the T cell clone. The infectious dengue virus titers in the culture supernatants 40 hrs p.i. were 3.2 x 10^3 PFU/ml for JK44, 6.1 x 10^3 PFU/ml for JK49, 2.7 x 10^4 PFU/ml for CB2.8 and 4.1 x 10^4 PFU/ml for CB6.17 cells.

It is generally accepted that monocytes are the cells which predominantly support a dengue virus infection *in vivo* (Halstead *et al.*, 1977). Dengue virus antigen-positive monocytes have been detected in humans with DHF (Boonpucknavig *et al.*, 1976). We have, however, previously reported that human T and B cell leukemia and lymphoma cell lines could be infected with dengue-2 virus *in vitro* (Kurane *et al.*, 1990). In the present study we showed that CD4⁺ and CD8⁺ human T cell clones, which are not leukemia or lymphoma cell lines, could also be infected with dengue-2 virus. Theofilopoulos *et al.* (1976) reported that T cells could not be infected with dengue-2 virus, 16681 strain, at MOI of 0.05 $TCID_{50}$/cell. Our success in infecting human T cell lines may be due to particular strains of dengue-2 virus or to a higher MOI. We used the New Guinea C strain at MOI of 20 PFU/cell in the present experiments.

Scott *et al.* (1980) has reported an isolation of dengue virus from a non-adherent fraction of PBMC of dengue patients. Our results along with those reported by Scott *et al.* (1980) suggest that activated human T lymphocytes support the replication of dengue-2 virus and probably other serotypes of dengue viruses. It has been reported that T lymphocytes are highly activated in PBMC of the patients with DHF (Kurane *et al.*, 1991b). Thus, these activated T lymphocytes may support dengue virus infection *in vivo* as well as monocytes/macrophages in patients with DHF. Furthermore, these activated, dengue virus-infected T lymphocytes may be lysed by dengue virus-specific cytotoxic T lymphocytes.

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