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Characterization with monoclonal antibodies of human lymphocytes active in natural killing and antibody-dependent cell-mediated cytotoxicity of dengue virus-infected cells

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SUMMARY

Non-immune human peripheral blood lymphocytes (PBL) lyse dengue virus-infected cells to a greater degree than uninfected cells. In the present study, the PBL active in lysing dengue virus-infected Raji cells are characterized using monoclonal antibodies and are compared to lymphocytes that lyse K562 cells. Leu11$^+$ cells lyse dengue virus-infected cells and K562 cells. Leu11$^-$ cells lyse dengue virus-infected cells, but not K562 cells. In the Leu11$^+$ fraction, Leu11$^+$Leu7$^-$ cells are more active than Leu11$^+$Leu7$^+$ cells in lysing dengue virus-infected cells. T3$^+$ cells also lyse dengue virus-infected cells, but they do not lyse K562 cells. T3$^-$ cells lyse both target cells. These results, along with the observation that Leu11$^+$ cells and T3$^+$ cells are different subsets of PBL, indicate that the PBL that are active in lysing dengue virus-infected cells are heterogeneous and are contained in Leu11$^+$ and T3$^+$ subsets. Leu11$^+$ cells are more active than T3$^+$ cells. Leu11$^+$ cells are active in lysing dengue virus-infected cells by antibody-dependent cell-mediated cytotoxicity, whereas T3$^+$ cells are not active.

INTRODUCTION

Dengue virus infection can cause a haemorrhagic fever (DHF) and shock syndrome (DSS), major health problems in subtropical and tropical areas of the world (Halstead, 1980). These complications are more commonly observed during secondary dengue virus infections caused by a dengue virus of another serotype than that which caused the primary infection (Halstead, 1981), and it is speculated that immune responses may cause these complications of dengue virus infection (Russell & Brandt, 1973; Halstead, 1981). Although it has been postulated that these complications may be due to the presence of antibodies that form immune complexes with viral antigens, resulting in complement activation (Russell & Brandt, 1973), or that enhance viral replication in macrophages (Halstead, 1979; Doughaday *et al.*, 1981), the mechanism has not been defined. Primary infection ordinarily occurs without haemorrhagic fever or shock, and most patients recover without complications (Halstead, 1980). This implies that host defence mechanisms usually effect recovery from dengue virus infection, but little is known about such mechanisms.

Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; FACS, fluorescence-activated cell sorter; NK, natural killer; PBL, peripheral blood lymphocytes; PBMC, peripheral blood mononuclear cells.

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Recently, we reported that dengue virus-infected cells were lysed by human peripheral blood mononuclear cells (PBMC) to a greater degree than uninfected cells in natural killer (NK) assays, and that the predominant effector cells were contained in the non-adherent fraction of PBMC (Kurane *et al.*, 1984). These observations raised questions that are addressed in this report. What are the characteristics of the lymphocytes that lyse dengue virus-infected cells? Are they similar to or different from NK cells that lyse K562 cells?

In this report, we define the lymphocytes active in killing dengue virus-infected cells in comparison with the NK cells that kill K562 cells, using a variety of monoclonal antibodies. We also determine whether the lymphocytes with NK activity are active in antibody-dependent cell-mediated cytotoxicity (ADCC) of dengue virus-infected cells. The results demonstrate that dengue virus-infected cells are lysed by heterogeneous subsets of human lymphocytes. The most active NK effector cells of dengue virus-infected cells have Leu11 antigen and are very active in lysing K562 cells. These cells are also active in lysing dengue-infected cells by ADCC. Other lymphocytes that have T3 antigen and do not lyse K562 cells are active in lysing dengue virus-infected cells; however, these T3$^+$ lymphocytes are not active in lysing dengue virus-infected cells by ADCC.

MATERIALS AND METHODS

Human peripheral blood mononucelar cells (PBMC)

Peripheral blood specimens were obtained from healthy donors

who did not have antibodies to dengue virus as determined by a plaque-neutralization test. PBMC were prepared by the Ficoll–Hypaque density gradient centrifugation method (Boyum, 1968) and cryopreserved until use.

Cell cultures

Raji cells (Pulvertaft, 1964), Raji cells persistently infected with the New Guinea C strain of dengue 2 virus (Kurane *et al.*, 1984) and K562 cells (Lozzio & Lozzio, 1975) were used. Raji cells and K562 cells were cultured in RPMI containing 10% FCS (RPMI/10%FCS). Dengue virus-infected Raji cells were cultured in RPMI/20%FCS. More than 90% of the dengue virus-infected Raji cells expressed virus-specific membrane and cytoplasmic antigens (Kurane *et al.*, 1984). Epstein–Barr virus (EBV) antigens were not detected on the membrane of Raji cells or dengue virus-infected Raji cells by using human serum positive for anti-EBV antibody.

Anti-dengue 2 antibody

Ascitic fluid from mice hyperimmunized with dengue virus type 2 was used as anti-dengue 2 antibody. This antibody was supplied by Dr Walter E. Brandt of Walter Reed Army Institute of Research, Washington, DC. The titre of this antibody was 1:1024 in a plaque-neutralization test. Hyperimmune ascitic fluid was heated at 56° for 30 min to destroy complement activity before use.

Monoclonal antibodies and other reagents

Phycoerythrin (PE)-conjugated anti-Leu1 (Engleman *et al.*, 1981), anti-Leu7 (Abo & Balch, 1981), biotin-conjugated anti-Leu7, fluorescein isothiocyanate (FITC)-conjugated anti-Leu11a (Lanier *et al.*, 1984) and anti-Leu11b (Thompson *et al.*, 1982) antibodies, and PE-conjugated avidin were purchased from Becton Dickinson Co. (Mountain View, CA). OKM1 (Bread *et al.*, 1980) and OKT3 (Kung *et al.*, 1979) antibodies were purchased from Ortho Diagnostic Co. (Raritan, NJ). FITC-conjugated sheep anti-mouse IgG antibody and FITC-conjugated sheep anti-mouse IgM antibody were purchased from Cappel Laboratories (West Chester, PA).

Two-colour immunofluorescence staining

Peripheral blood lymphocytes (PBL), 1×10^6 in 0·1 ml of RPMI/10% FCS, were incubated with 5 μl amounts of FITC-conjugated anti-Leu11a and PE-conjugated anti-Leu1 antibodies at 4° for 30 min. Cells were washed twice and resuspended in ml of RPMI/10% FCS.

For indirect fluorescence staining, 1×10^6 PBL in 0·1 ml of MEM/10% FCS were incubated with 5-μl amounts of FITC-conjugated anti-Leu11a and biotin-conjugated anti-Leu7 antibodies at 4° for 30 min. Cells were washed once, suspended in 0·1 ml of MEM/10% FCS, incubated with 5 μl of PE-conjugated avidin at 4° for 30 min, washed twice and resuspended in 1 ml of MEM/10% FCS.

Stained lymphocytes were analysed with a fluorescence-activated cell sorter (FACS 440, Becton-Dickinson Co.).

Cell separation by FACS

Adherent cells were depleted from PBMC by adherence to plastic dishes before cells were sorted. The percentage of monocytes contained in non-adherent fractions was 3% as determined by yeast ingestion (Kumagai *et al.*, 1979).

Non-adherent cells (PBL) were stained by indirect membrane immunofluorescence (Kurane *et al.*, 1984). Lymphocytes were sorted with a FACS 440. Cell viability after sorting exceeding 95% using a trypan blue dye exclusion test. The purity of each of the sorted populations was greater than 97%.

Treatment of PBL with monoclonal antibodies and complement

PBL were suspended, 1×10^7 in 1·0 ml of RPMI/1% FCS containing monoclonal antibody (1:10 dilution), and incubated at room temperature for 45 min. After being washed, PBL were incubated with rabbit complement (Cedarlane Laboratories, Ontario, Canada) diluted 1:3 in RPMI/1% FCS at 37° for 1 hr. The cells were then suspended in PBS at room temperature for 15 min, washed twice, resuspended in RPMI/10% FCS, and used as effector cells. After this treatment, less than 2% of residual cells reacted with the monoclonal antibody that was added with complement.

NK activity and ADCC assay

NK and ADCC functions were examined by a specific ^{51}Cr-release assay using dengue virus-infected Raji cells, uninfected Raji cells and K562 cells as targets. The methods and formula for calculating specific cytolysis have been described previously (Kurane *et al.*, 1984). The specific ^{51}Cr-release in this report was determined after 18 hr of cultivation. Spontaneous release from dengue virus-infected Raji cells and uninfected Raji cells was about 20% of maximum release. Specific lysis of the target cells by ADCC is expressed as ΔX. ΔX indicates the percentage lysis of target cells by PBL with antibody (ADCC assay) minus the percentage lysis of target cells by PBL without antibody (NK assay).

Statistical analysis

The significance of differences between values was examined by the Student's *t*-test and Fisher's exact probability test. Differences yielding P values of $< 0{\cdot}05$ were regarded as significant.

RESULTS

Characterization of effector cells using anti-Leu11 antibody

We previously reported that PBL of non-immune donors lyse dengue virus-infected cells to a greater degree than uninfected cells (Kurane *et al.*, 1984). The effector cells were first analysed using anti-Leu11 antibody because Leu11 antigen had been reported to be expressed on essentially all functional NK cells in the peripheral blood (Lanier *et al.*, 1984). Treatment of PBL with anti-Leu11b antibody and complement decreased the level of lysis of dengue virus-infected cells and K562 cells. The percentage decrease in the level of lysis of K562 cells (94% on average) was significantly greater than that in the level of lysis of dengue virus-infected cells (58% on average) ($P < 0{\cdot}002$). PBL depleted of Leu11$^+$ cells from most donors did not lyse K562 cells but did lyse dengue virus-infected cells to a low but significant level (Table 1, Exp. 1). FACS-sorted Leu11$^+$ cells were then used as effector cells. Leu11$^+$ cells were active in lysing dengue virus-infected cells and K562 cells. Leu11$^-$ cells did not lyse K562 cells but did lyse dengue virus-infected cells to some level (Table 1, Exp. 2). These results indicate that Leu11$^+$ PBL are the most active effector cells in lysing dengue virus-infected cells and K562 cells, and that the Leu11$^-$ fraction also contains some effector cells that are active in lysing dengue virus-infected cells but that are not active in lysing K562 cells.

Table 1. Lysis of dengue virus-infected cells by Leu11$^+$ PBL and Leu11$^-$ PBL

	Donor	Effector cells	E/T ratio	% specific ^{51}Cr-release†		
				Dengue-infected Raji	Uninfected Raji	K562
Experiment 1	D	C	40	64	11	39
			20	46	8	20
		Anti-Leu11+C	40	12***	0***	0***
			20	7***	0***	0***
	F	C	50	41	17	42
			25	27	9	27
		Anti-Leu11+C	50	28*	9*	1***
			25	14***	2**	1***
	G	C	30	34	19	54
			15	29	14	30
		Anti-Leu11+C	30	23*	9**	8***
			15	12*	7**	5***
	H	C	40	27	15	61
		Anti-Leu11+C	40	13*	4***	6***
	P	C	15	21	10	12
			7·5	11	2	8
		Anti-Leu11+C	15	7**	1*	1***
			7·5	6*	1****	1**
	Z	C	40	52	11	39
			20	40	7	17
		Anti-Leu11+C	40	17***	2*	2***
			20	12***	1***	0***
Experiment 2	F	Unfractionated	40	32	11	49
			20	21	7	31
		Leu11$^+$	40	ND	ND	ND
			20	16	6	62
		Leu11$^-$	40	10	3	1
			20	8***	2****	1***

*$P<0·05$.
**$P<0·01$.
***$P<0·001$.
****$P\geqslant 0·05$ (not significant).

† In Experiment 1, PBL were treated with complement (C) alone or anti-Leu11b antibody and C, and then used as effector cells. Significance was determined by Student's *t*-test between the levels of lysis by PBL treated with C alone and that by PBL treated with anti-Leu11b and C at the same E/T ratio on the same target cells. In Experiment 2, PBL were sorted on a FACS after staining with anti-Leu11b and FITC-labelled anti-mouse IgM. Significance was determined by Student's *t*-test between the level of lysis by Leu11$^+$ cells and that by Leu11$^-$ cells at the same E/T ratio on the same target cells.

Characterization of the most active Leu11$^+$ cells using anti-Leu7 antibody

We tried to characterize the Leu11$^+$ cells, which contained the most active effector cells, using anti-Leu7 antibody. We found using double-staining analysis with the FACS that Leu11$^+$ cells contained Leu7$^+$ cells and Leu7$^-$cells (data not presented) as previously reported by Lanier *et al.* (1983). FACS-sorted Leu7$^+$ cells and Leu7$^-$ cells lysed dengue virus-infected cells and K562 cells (Table 2, Exp. 1). PBL were also sorted into Leu11$^+$Leu7$^+$ and Leu11$^+$Leu7$^-$ fractions with the FACS. Although both Leu11$^+$Leu7$^+$ cells and Leu11$^+$Leu7$^-$ cells dengue virus-infected cells and K562 cells, Leu11$^+$Leu7$^-$ cells were significantly more active in lysing dengue virus-infected cells than Leu11$^+$Leu7$^+$ cells (Table 2, Exp. 2).

Lysis of dengue virus-infected cells by M1$^+$ PBL

We also characterized the effector cells using OKM1 antibody. M1 antigen has been reported to be expressed on NK cells (Ortaldo *et al.*, 1981). Treatment of PBL with OKM1 and complement decreased the level of lysis of dengue virus-infected cells and K562 cells (Table 3, Exp. 1). FACS-sorted M1$^+$ PBL lysed these target cells, but M1$^-$ PBL did not lyse the target cells (Table 3, Exp. 2).

Table 2. Lysis of dengue virus-infected cells by PBL sorted with anti-Leu11 and -Leu7 antibodies

	Donor	Effector cells	E/T ratio	% specific ^{51}Cr-release†		
				Dengue-infected Raji	Uninfected Raji	K562
Experiment 1	F	Unfractionated	20	30	5	29
			10	22	2	21
		Leu7$^+$	20	14	2	29
			10	ND	ND	ND
		Leu7$^-$	20	31**	4*	32****
			10	23	3	19
Experiment 2	B	Unfractionated	20	21	9	77
			10	16	6	59
		Leu11$^+$Leu7$^+$	20	ND	ND	ND
			10	18	8	87
		Leu11$^+$Leu7$^-$	20	43	19	95
			10	36***	15**	94****

*$P<0.05$.
**$P<0.001$.
***$P<0.001$.
****$P\geqslant 0.05$ (not significant).
† Significant was determined by Student's t-test between the level of lysis of target cells by Leu7$^+$ cells and that by Leu7$^-$ cells, and between the level of lysis by Leu11$^+$Leu7$^+$ cells and by Leu11$^+$Leu7$^-$ cells at the same E/T ratio on the same target cells.

Table 3. Lysis of dengue virus-infected cells by M1$^+$ PBL

	Donor	Effector cells	E/T ratio	% specific ^{51}Cr-release†		
				Dengue-infected Raji	Uninfected Raji	K562
Experiment 1	E	C	35	23	14	45
		OKM1+C	35	4***	2***	1***
	P	C	40	26	5	41
		OKM1+C	40	11***	0*	10***
Experiment 2	A	Unfractionated	10	11	3	4
			5	6	2	3
		M1$^+$	10	21	5	26
			5	12	4	13
		M1$^-$	10	3***	1****	1***
			5	2***	1****	1***
	F	Unfractionated	20	15	5	19
		M1$^+$	20	15	9	37
		M1$^-$	20	2**	0***	1***

*$P<0.05$.
**$P<0.01$.
***$P<0.001$.
****$P\geqslant 0.05$ (not significant).
† In Experiment 1, PBL were treated with complement (C) alone or OKM1 and C, and then used as effector cells. Significance was determined by Student's t-test between the level of lysis by PBL treated with C alone and that by PBL treated with OKM1 and C at the same E/T ratio on the same target cells. In Experiment 2, PBL were sorted on a FACS after staining with OKM1 and FITC-labelled anti-mouse IgG. Significance was determined by Student's t-test between the level of lysis by M1$^+$ cells and that by M1$^-$ cells at the same E/T ratio on the same target cells.

Table 4. Lysis of dengue virus-infected cells by T3$^+$ cells and T3$^-$ cells

	Donor	Effector cells	E/T ratio	% specific ^{51}Cr-release† Infected Raji	Uninfected Raji	K562
Experiment 1	A	Unfractionated	10	20	5	9
			5	15	2	3
		T3$^+$	10	16‡	1	1‡
			5	7‡	1	1‡
		T3$^-$	10	18****	5*	33***
			5	14****	3****	21***
Experiment 2	F	Unfractionated	20	20	9	34
			10	16	4	27
		T3$^+$	20	8‡	4	0‡
			10	6‡	1	0‡
		T3$^-$	20	33***	18**	74***
			10	ND	ND	ND

*$P<0.05$.
**$P<0.01$.
***$P<0.001$.
****$P\geqslant 0.05$ (not significant).
† Significance was determined by Student's t-test between the level of lysis by T3$^+$ cells and that by T3$^-$ cells at the same E/T ratio on the same target cells.
‡ The level of lysis of dengue-infected Raji cells by T3$^+$ cells was higher than that of K562 cells by T3$^+$ cells determined by Fisher's exact probability test ($P<0.02$).

Lysis of dengue virus-infected cells by T3$^+$ cells and T3$^-$ cells

The previous experiments using anti-Leu11 antibody gave results that indicated heterogeneity of the cytotoxic lymphocytes, i.e. Leu11$^+$ cells are active in lysing both K562 and dengue virus-infected cells and Leu11$^-$ cells are not active in lysing K562 cells but active in lysing dengue virus-infected cells (Table 1). These results stimulated us to characterize further the effector lymphocytes in the Leu11$^-$ subset.

We demonstrated that Leu11$^+$ cells did not possess a pan T antigen (Leu1) by double-staining analysis (data not presented), as previously reported by Lanier *et al.* (1983). This result indicates that the Leu11$^-$ subset contained all of the T cells that may have contributed to the lysis of dengue virus-infected cells by Leu11$^-$ cells. We sorted PBL with the FACS using OKT3 antibody, which also recognize a pan T antigen, and used them as effector cells (Table 4). T3$^-$ cells, which contain Leu11$^+$ cells, lysed dengue virus-infected cells and K562 cells. T3$^+$ cells did not lyse K562 cells; however, they lysed dengue virus-infected cells to a low but significant degree ($P<0.02$). These results indicate that T3$^+$ cells contribute to the lysis of dengue virus-infected cells by Leu11$^-$ cells shown in Table 1.

ADCC activity of Leu11$^+$ cells and T3$^+$ cells

We reported that PBL lyse dengue virus-infected cells by ADCC (Kurane *et al.*, 1984). Anti-dengue sera increased the level of lysis of dengue virus-infected cells by PBL, but did not increase that of uninfected cells. Normal mouse ascitic fluid did not increase the level of lysis of dengue virus-infected cells. Human serum positive for anti-EBV antibody but negative for anti-dengue 2 antibody did not augment the lysis of dengue-infected cells (data not presented). Therefore, the lysis by ADCC is specific for dengue virus-infected cells.

We tried to learn whether Leu11$^+$ cells and T3$^+$ cells active in lysing dengue virus-infected cells are also active in ADCC against dengue virus-infected cells. Treatment of PBL with anti-Leu11b antibody and complement decreased the level of lysis by ADCC (61% on average), and PBL from some donors were less active in ADCC after depletion of Leu11$^+$ cells (Table 5). The FACS-sorting experiments confirmed the results of complement-dependent cell-mediated experiments. FACS-sorted Leu11$^+$ cells were active in ADCC, and Leu11$^-$ cells were less active (data not presented). In experiments using FACS-sorted T3$^+$ and T3$^-$ cells as effector cells, T3$^+$ cells were not active in killing dengue virus-infected cells by ADCC, but T3$^-$ cells were active (Table 6). These results indicate that Leu11$^+$ cells are active in ADCC against dengue virus-infected cells, but T3$^+$ cells are not active in ADCC.

DISCUSSION

In this report, we have described the lymphocytes that are active in lysing dengue virus-infected target cells [NK(DV)], in comparison with the lytic activity of the same effector cells for K562 cells. Effector cells contained in Leu11$^+$, M1$^+$ and T3$^-$ fractions are the most active in lysing both dengue virus-infected cells and K562 cells. In addition to these effector cells, the T3$^+$ fraction, which does not lyse K562 cells, contains some effector cells that are active in lysing dengue virus-infected cells.

These results indicate the heterogeneity of the PBL that lyse dengue virus-infected cells. Heterogeneity of NK cells has been

Table 5. Lysis by ADCC of dengue virus-infected cells by Leu11$^+$ cells

Donor	Effector cells	E/T ratio	% specific ^{51}Cr-release†		
			+Antibody‡	−Antibody	ΔX§
E	C	50	29	19	10*
		25	23	16	7*
	Anti-Leu11+C	50	10	5	5*
		25	7	5	2****
F	C	50	53	41	12**
		25	49	27	22***
	Anti-Leu11+C	50	32	28	4****
		25	28	14	14**
G	C	30	53	34	19**
	Anti-Leu11+C	30	35	23	12*
K	C	40	25	17	8***
	Anti-Leu11+C	40	9	7	2****
Z	C	20	51	40	11*
		10	40	30	10***
	Anti-Leu11+C	20	14	12	2****
		10	8	5	3****

*$P<0.05$.
**$P<0.01$.
***$P<0.001$.
****$P\geqslant 0.05$ (not significant).

† Significant was determined by Student's t-test between the level of lysis of target cells by PBL with anti-dengue 2 antibody and that by PBL without antibody at the same E/T ratio.

‡ Hyperimmune mouse ascitic fluid was used as a source of anti-dengue 2 antibody at a 1:20 dilution.

§ ΔX indicates the percentage lysis of target cells by PBL with antibody minus the percentage lysis of target cells by PBL without antibody. Therefore, ΔX indicates the specific lysis of the target cells by ADCC.

reported previously using herpes simplex virus-infected target cells. Fitzgerald *et al.* reported that NK cells that lyse HSV-1-infected target cells [NK(HSV)] have somewhat different characteristics from NK cells that lyse K562 cells [NK(K562)], and that NK (HSV) were Leu7$^{+/-}$, Leu1$^-$ and Leu4$^-$ (Fitzgerald, Kirkpatrick & Lopez, 1982; Fitzgerald *et al.*, 1983). Hendricks & Sugar (1984) reported that NK (HSV) were Leu7$^+$ and T3$^+$. Since we may regard Leu1 and Leu4 as T3, the phenotypes of the predominant NK (DV) are generally consistent with those of the NK (HSV) reported by Fitzgerald *et al.* However, we found that T3$^+$ cells were also active in lysing dengue virus-infected cells using highly purified lymphocyte subsets sorted with the FACS. In addition, we observed that the most active NK (DV) are Leu11$^+$ and M1$^+$ cells, and using a double-staining technique we found that Leu11$^+$Leu7$^-$ cells are more lytic to dengue virus-infected cells than Leu11$^+$Leu7$^+$ cells. We recently described the human PBL active in lysing cells persistently infected with hepatitis A virus in the absence of detectable surface antigens on the cell membrane (Kurane *et al.*, 1985). The lysis was due to effector cells in the Leu11$^+$ subset, and T3$^+$ cells did not contribute to the lysis of the hepatitis A virus-infected cells. Thus, the lysis was accomplished by a homogeneous subset of Leu11$^+$ cells similar to the subset of the lymphocytes that kill K562 cells, and unlike the heterogeneous subsets of lymphocytes that are responsible for lysis of dengue virus-infected cells.

The mechanisms of increased lysis of dengue virus-infected cells remain to be elucidated. We reported results that indicated that IFN did not appear to mediate increased killing of dengue virus-infected cells by PBL (Kurane *et al.*, 1984). It has been reported that glycoprotein of measles virus and lymphocytic choriomeningitis virus induce non-specific cell-mediated cytotoxicity without induction of IFN (Casali *et al.*, 1981; Casali & Olstone, 1982). The HN glycoprotein of Sendai virus (gp 71) and the haemagglutinin and neuraminidase glycoproteins of influenza virus have also been reported to induce cell-mediated cytotoxicity (Alsheikhly *et al.*, 1983; Arora *et al.*, 1984). Recently, Alsheikhly *et al.* reported that T3$^+$ cells acquired cytolytic activity after treatment with UV-inactivated mumps virions (Alsheikhly, Andersson & Perlmann, 1985). These results suggest the possibility that glycoproteins of dengue virus expressed on the dengue virus-infected cells may be the reason that dengue virus-infected cells are lysed to a greater extent than uninfected cells, and that T3$^+$ cells, which are not active in killing K562 cells or uninfected cells, are active in killing dengue virus-infected cells.

The relationship between antibody-dependent killer (K) cells and NK cells is also a topic of interest. Most reports

Table 6. Lysis by ADCC of dengue virus-infected cells by PBL contained in $T3^-$ fraction, but not in $T3^+$ fraction

Donor	Effector cells	E/T ratio	% specific ^{51}Cr-release†		
			+Antibody‡	−Antibody	ΔX§
A	Unfractionated	10	25	20	5*
		5	15	14	1****
	$T3^+$	10	14	16	0****
		5	8	7	0****
	$T3^-$	10	26	18	8*
		5	21	14	7*
F	Unfractionated	20	30	20	10**
		10	24	16	8*
	$T3^+$	20	5	8	0****
		10	4	6	0****
	$T3^-$	20	48	33	15*
		10	ND	ND	ND

*$P<0.05$.
**$P<0.01$.
***$P<0.001$.
****$P\geq 0.05$ (not significant).

† Significance was determined by Student's t-test between the level of lysis of target cells by PBL with anti-dengue 2 antibody and that by PBL without antibody at the same E/T ratio.

‡ Hyperimmune mouse ascitis fluid was used as a source of anti-dengue 2 antibody at a 1:20 dilution.

§ ΔX indicates the percentage lysis of target cells by PBL with antibody minus the percentage lysis of target cells by PBL without antibody. Therefore, ΔX indicates the specific lysis of the target cells by ADCC.

indicate that K cells are the same cells as NK cells (Timonen, Ortaldo & Herberman, 1981; Bradley, 1982), but another report has suggested that K cells are different from NK cells (Neville, 1980) using tumour cells as targets. Our results indicate that most of the PBL that are active in the ADCC against dengue virus-infected cells are contained in the same subsets as PBL active in the NK assay; however, some PBL that are contained in $T3^+$ fraction and active in the NK assay are not active in the ADCC assay.

The heterogeneity of lymphocytes that are cytotoxic to dengue virus-infected cells suggests the possibility that some subsets of NK(DV) may be more important than others in recovery from dengue virus infections or in the pathogenesis of DHF/DSS. There is some variation in the lytic activity of PBL to dengue virus-infected cells between donors (Kurane *et al.*, 1984), and it is possible that this variation may contribute to the susceptibility of certain individuals to dengue virus infection; however, there have not been any reports of correlations between levels of cytolytic activity and the outcome of dengue virus infection. Lymphocytes that are active in HLA-restricted, cytotoxic T lymphocyte response remain to be defined in dengue infections. These investigations on the nature of lymphocytes active in lysing dengue virus-infected cells should improve the understanding of the mechanisms of the recovery from and the pathogenesis of dengue virus infections.

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