Clin. exp. Immunol. (1985) **62,** 515–524.

Cytomegalovirus infection in cardiac transplant recipients associated with chronic T cell subset ratio inversion with expansion of a Leu-7$^+$ $T_{S\text{-}C}{}^+$ subset

PATRICIA MAHER,* CAROL M. O'TOOLE,*† T. G. WREGHITT,‡ D. J. SPIEGELHALTER,§ & T. A. H. ENGLISH† * *Division of Immunology, Department of Pathology, University of Cambridge,* † *British Heart Foundation Transplant Research Group, Papworth Hospital, Papworth Everard,* ‡ *Clinical Microbiology and Public Health Laboratory, Addenbrooke's Hospital,* § *MRC Biostatistics Unit, Cambridge, UK*

(Accepted for publication 18 June 1985)

SUMMARY

Lymphocyte subsets were analysed in 18 patients during the first 3 years after cardiac transplantation. The patients received Cyclosporin A and prednisolone for maintenance immunosuppression. Serological evidence of active cytomegalovirus (CMV) infection was found in 13 cases (72%), and in 12 of these an inversion of the T helper/T suppressor-cytotoxic ratio ($T_H/T_{S\text{-}C}$) was detected. T subset inversion usually preceded the diagnostic rise in CMV antibody titre. In 69% of patients with CMV the $T_H/T_{S\text{-}C}$ ratio remained inverted throughout follow-up (245–951 days). Persistent T subset inversion was not found in all five patients who lacked serological evidence of active CMV. Chronic inversion consisted of an average increase in $T_{S\text{-}C}$ of 152% and an average decline in T_H cells of 31% as compared to CMV negative patients. The proportion of lymphoid cells reacting with a phenotypic marker for natural killer (NK) cells (Leu-7) was increased by 83%. These alterations were also reflected in the absolute numbers of cells with these markers. Two-colour immunofluorescence analysis revealed that the expanded $T_{S\text{-}C}$ population present during chronic inversion was predominantly Leu-7$^+$. As $T_{S\text{-}C}{}^+$ Leu-7$^+$ cells in healthy persons may be hyporesponsive NK cells, a sustained increase in this cell type in allograft recipients could further reduce immunocompetence, thereby predisposing to superinfection or malignancy.

Keywords immunosuppression chronic CMV infection $T_H/T_{S\text{-}C}$ inversion $T_{S\text{-}C}{}^+$ Leu-7$^+$ subset

INTRODUCTION

Two major subsets of T lymphocytes have been defined by their surface markers and functions *in vitro* (Reinherz, Meuer & Schlossman, 1983). The $T_{S\text{-}C}$, T8$^+$ or Leu-2a$^+$, subset includes cytotoxic cells functionally restricted by MHC Class I molecules; and cells which suppress T cell proliferation and help for Ig production by B cells. The T_H, T4$^+$ or Leu-3a$^+$, subset includes cells with proliferative and cytotoxic potentials restricted by MHC Class II antigens (Engleman *et al.*, 1981). T4$^+$ cells can also have amplifying or suppressing effects in T-T and T-B cell interactions (Meuer *et al.*, 1983).

Unlike $T_{S\text{-}C}$ or T_H cells the NK subset exhibits non-MHC restricted cytotoxicity. NK cells have

Correspondence: Dr C. M. O'Toole, Division of Immunology, Laboratories Block, Level 3, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QQ, UK.

been proposed as an important first line of defence against viral infection and malignancy (Herberman, 1982). In the blood of healthy adults a minority of Leu-7$^+$ cells co-express the T cell markers T8 or Leu-2a. The functional role of this cell type is unclear, however, some authors have found T8$^+$ Leu-7$^+$ cells to have weaker NK activity than T8$^-$ Leu-7$^+$ cells. As most Leu-7$^+$ cells in bone marrow also express the T8 marker it has been suggested that the T8$^+$ Leu-7$^+$ cell represents an immature NK cell (Abo, Cooper & Balch, 1982; Abo *et al.*, 1983; Tilden, Abo & Balch, 1983). However, others have found T8$^+$ cells with an NK phenotype to have comparable NK lytic activity to T8$^-$ NK$^+$ cells (Perussia, Fanning & Trinchieri, 1983). T8$^+$ Leu-7$^+$ cells have also been shown to suppress B cell differentiation (Clement, Grossi & Gartland, 1984).

Acute CMV or Epstein-Barr virus (EBV) infections are accompanied by a reversal of the normal $T_H/T_{S\text{-}C}$ ratio in the blood. This has been established in otherwise healthy persons and immunosuppressed allograft recipients (Carney, Iacoviello & Hirsch, 1983; Crawford *et al.*, 1981; Dummer *et al.*, 1984; Reinherz *et al.*, 1980; Rubin *et al.*, 1981; Schooley *et al.*, 1983; Verdonck & DeGast, 1984). T subset ratio inversion is comprised of a major increase in the number and proportion of T8$^+$ cells and a smaller fall in T4$^+$ cells. The majority of the expanded T8$^+$ subset also express HLA-DR (Ia-like) antigens suggesting a state of activation (Evans *et al.*, 1978; Winchester & Kunkel, 1979). *In vitro* the T8$^+$ Ia$^+$ subset is hyporesponsive to mitogens and alloantigens but retains suppressor activity. In convalescence the $T_H/T_{S\text{-}C}$ ratios usually revert toward normality and T8$^+$ cells regain full functional activities. However, in some allograft recipients a prolonged T subset inversion has been noted and linked to persistence of viral infection (Rubin *et al.*, 1981).

We examined the incidence and duration of $T_H/T_{S\text{-}C}$ inversion in cardiac transplant recipients. In a retrospective study persistent inversion was found exclusively in patients with serological evidence of active CMV infection. CMV infection was frequently associated with superinfection particularly with EBV. Dual marker analysis showed that in patients with chronic $T_H/T_{S\text{-}C}$ ratio inversion, the majority of cells in the expanded T8$^+$ subset expressed the NK cell marker Leu-7.

MATERIALS AND METHODS

Patients. Lymphocyte marker analysis was performed in 18 patients: 17 had received heart and one a combined heart and lung graft. Donors and recipients were unmatched for HLA Class I and Class II antigens. There were three females and 15 males with ages ranging from 27–52 years, with a mean of 41 years. All patients were maintained on Cyclosporin A, 6–10 mg/kg and prednisolone 0·3 mg/kg daily.

Controls. 19 healthy persons of comparable ages and sex with the patient group were studied.

Microbiological Tests. Serological tests were done pre-operatively; weekly for the first 3 months and then at 3–6 month intervals after transplantation. CMV and herpes simplex (HSV) antibodies were measured by complement fixation tests (Bradstreet & Taylor, 1962). EBV IgG antibodies directed against the viral capsid antigen were assessed in an indirect immunofluorescence assay (Edwards, Taylor & Tomlinson, 1982). *Toxoplasma gondii* antibody was measured by latex agglutination using the EIKEN TOXO reagent. A four-fold or greater increase in antibody titre was considered as indicative of active infection. *Pneumocystis carinii* infection was diagnosed by chest X-ray and clinical symptoms and confirmed histologically on lung biopsy. Toxoplasma was also confirmed histologically in a cardiac biopsy.

Lymphocyte marker analysis. Mononuclear cells were isolated from defibrinated blood on Ficoll Hypaque. Adherent cells were removed during incubation on tissue culture grade plastic plates. Monoclonal antibodies of the following specificities were purchease from Becton Dickinson: T cells (Leu-4), T_H (Leu-3a), $T_{S\text{-}C}$ (Leu-2a), NK (Leu-7) and HLA-DR. For single-marker analysis lymphocytes were incubated with monoclonal antibody on ice for 45 min then washed once in cold PBS containing 1% heat-inactivated foetal calf serum (FCS) and 0·02% sodium azide. Cells were then reacted for 30 min on ice with fluorescein (FITC) conjugated rabbit antibodies to mouse IgG or IgM (Nordic Laboratories, Tilburg, The Netherlands). To detect dual marker expression lymphoid cells were incubated with the following pairs of monoclonal antibodies: Leu-2a FITC and Leu-3a biotinylated (B); Leu-2a FITC and Leu-7 B; or Leu-3a B and Leu-7 unlabelled. Biotin conjugated

antibodies were reacted with avidin phycoerythrin. Leu-7 staining was identified by a FITC-labelled antibody to mouse IgM Fc. Cells were counted using a Leitz microscope equipped with Ploemopak fluorescence from a mercury vapour source. Exciting filters at 450–490 and 530–560 were used for green (FITC) and red (phycoerythrin) fluorescence respectively.

Statistical analysis. The lymphocyte composition for each phenotypic marker was calculated as a percentage and absolute number. Absolute numbers were converted to a log scale to compensate for skewness. The patient groups were compared using a one-way analysis of variance and *t*-tests.

RESULTS

The opportunistic infections detected in the cardiac transplant recipients are summarized in Table 1. The pre-eminent cause of infection was CMV which occurred in 13 (72%) of patients. A pre-operative CMV antibody titre of $< 1:8$ was considered to indicate lack of previous exposure to the virus. There were seven cases with primary and six with secondary CMV infection. CMV infection occurred between 30 and 240 days after transplant (mean 62 days). Superinfection was found in nine patients with CMV. Six patients with primary CMV has reactivation of EBV. Secondary CMV was associated with *Pneumocystis carinii* in two cases and primary *Toxoplasma gondii* in one patient. Localized reactivation of HSV was found in four patients, two of whom had concurrent CMV infection. The absolute lymphocyte counts per mm^3 (mean $\pm$ s.d.) in patients with serological evidence of active CMV were 1861 ± 1063 at 200 days; 1806 ± 776 at 300 days; and 2891 ± 1725 at 600 days after transplantation. The comparable values for CMV free patients were 985 ± 408;

Table 1. Opportunistic infections and $T_H/T_{S\text{-}C}$ ratio inversion in cardiac transplant recipients

	Days after transplantation						
Patient	Lymphocyte marker monitoring	$T_H/T_{S\text{-}C}$ Inversion detected	CMV*	EBV*‡	Pneumocystis	Toxoplasma*	HSV*‡
36	275–597	275					
45	15–622	None					85
47	12–652	None					
50	12–457	None					21
54	14–396	None					
30	670–734	670	240†	550			
31	566–951	566¶	70†	83			
34	605–920	None	60‡				
35	502–734	502¶	56†	68			
37	619–734	619¶	53†	136,700			
38	258–678	258¶	40‡				
42	93–448	98–100	30‡		110		
46	16–646	32¶	39†§	39			
48	14–304	26–28, 175¶	44‡§		139		
49	12–653	50¶	53†§	60			
51	14–547	22¶	34‡§				
52	14–566	26–61	33‡§			33†	40
HL-1	14–245	47¶	50†§				15

* Infection diagnosed by $\geqslant$4-fold increase in antibody titre.

† Primary infection.

‡ Secondary infection.

§ $T_H/T_{S\text{-}C}$ ratio inversion preceded diagnostic increase in CMV antibody titre.

¶ Chronic inversion throughout remaining follow-up.

Table 2. Lymphocyte markers (% composition) at maximum follow up time in relation to CMV infection; and duration of $T_H/T_{S\text{-}C}$ ratio inversion.

Patient	CMV	Marker analysis postoperative day	Percentage of cells with markers						Known duration of inversion in days
			T	T_H	$T_{S\text{-}C}$	Leu-7	HLA-DR	$T_H/T_{S\text{-}C}$	
Group I									
36	None	597	51	25	25	14	2	1·4	<322
45	None	622	54	32	7	23	0	4·6	None
47	None	652	45	22	15	33	7	1·5	None
50	None	457	67	48	19	26	3	2·5	None
54	None	396	86	44	39	52	2	1·1	None
($n=5$)		(mean ± s.d.)	(61 ± 16)	(36 ± 10)	(21 ± 12)	(30 ± 14)	(3 ± 2·6)		
Group II									
30	Primary	734	93	53	35	24	4	1·5	<64
34	Secondary	920	81	39	23	35	8	1·7	None
42	Secondary	448	44	29	21	50	3	1·4	<2
52	Secondary	566	76	35	29	40	11	1·2	<35
($n=4$)		(mean ± s.d)	(74 ± 21)	(36 ± 10)	(27 ± 6)	(37 ± 11)	(7 ± 3)		
Group III									
31	Primary	951	86	26	67	66	29	0·4	385
35	Primary	734	84	36	56	48	2	0·6	232
37	Primary	734	98	16	66	72	22	0·2	115
38	Secondary	930	69	30	43	50	7	0·7	357
46	Primary	646	82	24	45	58	5	0·5	614
48	Secondary	304	78	33	53	40	11	0·6	280
49	Primary	653	89	26	52	50	16	0·5	603
51	Secondary	547	77	24	61	47	6	0·4	527
HL-1	Primary	245	56	9	37	63	6	0·2	202
($n=9$)		(mean ± s.d.)	(80 ± 12)	(25 ± 8)	(53 ± 10)	(55 ± 10)	(12 ± 9)		
Healthy controls ($n=19$)		(mean ± s.d)	(73 ± 9)	(46 ± 8)	(23 ± 4)	(12 ± 6)	(9 ± 4)	2·1	
Analysis of variance between groups				$P<0·03$	$P<0·001$	$P<0·005$			
Significance of differences between groups (*t*-test)									
I versus II			no significant difference						
I versus III			$P<0·05$	$P<0·05$	$P<0·001$	$P<0·001$	$P<0·05$		
II versus III				$P<0·05$	$P<0·001$	$P<0·05$			

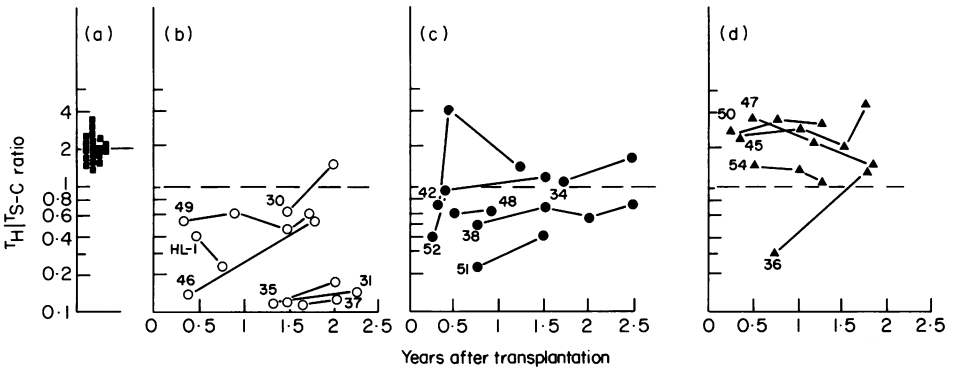

Fig. 1. Summary of T_H/T_{S-C} cell ratios in normal healthy persons and those found in serial tests in cardiac transplant recipients with and without CMV infections. (a) Normals ($n=19$), (b) Primary CMV ($n=7$), (c) Secondary CMV ($n=6$), (d) CMV negative ($n=5$).

955 ± 244; and 830 ± 245. The differences between CMV negative and positive groups were significant ($P < 0{\cdot}05$ and $P < 0{\cdot}001$), at 300 and 600 days respectively, using a two-sided t-test based on the logarithm of the values.

T cell subset ratios

The percentage composition (mean $\pm$ s.d.) of cells with T, T_H, T_{S-C} Leu-7 and HLA-DR markers in 19 healthy individuals is given in Table 2; the T_H/T_{S-C} ratio averaged 2·1. For comparison Fig. 1 summarizes the T_H/T_{S-C} ratios found in serial tests on cardiac transplant recipients at 200–900 days after operation. Prior to transplant no patient had T cell subset ratio inversion (T_H/T_{S-C} ratio < 1), data not shown. After transplantation inversion was found in 12 of 13 patients with serological evidence of active CMV but in only one of five cases who had no change in CMV antibody titre. The temporary inversion seen in Case 36 could not be linked to any known infection. Failure to detect inversion in Case 34 was probably due to the interval of 545 days between the increase in titre to CMV and lymphocyte marker analysis. Figure 1 shows that T_H/T_{S-C} ratio inversion persisted throughout follow-up in six of seven patients with primary and three of six patients with secondary CMV.

When T cell subsets were monitored from 2 or 3 weeks onwards after transplantation inversion was always found to precede the diagnostic increase in CMV antibody titre (Table 1). This is illustrated for four patients in Fig. 2, two of whom had primary and two with secondary CMV infection.

Single marker analysis of lymphocyte subsets

The percentages of cells expressing T, T_H, T_{S-C} Leu-7 and HLA-DR in each patient tested at the longest interval after transplantation are shown in Table 2. The patients could be separated into three groups: Group I had no serological evidence of active CMV and only one patient showed a temporary T cell subset inversion; Group II developed CMV and a temporary T_H/T_{S-C} ratio inversion was found in three of the four patients; while in Group III all patients had pesistent inversion following CMV infection. Since Groups II and III are selected on the basis of their T_H and T_{S-C} levels, a difference between these groups in relation to these subsets is inevitable. However, differences in relation to other markers were also found.

Analysis of variance showed that overall the three groups differed significantly in their proportion of T_H, T_{S-C} and Leu-7$^+$ but not total T or HLA-DR$^+$ cells. When groups were compared pairwise, no significant differences were found between Groups I and II for any marker. Group III had significantly increased proportions of T_{S-C} and Leu-7$^+$ and decreased T_H cells as compared with either Group I or Group II. In addition, Group III patients had a significant increase in total T cells and HLA-DR$^+$ cells as compared with Group I patients. It should be noted, however, that the

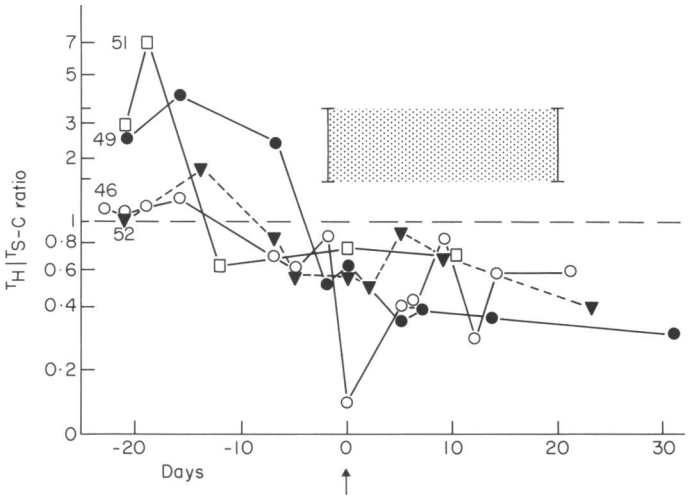

Fig. 2. Frequent monitoring showed T cell subset inversion (T_H/T_{S-C} ratio < 1) preceded the diagnostic ($\geq$ 4-fold) increase in CMV antibody titre in both primary (cases 46 and 49) and secondary (cases 51 and 52) CMV infection. Shaded area shows range for T_H/T_{S-C} ratios in normal healthy persons. (↑) indicates CMV antibody rise.

differences marked as $P < 0{\cdot}05$ are somewhat marginal as they no longer retain their 5% level when allowance is made for multiple testing. Patients with chronic T_H/T_{S-C} ratio inversion (Group III) had an average increase in T_{S-C} and Leu-7$^+$ cells of 152% and 83% respectively and a fall in T_H of 31% as compared to CMV negative patients (Group I).

The average absolute values for cells with each marker for three patients groups are given in Table 3. Overall the groups differed significantly from each other for each marker. Group I patients had significantly lower levels of T, T_H, T_{S-C}, Leu-7$^+$ and HLA-DR$^+$ cells than Groups II and III. Group III patients differed from Group II in having increased numbers of T_{S-C} cells and marginally increased Leu-7$^+$ cells.

Dual marker analysis of lymphocyte subsets

Lymphoid cells from cardiac transport recipients and healthy controls were examined for co-expression of T_H, T_{S-C} and Leu-7 (NK) markers. The results for two patients and one control are shown in Table 4. No cells expressing both Leu-2a (T_{S-C}) and Leu-3a (T_H) markers were detected in any samples. In both patients and normals a minority ($\leq 5\%$) of total lymphoid cells expressed both the Leu-7 and Leu-3a markers. However, major differences were apparent between CMV-infected cardiac transplant recipients and uninfected patients or controls in the levels of cells expressing both Leu-7 and Leu-2a markers. In patient 31 with chronic $T_{H/S-C}$ ratio inversion the majority of cells from both the Leu-7$^+$ and Leu-2a$^+$ subsets shared these markers (Table 4). In contrast, in patient 45 without CMV infection and normal healthy donor DT a minority of cells co-expressed Leu-7 and Leu-2a (Table 4).

The results of dual marker analysis in 15 subjects using the Leu-2a and Leu-7 monoclonal antibodies are given in Table 5. There were four groups, transplant recipients with primary CMV, those with secondary CMV, and those without evidence of CMV; and normal healthy controls. An overall difference between these groups was found in the percentage of cells co-expressing Leu-7 and Leu-2a as well as a difference in the proportions of Leu-2a$^+$ cells expressing Leu-7; and of Leu-7$^+$ cells expressing Leu-2a$^+$. Allowing for multiple testing, the patients with chronic T_H/T_{S-C} ratio inversion following primary CMV infection had significantly increased numbers of dual marked cells as compared to healthy donors. These patients has a significantly greater proportion of cells in the Leu-7$^+$ subset which expressed Leu-2a, as compared to healthy donors or CMV negative transplant recipients.

Table 3. Absolute lymphocyte marker values per mm³ (mean ± s.d.) in cardiac transplant recipients with and without CMV infection

Patient group*	T	T_H	$T_{S\text{-}C}$	Leu-7	HLA-DR
I	474 ± 130	292 ± 107	166 ± 99	218 ± 79	20 ± 15
II	1367 ± 254	750 ± 228	525 ± 165	794 ± 574	115 ± 48
III	2570 ± 1799	751 ± 379	1727 ± 1228	1788 ± 1341	372 ± 50
Analysis of variance between groups					
	$P<0.001$	$P<0.02$	$P<0.0001$	$P<0.0001$	$P<0.001$
Significance of differences between groups based on log. (absolute counts)					
I versus II	$P<0.01$	$P<0.02$	$P<0.01$	$P<0.01$	$P<0.01$
I versus III	$P<0.001$	$P<0.01$	$P<0.001$	$P<0.001$	$P<0.001$
II versus III			$P<0.01$	$P<0.05$	

Differences marked as $P<0.05$ do not retain the level of significance when allowance is made for multiple testing. All other comparisons are still significant at least at the 5% level.

* CMV status as in Table 2.

Table 4. Dual marker expression by T cell subsets in cardiac transplant recipients.

Reactivity with pairs of Leu series monoclonal antibodies	Percentages of cells with dual markers		
	Case 31 primary CMV	Case 45 CMV negative	DT normal
$2a^+3a^+$	0	0	0
$2a^+3a^-$	63	14	30
$2a^+7^+$	44	4	4
$2a^+7^-$	13	12	29
$2a^-7^+$	17	25	5
$2a^-3a^+$	26	34	55
$3a^+7^+$	4	1	2
$3a^+7^-$	25	22	43
$3a^-7^+$	58	35	7

The lymphocyte subset composition for patients 31 and 45 determined by single marker analysis is presented in Table 2.

If the expression of Leu-2a and Leu-7 were independent events it could be expected that the chances of their dual expression would increase if either Leu-7^+ or Leu-$2a^+$ bearing cells became more frequent in the lymphocyte population. A comparison of the observed percentages of cells with both markers with that expected under the independence hypothesis was carried out using a chi-square test. The results show (Table 5) that CMV infected patients with chronic $T_H/T_{S\text{-}C}$ ratio inversion often have an increased dual expression of these markers above that attributable to the increases in proportions of cells with each of the markers. Although the percentage of Leu-$2a^+$ cells which co-expressed the Leu-7 marker were higher in patients with chronic $T_H/T_{S\text{-}C}$ inversion this increase was not significant due to the wide variation in this subset in healthy individuals.

Table 5. Summary of dual marker analysis using monoclonal antibodies Leu-7 (NK) and leu-2a ($T_{S\text{-}C}$)

Patient group	Percentage of cells expressing both Leu-7+ and 2a+		Percentage of 2a+ cells which are also Leu-7+	Percentage of Leu-7+ cells which are also Leu-2a+
A: *Primary CMV*		(Expected)†		
49‡	44	(27·4***)	79	90
HL-1‡	21	(19·5)	70	32
46‡	35	(28·6*)	63	69
31‡	44	(34·8***)	77	72
37‡	54	(49·8)	77	76
B: *Secondary CMV*				
51‡	47	(36·5)	82	73
42	8	(4·4)	40	36
38‡	35	(24·0***)	73	70
34	15	(8·4**)	50	45
52	13	(11·1)	48	32
C: *No CMV*				
45	4	(4·6)	25	14
47	7	(5·6)	44	20
D: *Normal Controls*				
DT	4	(3·0)	44	12
DB	1	(1·2)	16	5
JP	5	(1·7**)	71	21
Analysis of variance between groups				
	$P<0·01$,		$P=0·06$	$P<0·01$
Differences between groups significant at 5% level after allowance for multiple testing				
	AvsD			AvsD AvsC

† 'Expected' shows the percentage of cells with both markers under hypothesis of independent expression (*$P<0·05$; **$P<0·01$; ***$P<0·001$).

‡ Chronic $T_H/T_{S\text{-}C}$ ratio inversion.

DISCUSSION

Inversion of the $T_H/T_{S\text{-}C}$ cell ratio occurs during acute CMV or EBV infection in both normal persons and immunosuppressed allograft recipients. This is due to a large increase in the proportion and absolute number of $T_{S\text{-}C}$ cells and a minor decline in T_H cells (Carney *et al.*, 1983; Crawford *et al.*, 1981; Reinherz *et al.*, 1980; Schooley *et al.*, 1983). The expanded $T_{S\text{-}C}$ subset is unresponsive to alloantigens and mitogens but retains suppressor activity towards T cell proliferative and help responses. In otherwise non-immunosuppressed persons T cell subset inversion is temporary and the ratio and $T_{S\text{-}C}$ functions revert to normality during convalescence. However, chronic inversion of the $T_H/T_{S\text{-}C}$ ratio has been reported in some renal allograft recipients for up to 4 years after CMV or EBV infections (Rubin *et al.*, 1981) and this has been linked to persistence of viral infection. It should be noted that failure to clear CMV infection appears to be common in renal allograft recipients who may continue to excrete the virus for several years after infection (Cheeseman *et al.*, 1979).

In this survey of 18 cardiac allografts recipients T cell subset inversion was observed in 12 of 13 patients with CMV infection. When T cell markers were monitored throughout the post-operative

period inversion was always found to precede the diagnostic rise in CMV antibody titre. $T_H/T_{S\text{-}C}$ ratio inversion persisted throughout follow-up ($\leqslant 3$ years) in 69% of patients with CMV and consisted of a large increase in $T_{S\text{-}C}$ cells with a minor fall in T_H cells. Chronic T cell subset inversion was not observed in patients with no serological evidence of active CMV infection. Dual marker studies revealed that for all seven patients with chronic inversion $\geqslant 60\%$ of cells in the expanded $T_{S\text{-}C}$ subset expressed the NK cell marker Leu-7. In normal adults a minority of Leu-7$^+$ cells in the blood coexpress the $T_{S\text{-}C}$ marker T8, but in bone marrow all Leu-7$^+$ cells are T8$^+$ (Abo *et al.*, 1982). In NK function tests T8$^+$ Leu-7$^+$ cells from blood were much less efficient than T8$^-$ Leu-7$^+$ (Tilden *et al.*, 1983). These results prompted the suggestion that the T8$^+$ Leu-7$^+$ cell may be an immature stage in the NK lineage (Abo *et al.*, 1983). However, others have reported that T8$^+$ cells which share NK markers are as efficient as T8$^-$ NK$^+$ cells in both NK and K cell activity (Perussia *et al.*, 1983). T8$^+$ Leu-7$^+$ cells can also act as suppressors of B cell differentiation *in vitro* (Clement *et al.*, 1984). To our knowledge, the T8$^+$ Leu-7$^+$ subset has only been studied in healthy individuals. The expansion of this subset in cardiac transplant recipients with CMV infection suggests that this may be a response to chronic antigenic stimulation. If this is the case a major increase in T8$^+$ Leu-7$^+$ cells in the blood would be expected to occur in other chronic infections.

As both IgM and IgG antibody responses to CMV are relatively efficient in allograft recipients (Glenn, 1981; O'Toole *et al.*, 1984) failure to clear viral infection is generally considered to be due to drug-induced suppression of cellular immunity. The predominance of the T8$^+$ Leu-7$^+$ subset following CMV infection could provide a basis for cellular hyporesponsiveness, particularly if NK function is impaired. Our results suggest that this T cell subset imbalance could be germane to the increased susceptibility of allograft recipients to superinfection and malignancy (Penn, 1984). However, verification of this must await a functional analysis of the T8$^+$ Leu-7$^+$ subset from patients with chronic T cell subset inversion. Long-term follow-up studies on cardiac transplant recipients will be necessary to show if expansion of the T8$^+$ Leu-7$^+$ subset is associated with an increased incidence of neoplasia.

This study was funded by The British Heart Foundation grant 82/18. We express thanks to Mr Peter Free and Mr F. Howlett, Department of Haematology, Papworth Hospital for obtaining clinical samples; and to the staff of the Virology Laboratory, Clinical Microbiology and Public Health Laboratory, Addenbrooke's Hospital, Cambridge for microbiological tests.

REFERENCES

ABO, T., COOPER, M.D. & BALCH, C.M. (1982) Characterization of HNK-1$^+$ (Leu-7) human lymphocytes. I. Two distinct phenotypes of human NK cells with different cytolytic capability. *J. Immunol.* **129,** 1752.

ABO, T., MILLER, C.A., GARTLAND, G.L. & BALCH, C.M. (1983) Differentiation stages of human natural killer cells in lymphoid tissues from fetal to adult life. *J. exp. Med.* **157,** 273.

BRADSTREET, C.M.P. & TAYLOR, C.E.D. (1962) Technique of the complement fixation test applicable to the diagnosis of virus diseases. *Month. Bull. Min. Health & Public Health Lab. Service*, **21,** 96.

CARNEY, W.P., IACOVIELLO, V. & HIRSCH, M.S. (1983) Functional properties of T lymphocytes and their subsets in cytomegalovirus mononucleosis. *J. Immunol.* **130,** 390.

CHEESEMAN, S.H., STEWART, J.A., WINKLE, S., COSIMI, A.B., TOLKOFF-RUBIN, N.E., RUSSELL, P.S., BAKER, G.P., HERRIN, J. & RUBIN, R.H. (1979) Cytomegalovirus excretion 2–14 years after renal transplantation. *Transplant. Proc.* **XI,** 71.

CLEMENT, L.T., GROSSI, C.E. & GARTLAND, G.L. (1984) Morphologic and phenotypic features of the subpopulation of Leu-2$^+$ cells that suppress B cell differentiation. *J. Immunol.* **133,** 2461.

CRAWFORD, D.H., BRICKELL, P., TIDMAN, N., MCCONNELL, I., HOFFBRAND, A.V. & JANOSSY, G. (1981) Increased numbers of cells with suppressor T cell phenotype in peripheral blood of patients with infectious mononucleosis. *Clin. exp. Immunol.* **43,** 291.

DUMMER, J.S., HO, M., RABIN, B., GRIFFITH, B.P., HARDESTY, R.L. & BAHNSON, H.T. (1984) The effect of cytomegalovirus and Epstein-Barr virus infection on T lymphocyte subsets in cardiac transplant recipients on cyclosporin A. *Transplant*, **38,** 433.

EDWARDS, J.M.B., TAYLOR, C.E.D. & TOMLINSON, A.H. (1982) Immunofluorescence techniques in diagnostic microbiology. Public Health Lab. Service Mono Series, HMSO London.

ENGLEMAN, E., BENIKE, C.J., GRUMET, F.C. & EVANS, R.L. (1981) Activation of human lymphocyte subsets: Helper and suppressor/cytotoxic T cells recognise and respond to distinct histocompatibility antigens. *J. Immunol.* **127,** 2124.

EVANS, R.L., FALDETTA, T.J., HUMPHREYS, R.E., PRATT, D., YUNIS, E.J. & SCHLOSSMAN, S.F. (1978)

Peripheral human T cells sensitized in mixed leukocyte culture synthesise and express Ia-like antigens. *J. exp. Med.* **148,** 1440.

GLENN, J. (1981) Cytomegalovirus infections following renal transplantation. *Rev. Infec. Dis.* **3,** 1151.

HERBERMAN, R.B. (1982) *NK Cells and Other Natural Effector Cells*, Academic Press, New York.

MEUER, S.C., COOPER, D.A., HODGDON, J.C., HUSSEY, R.E. MORIMOTO, C., SCHLOSSMAN, S.F. & REINHERZ, E.L. (1983) Immunoregulatory human T lymphocytes triggered as a consequence of viral infection: Clonal analysis of helper, suppressor inducer and suppressor effector cell populations. *J. Immunol.* **131,** 1167.

O'TOOLE, C.M., CARVALHO, G.S., CRANAGE, M.P. & LARGE, S. (1984) Immune responses in cardiac transplantation. Detection of activated TIa$^+$ cells in the blood during herpes virus infections. *Clin. exp. Immunol.* **57,** 671.

PENN, I. (1984) Cancer in immunosuppressed patients. *Transplant Proc.* **XVI,** 492.

PERUSSIA, B., FANNING, V. & TRINCHERI, G. (1983) A human NK and K cell subset shares with cytotoxic T cells expression of the antigen recognised by antibody OKT8. *J. Immunol.* **131,** 223.

REINHERZ, E.L., O'BRIEN, C., ROSENTHAL, P. & SCHLOSSMAN, S.F. (1980) The cellular basis for viral induced immunodeficiency: Analysis by monoclonal antibodies. *J. Immunol.* **125,** 1269.

REINHERZ, E.L., MEUER, S.C. & SCHLOSSMAN, S.F. (1983) The delineation of antigen receptors on human T lymphocytes. *Immunol. Today*, **4,** 5.

RUBIN, R.H., CARNEY, W.P., SCHOOLEY, R.T., COLVIN, R.B., BURTON, R.C., HOFFMAN, R.A., HANSEN, W.P., COSIMI, A.B., RUSSELL, P.S. & HIRSCH, M.S. (1981) The effect of infection on T lymphocyte subpopulations A preliminary report. *J. Immunopharmac.* **3,** 307.

SCHOOLEY, R.T., HIRSCH, M.S., COLVIN, R.B., COSIMI, A.B., TOLKOFF-RUBIN, N.E., MCCLUSKEY, R.T., BURTON, R.C., RUSSELL, P.S., HERRIN, J.T., DELMONICO, F.L., GIORGI, J.V., HENLE, V. & RUBIN, R.H. (1983) Association of herpes virus infections with T lymphocyte subset alterations, glomerulopathy and opportunistic infections after renal transplantation. *New Engl. J. Med.* **308,** 307.

TILDEN, A.B., ABO, T. & BALCH, C.M. (1983) Suppressor cell function of human granular lymphocytes identified by HNK-1 (Leu-7) monoclonal antibody. *J. Immunol.* **130,** 1171.

VERDONCK, F.L. & DEGAST, G.C. (1984) Is cytomegalovirus infection a major cause of T cell alterations after (autologous) bone-marrow transplantation? *Lancet*, **i,** 932.

WINCHESTER, R.J. & KUNKEL, H.G. (1979) The human Ia system. *Adv. Immunol.* **28,** 221.