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Inverted Vδ1/Vδ2 ratio within the T cell receptor (TCR)-$\gamma\delta$ T cell population in peripheral blood of heart transplant recipients

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SUMMARY

We investigated the levels of TCR-$\gamma\delta$ T cells and their subpopulations Vδ1 and Vδ2 in the peripheral blood lymphocytes (PBL) of 28 heart transplant (HTx) patients. Patients ($n = 10$) receiving cyclosporin A (CsA) for treatment of a nephrotic syndrome (NS) and 10 healthy individuals served as controls. There was no difference in levels of TCR-$\gamma\delta$ T cells between the different groups. However, an elevated proportion of V$\delta1^+$ $\gamma\delta$ T cells was found in the PBL of HTx patients, especially when these cells were present in their graft-infiltrating lymphocyte (GIL) cultures. V$\delta1^+$ $\gamma\delta$ T cells of HTx patients showed normal expression of CD45RO and lacked the activation markers CD25 and HLA-DR. After expanding in IL-2-containing medium, PBL cultures of HTx patients more often were dominated by Vδ1 cells than PBL cultures of controls, in which Vδ2 cells were predominantly grown. The aberrant composition of the TCR-$\gamma\delta$ population in HTx patients was not a result of immunosuppressive medication, since the proportion V$\delta1^+$ $\gamma\delta$ T cells was normal in the PBL of the NS patients receiving a similar dose of CsA. It is postulated that long-term antigenic stimulation by the graft, at low level, might be responsible for the altered composition of the $\gamma\delta$ pool in the HTx patients. Since no donor HLA-specific $\gamma\delta$ T cells have been detected, other ligands, such as heat shock proteins, may be involved.

Keywords $\gamma\delta$ cells Vδ1/Vδ2 ratio heart transplantation

INTRODUCTION

The TCR $\gamma\delta$ is expressed in 0·5–10% of human CD3$^+$ peripheral blood lymphocytes (PBL) [1–3]. They can be divided into two major, mutually exclusive, subsets. One expresses a TCR composed of a Vγ9-JγP-Cγ1-positive γ-chain in association with a V$\delta2^+$ δ-chain, and can be identified by the MoAbs TiγA, recognizing the Vγ9 gene product, and by BB3 or 15D recognizing the Vδ2 product [4–8]. The second subset of $\gamma\delta$ cells carries a TCR using Vδ1 gene products rearranged to Jδ1 in association with members of the VγI gene family, to be identified by the MoAb δTCS-1 [5,9].

In the post-natal thymus $\approx$ 15% of all $\gamma\delta$ cells are of the Vδ2 (V$\gamma9^+$/V$\delta2^+$) subset and 80% of the Vδ1 (V$\gamma9^-$/V$\delta1^+$) type. These proportions remain constant throughout adult life [10,11]. In the peripheral blood, however, the V$\delta2^+$ population gradually expands with age from $\approx$ 20% in cord blood to 70% in the peripheral blood of most adults. Consequently, the proportion of V$\delta1^+$ cells decreases from about 45% in cord blood to 20% in the blood of adults [10,11]. The increase of the proportion V$\delta2^+$ cells is paralleled by the acquisition of CD45RO by these cells. In cord blood, 10% of both Vδ1 and Vδ2 cells are strongly positive for CD45RO [10]. During adult life about 60% of Vδ2 cells express CD45RO at high intensity, whereas maximal 20% of Vδ1 cells are brightly stained for this antigen [10,12,13], which is thought to be a marker for activated T cells [14]. The expansion of CD45RO$^+$ Vδ2 cells might be driven by antigens of intestinal flora or of opportunistic pathogens [10].

The physiological and pathological role of $\gamma\delta$ cells is poorly understood. Several reports show that Vδ2 cells are increased in the PB of patients with infectious diseases such as malaria [15], toxoplasmosis [16], and infectious mononucleosis [17]. Vδ1 cells might be of pathogenic significance since they are found in inflamed tissues in autoimmune diseases such as rheumatoid arthritis (RA) [18–22], systemic lupus erythematosus (SLE) [23], coeliac disease [24], multiple sclerosis [25,26], autoimmune chronic liver disease [27] as well as in infectious diseases like HIV [28]. We reported the presence of Vδ1 cells within cardiac transplants especially more than 1 year after transplantation [29], which has recently been confirmed by others [30]. Now we report an aberrant ratio between the two $\gamma\delta$ subsets, and a different

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proliferation pattern in response to IL-2 in the PBL of these heart transplant patients compared with healthy individuals.

PATIENTS AND METHODS

Heart transplant patients and controls

Peripheral blood from 28 heart transplant (HTx) patients was taken at the time of endomyocardial biopsy (EMB) procedures. All HTx recipients studied had received preoperative blood transfusions and were under cyclosporin A (CsA) medication in a dosage sufficient to keep plasma 12 h-specific trough levels (between 50 and 120 ng/ml) and low dose prednisolone (10 mg/day); none of the patients recieved azathioprine. Mean number of HLA-A, HLA-B and HLA-DR mismatches was 1·3, 1·6 and 1·4, respectively. The reason for transplantation was ischaemic heart disease (n = 13), myocardiopathy (n = 13) and valvular heart disease (n = 2). None of them was transplanted because of autoimmune disease.

We had 15 PBL samples available of 15 HTx patients who had $\gamma\delta$ cells in graft-infiltrating lymphocyte (GIL) cultures propagated from the EMB concurrently taken with the PBL sample. Another 13 PBL samples were derived from HTx patients (n = 13) who had no $\gamma\delta$ cells in GIL cultures propagated from the concurrently taken EMB. The time point post-HTx when the PBL samples were taken did not differ significantly between the two HTx subgroups. For the patients with $\gamma\delta$ cells in the graft this was 336 days (median, range 28–1321 days) and for the other subgroup it was 257 days post-HTx (median, range 8–1460 days; P = 0·5 (Mann–Whitney U-test)).

Ten patients with an idiopathic nephrotic syndrome (NS) served as control group, receiving comparable doses of CsA as HTx patients but no prednisolone, and 10 healthy individuals. There were no significant differences in age between the four groups (P = 0·1501; Kruskal–Wallis ANOVA) for HTx with $\gamma\delta$ in GIL: median 48·5 years, range 15–57 years; HTx without $\gamma\delta$ in GIL: median 50 years, range 19–50 years; healthy controls (HC): median 37 years, range 26–52 years; and NS patients: median 37·5 years, range 24–69 years. There was also no difference in distribution of gender (male/female) between the groups: HTx group (21/7), *versus* HC (6/4) (P = 0·4318; Fisher's exact test); HTx group *versus* NS patients (6/4) (P = 0·4318); Htx group with $\gamma\delta$ in GIL (10/5) *versus* Htx group without $\gamma\delta$ in GIL (11/2) (P = 0·3955).

Cell preparations

Mononuclear cells (PBMC) were prepared from heparinized blood by centrifugation over a Ficoll–Hypaque (Pharmacia, Woerden, Netherlands) density gradient. Samples from HTx patients were cryopreserved and kept in liquid nitrogen until analysis. Frozen samples were rapidly thawed, washed in Hanks' balanced salt solution (HBSS), resuspended in RPMI 1640 and counted. Fresh samples were resuspended in RPMI 1640 directly after isolation and counted. For phenotype assessment 3×10^6 cells were taken into staining medium (HBSS supplemented with 1% bovine serum albumin (BSA) and 0·1% sodium azide). The remaining cells were resuspended in culture medium (CM), which consisted of RPMI 1640-Dutch modification (GIBCO, Paisley, UK) supplemented with 10% human serum, 4 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin.

Monoclonal antibodies

The MoAb anti-TCR-γ/δ-1 (clone 11F2), specific for human TCR γ-chains [31], anti-Leu-4 (CD3) and WT31, reactive with the human TCR-$\alpha\beta$ chain, were purchased from Becton Dickinson (San Jose, CA), the MoAb δTCS1, specific for a Vδ1Jδ1-encoded epitope on the δ-chain of human TCR-$\gamma\delta$ [9] and the MoAb 15D against the Vδ2 gene product [8] were obtained from T Cell Sciences (Cambridge, MA). Anti-CD45RO (clone UCHL1) and isotype control MoAb were obtained from Immunotech (Marseille, France). All MoAbs were directly conjugated to FITC or PE.

Immunofluorescence analysis

PBMC, T cell lines and clones were analysed for the expression of cell surface antigens with the MoAbs defined above. Cells (5×10^5) were incubated with fluoresceinated MoAb for 30 min at room temperature and subsequently washed in PBS, and from each PBL sample at least 10 000 cells were analysed in a lymphocyte gate on a FACScan (Becton Dickinson). Lymphocyte gate was set on forward (FSC) and sideways (SSC) light scatter, while cell debris was gated out by a threshold on FSC. For the expression of the activation markers CD45RO, CD25 or HLA-DR on the Vδ1 and Vδ2 population, cells were stained with anti-CD45RO, -CD25, -HLA-DR, respectively, conjugated to PE, in combination with δTCS1 or 15D conjugated to FITC. FITC-negative cells were gated out electronically by a threshold on FITC channel number 250. In this way all V$\delta1^-$ or V$\delta2^-$ cells were gated out and we were able to accumulate at least 500 V$\delta1^+$ or V$\delta2^+$ cells, and analyse them for the expression of PE-stained activation markers. Limits for 'non-activated' cells were set by PE-labelled isotype control MoAbs.

Generation of T cell lines in IL-2

PBL were cultured in CM supplemented with 30–50 U rIL-2 (Biotest, Dreieich, Germany). Cultures were grown in 24-well plates (Costar, Cambridge, MA) in 2 ml culture medium at a concentration of 10^6 cells/ml at 37°C in a humidified 5% CO_2 incubator. Half of the culture medium was changed three times a week. When a well was grown confluently, the cells were resuspended and divided over two or three wells. Cultures were analysed for phenotype expression at the start of the culture and thereafter once a week during a period of 2–3 weeks.

Statistical analysis

Differences in $\gamma\delta$ and Vδ1 levels between the groups were tested for significance with the Kruskal–Wallis non-parametric one-way analysis of variance (ANOVA) test, corrected for ties. If significant differences were found, this was narrowed down using 2×2 comparisons in the Mann–Whitney U-test. Fischer's exact test with Yates' correction was used to test for differences in gender, and to test for differences between patient groups for the number of PBL cultures with expanding Vδ1 or Vδ2 T cells. All calculations were performed with InStat software (GraphPad Software, Inc., San Diego, CA).

RESULTS

Levels of $\gamma\delta$ T cells in HTx patients and controls

The total level of $\gamma\delta^+$ T cells in PBL was determined with the MoAb 11F2 (anti-TCR-γ/δ-1) and expressed as percentage of CD3$^+$ T cells (Fig. 1). The levels of $\gamma\delta^+$ T cells in HTx patients (median 5·2%, range 0·5–17·1%) were not different from those of the HC group (median 2·6%, range 1·0–14·6%) or CsA-treated NS

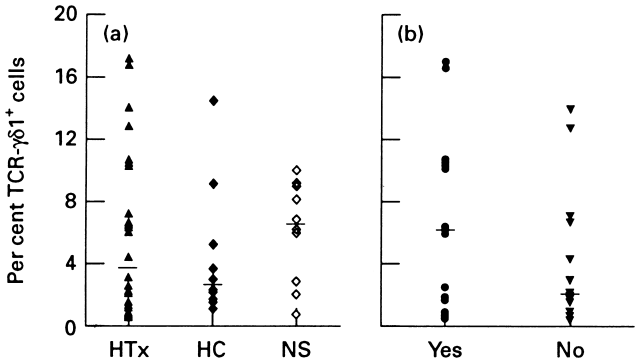

Fig. 1. (a) The proportion TCR-$\gamma\delta^+$ T cells expressed as percentage of all $CD3^+$ (T) cells in peripheral blood lymphocyte (PBL) samples of all heart transplant patients (HTx), healthy controls (HC) and patients who received cyclosporin A (CsA) because of a nephrotic syndrome (NS) are compared. (b) HTx patients are separated into a group with (yes) and without (no) $\gamma\delta$ T cells in their endomyocardial biopsy (EMB)-derived graft-infiltrating lymphocyte (GIL) cultures at the time the PBL sample was taken. No significant differences were found between the various groups.

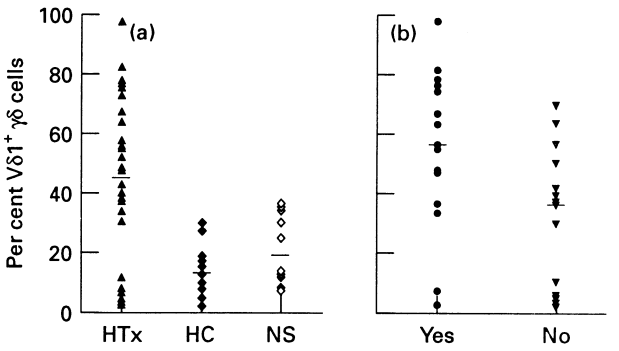

Fig. 2. (a) The proportion $V\delta1^+$ T cells expressed as percentage of all TCR-$\gamma\delta$-1^+ cells in peripheral blood lymphocyte (PBL) samples of all heart transplant patients (HTx), healthy controls (HC) and patients who received cyclosporin A (CsA) because of a nephrotic syndrome (NS) are compared. In PBL of HTx a significantly higher proportion $V\delta1^+$ $\gamma\delta$ cells was present than in HC (P = 0·023) and NS (P = 0·019). There was no difference between HC and NS. (b) HTx patients with (yes) and without (no) $\gamma\delta$ T cells in their endomyocardial biopsy (EMB)-derived graft-infiltrating lymphocyte (GIL) cultures at the time the PBL sample was taken are separately depicted. In PBL from HTx patients with $\gamma\delta$ cells in the GIL cultures a significantly higher proportion of $\gamma\delta$ cells were $V\delta1^+$ than in other HTx patients (P = 0·012).

patients (median 6·5%, range 0·6–10%). No differences were found for total levels of $\gamma\delta$ cells between the two HTx subgroups, HC and NS patients (Fig. 1b) (P = 0·4856; Kruskal–Wallis ANOVA). Since absolute numbers may give more information about differences in $\gamma\delta^+$ T cell populations between the several groups we determined the amount $\gamma\delta$ cells/μl. In the HTx group 29·5 $\gamma\delta/\mu$l (median, range 4–281) were present. This was significantly different neither from HC (median 28, range 9–122 $\gamma\delta/\mu$l), nor from NS patients (median 63, range 9–161 $\gamma\delta/\mu$l) (P = 0·5594 as tested with Kruskal–Wallis ANOVA). There were also no differences between the two HTx subgroups, HC and NS patients (P = 0·4712; Kruskal–Wallis). HTx patients with $\gamma\delta$ in GIL had 46 $\gamma\delta/\mu$l (median), range 4–261, and HTx patients without $\gamma\delta$ in GIL had 22, range 6–281 $\gamma\delta/\mu$l in their PBL.

The levels of $V\delta1^+$ $\gamma\delta$ T cells (δTCS1$^+$) were expressed as percentage of all $\gamma\delta^+$ T cells (Fig. 2a). In PBL of HTx patients significantly (P = 0·023) more $V\delta1^+$ $\gamma\delta$ cells (48%, 2·8–99·9%) were present compared with HC (21·7%, 6·0–38·0%).

The proportion of $V\delta1^+$ $\gamma\delta$ T cells in NS patients was comparable to HC (median 19·5%, range 6·0–36·5%). In PBL samples of all HC and all NS patients $V\delta2$ cells were the predominant subpopulation, resulting in a $V\delta1/V\delta2$ ratio of < 1·0. In PBL samples from 11/28 HTx patients, the $V\delta1/V\delta2$ ratio was > 1·0. Nine of these 11 PBL samples were from patients that had $\gamma\delta$ cells in the GIL cultures propagated from the EMB taken at the same time as the PBL sample. Comparison of the two subgroups of HTx patients, HC and NS patients, with Kruskal–Wallis ANOVA test, revealed a significant difference between the groups (P = 0·0024). Subsequently the groups were compared 2 × 2 in a Mann–Whitney *U*-test, which showed that indeed in HTx patients with TCR-$\gamma\delta^+$ cells in their GIL cultures, the proportion of $V\delta1^+$ cells in PBL was significantly higher than in PBL of HTx patients without $\gamma\delta$ cells in their GIL cultures (P = 0·012). In the HTx group with $\gamma\delta$ cells in the GIL cultures 56% (median; range 2·8–99·9%) of the $\gamma\delta$ cells in PBL were $V\delta1^+$ (Table 1), while in the HTx group, without $\gamma\delta$ cells in their GIL cultures, 37·0% (median; range 2·8–73·2%) of the $\gamma\delta$ cells in the PBL were $V\delta1^+$ (Fig. 2b). The latter was comparable to the percentages found in HC (P = 0·4) and NS patients (P = 0·3).

We also attempted to correlate the existence of an inverted $V\delta1/V\delta2$ ratio with clinical features such as transplant coronary artery disease, acute rejection and the occurrence of infectious diseases. A correlation with these clinical features, however, was not found (data not shown).

Expression of activation markers on Vδ1 and Vδ2 T cells

$V\delta1$ and $V\delta2$ cells were analysed for expressing CD45RO strongly (bright), weakly (dim), or not at all (neg) (Table 1). No difference was found between the various groups with respect to the proportion of $V\delta1^+$ and $V\delta2^+$ $\gamma\delta$ cells expressing CD45RO. In HC the proportion (median (range)) of CD45RO (bright and dim)-positive $V\delta1^+$ cells was 42% (28–56%), for $V\delta2$ positivity this was 92·5% (87–100%); in NS patients 41% (29–50%) for $V\delta1^+$ cells and 93% (87–97%) for $V\delta2^+$ cells. In HTx patients with $\gamma\delta$ cells in the GIL cultures 31·6% (13–78%) of the $V\delta1^+$ cells and 83·4% (12·8–99·2%) of the $V\delta2^+$ cells were CD45RO$^+$, in HTx patients without $\gamma\delta$ in the GIL cultures this was 43·6% (24·8–77·9%) and 90% (79–90·9%), respectively. There was also no significant difference in the intensity of CD45RO expression, either on $V\delta1^+$ cells or on $V\delta2^+$ cells (Table 2).

The percentage of $V\delta1^+$ and $V\delta2^+$ cells in the PBL bearing HLA-DR was very low, only weakly positive, in all patient groups tested. For $V\delta1$ this was 7·5% (1–16% dim) (median (range)), 9% (7–13%) and 8% (5–15%) for HTx with $\gamma\delta$, HTx without $\gamma\delta$ and HC, respectively. Comparable results were obtained for the $V\delta2$ population.

The IL-2 receptor (CD25) was detectable neither on $V\delta1^+$ nor on $V\delta2^+$ cells, except for one patient, in which 20% of the $V\delta1^+$ PBL coexpressed CD25 on their surface at low intensity.

Proliferation after stimulation with IL–2

We also tested whether $\gamma\delta$ cells in PBL proliferated in response to 30–50 U rIL-2, and the results are summarized in Table 3. Cultures were scored as expanders when the proportion of $\gamma\delta$ T cells had increased five times or more after 3 weeks in culture. Compared

Table 1. Per cent $\gamma\delta^+$ T cells and V$\delta1^+$ subpopulation in peripheral blood and graft-infiltrating lymphocyte (GIL) culture propagated from endomyocardial biopsy (EMB) concurrently taken from heart transplant patients

	GIL culture		PBL sample	
ID*	$\gamma\delta$ T cells""	V$\delta1$~~	$\gamma\delta$ T cells""	V-$\delta1$~~
RO	41	100	0·8	48·2
LU	8	50	0·6	77·9
GI	30	90	1·0	99·9
BN	8	100	10·3	7·6
SC	34	85	10·3	82·7
OO	21	90	1·8	37·0
DR	12	92	10·9	55·5
BE	33	66	10·3	75·3
BA	92	100	17·1	64·2
JU	49	100	6·3	55·9
VU	83	100	6·5	33·8
ZE	35	86	6·0	77·1
VS	81	93	2·5	47·3
GR	53	70	16·8	2·8
MO	78	100	11·1	67·7

*Patient identification.
† $\gamma\delta^+$ T cells as per cent of CD3$^+$ cells.
‡ V$\delta1^+$ T cells as per cent of all $\gamma\delta$ cells.

with HC, more PBL samples obtained from HTx patients showed V$\delta1^+$ cells as the major expanding $\gamma\delta$ population after stimulation with IL-2 (P = 0·011). In nearly all HTx patients with $\gamma\delta$ T cells in the graft, PBL samples that showed expansion of the $\gamma\delta$ cells, the V$\delta1^+$ population dominated, whereas in all PBL samples with expanding $\gamma\delta$ cells taken from HC, V$\delta2^+$ cells became predominant. This difference was statistically significant (P = 0·015). There was no significant difference between the two HTx groups. Results in the CsA-treated NS patients were inconclusive. However, in contrast to the HC, some V$\delta1$ cell-dominated PBL

Table 3. Proliferation of peripheral blood lymphocyte (PBL) $\gamma\delta$ T cells in IL-2

	*n**	V$\delta1$†	V$\delta2$†
HTx with $\gamma\delta$ in GIL	14	6	1
HTx without $\gamma\delta$ in GIL	12	3	1
Patients with nephrotic syndrome	10	2	3
Healthy controls	10	0	4

*Number of PBL samples cultured.
† Number of cultures in which predominantly V$\delta1$ or V$\delta2$ cells expanded. Expansion was defined as an increase of five times or more from the particular $\gamma\delta$ population during the culture period of 3 weeks in 30–50 U IL-2.
HTx, Heart transplant; GIL, graft-infiltrating lymphocyte.

cultures were found after expansion in IL-2 (Table 3). The percentage of CD25$^+$ $\gamma\delta$ cells in the expanded populations was variable, but not different in the groups tested. For HTx patients the median percentage was 54·8% (range 34·9–88·8%). For the HC this was 56·7% (range 44·9–93·6%) after 3 weeks of culture.

DISCUSSION

In PBL of heart transplant patients V$\delta1^+$ $\gamma\delta$ cells were more often the predominant $\gamma\delta$ subpopulation compared with PBL of HC. Especially in HTx patients from whose endomyocardial biopsy $\gamma\delta^+$ T cells could be propagated, PBL contained significantly more V$\delta1^+$ $\gamma\delta$ T cells than PBL of HTx patients in whose biopsy-derived GIL cultures no $\gamma\delta$ cells were present. This resulted in a reversed V$\delta1$/V$\delta2$ ratio compared with HC, since the MoAbs δTCS1 and 15D identify two distinct, non-overlapping subpopulations of $\gamma\delta$ T cells and represent ≈ 95% of $\gamma\delta$ cells in the peripheral blood of healthy individuals [6,8,10]. In the PBL of HC the V$\delta2$ (15D$^+$) T cells predominated, comprising 65% or more of $\gamma\delta$ T cells, while the remaining $\gamma\delta$ cells were nearly all of the V$\delta1$ (δTCS1$^+$) subpopulation. This distribution in PBL of HC was similar to that reported by several other investigators [6,10,11]. As far as we

Table 2. CD45RO expression on V$\delta1$ and V$\delta2$ cells

	V$\delta1$			V$\delta2$		
	Bright	Dim	Neg	Bright	Dim	Neg
HTx with $\gamma\delta$ in GIL	10* (0·8–53)†	26·1 (3·9–38)	67·7 (22–86)	45·5 (8–84)	19·1 (5–48)	15·7 (0–87)
HTx without $\gamma\delta$ in GIL	31·1 (8–44)	18·4 (10–32)	54·1 (23–75)	55 (31–70)	32·9 (20–47)	9·4 (7–19)
Patients with nephrotic syndrome	11 (6–17)	28·5 (14–42)	59 (52–71)	75 (56–85)	18 (10–37)	7 (2–13)
Healthy controls	11 (6–14)	29·5 (19–42)	60·5 (44–72)	64 (32–87)	27·5 (8–62)	8 (0–13)

Percentage V$\delta1^+$ and V$\delta2^+$ cells expressing CD45RO in PBL of heart transplant (HTx) patients, healthy controls and patients receiving CsA because of nephrotic syndrome.
*Median percentage.
† Range.
GIL, Graft-infiltrating lymphocyte.

know, no study has determined the ratio between Vδ1 and Vδ2 in the PBL of transplant patients. PBL of patients with autoimmune disease are studied more intensively in this respect. As in GIL cultures propagated from the EMB, particularly in those taken more than 1 year after HTx [29,30], $V\delta1^+$ cells are the predominant γδ T cells in rheumatoid joints [18–21], in autoimmune chronic active hepatitis [27], and in the affected muscles of patients with polymyositis [32]. Several studies in these patients showed a similar, reversed, Vδ1/Vδ2 ratio, as we now report for PBL of HTx patients [20,21,23,27].

The total level of γδ T cells in the PBL of both HTx groups had not changed compared with healthy controls, and was concordant with results published by others for large series of healthy individuals [1,10]. In patients with autoimmune chronic active hepatitis and primary sclerosing cholangitis, showing reversed Vδ1/Vδ2 ratios, Wen *et al.* found elevated levels of total γδ T cells [27]. For patients with rheumatoid arthritis (RA), conflicting results have been reported, both decreased percentages of total circulating γδ cells [18–20] as well as increased [33,34] or normal levels [34,35] being found. The reason for these discrepancies might be due to marked variations in median levels of the control and patient groups, because of small sample sizes. In most studies the given data for patients are within the range for normal individuals.

Normal expression of CD45RO, and the lack of activation markers CD25 and HLA-DR on $V\delta1^+$ PBL in HTx patients of our study, in combination with a normal level of total γδ T cells, argue against peripheral expansion of the Vδ1 population due to activation. Nevertheless, the γδ population in HTx patients seems to be distinct from that in HC, showing different *in vitro* expansion patterns. When cultured in 30–50 U IL-2, a significant difference became apparent between HC and HTx patients. In the HTx patients with $V\delta1^+$ γδ T cells in their GIL cultures, predominantly $V\delta1^+$ γδ T cells expanded, in contrast to the HC, where only $V\delta2^+$ γδ T cells expanded. This finding in HC was in harmony with the results of Orsini *et al.* [36], who found that in 12/30 HC selectively the $V\delta2^+$ γδ T cells expanded in IL-2, whereas in their other 18 cultures γδ T cells did not expand. NS patients seemed to be in a more intermediate position. This group of 10 patients with nephrotic syndrome received a comparable dose of CsA to the HTx patients. Since $V\delta2^+$ cells are thought to be activated γδ cells [10,11], CsA might have reduced this population and in this way the Vδ1/Vδ2 ratio might have been reversed. However, the results showed no significant difference between the HC and NS groups, neither in the percentage of $CD45RO^+$ Vδ2 cells nor in the level of CD45RO expression on Vδ2 cells. CD45RO expression on Vδ2 and on Vδ1 cells was the same in all groups and very similar to the results of Parker *et al.* for normal adults [9]. Obviously CsA did not alter the Vδ1/Vδ2 ratio by inhibiting the $V\delta2^+$ γδ T cell population, but might have diminished the capacity of Vδ2 cells to proliferate in response to IL-2. However, definitive proof for this assumption is lacking. Kjeldsen-Kragh *et al.* [37] gave evidence for the expansion of resting γδ T cells, only expressing the intermediate affinity IL-2Rβ chain. Orsini *et al.* [36], however, postulated that especially γδ cells previously activated by a hitherto unknown ligand expand in IL-2.

The cause of the reversed Vδ1/Vδ2 ratio in PBL of HTx patients remains an open question. It is possible that Vδ1 cells slowly proliferate in response to chronic antigenic stimulation. However, the responsible ligand must be something other than donor HLA-antigen, since in previous studies no donor-reactive Vδ1 cells were found in the grafts of these patients [29,30]. A group of ligands responsible for this activation might be formed by heat shock proteins (hsp). Hsp, also called stress proteins, can be expressed in the graft due to inflammation processes, since injurious stimuli to cells induce an increased production and subsequent expression of hsp on the cell surface (reviewed by Harboe & Quayle [39]). Moliterno *et al.* [30] showed that both TCR α/β and TCR γ/δ cells responsive to hsp could be propagated from EMB. The γδ T cells were predominantly of the Vδ1 subpopulation, and they could be propagated more often from long-term transplant biopsies, and in line with our results, they did not find a relation with acute rejection. These hsp may be transported to the lymphoid organs and activate γδ T cells which subsequently migrate and home in the graft. The lack of activation markers on the Vδ1 cells in PBL represents a problem for this concept. However, these Vδ1 cells may express the intermediate-affinity IL-2 receptor, and may slowly expand in the graft due to IL-2 produced by activated αβ T cells, as suggested by Kjeldsen-Kragh *et al.* in their report on the expansion of γδ T cells in RA lesions [37]. IL-2-producing αβ cells are indeed present in the graft in the period between 90 and 1770 days after HTx cells [40], when γδ cells can be cultured most frequently [29,30]. Finally, since we did not find a relation with acute and chronic rejection and no known donor-specific ligand is involved, $V\delta1^+$ cells might play a role in a more aspecific down-regulation of the immune response, as we postulated before [29].

REFERENCES

1 Groh V, Porcelli S, Fabbi M *et al.* Human lymphocytes bearing T cell receptor γ/δ are phenotypically diverse and evenly distributed throughout the lymphoid system. J Exp Med 1989; **169**:1277–94.

2 Bucy RP, Chen C, Cooper MD. Tissue localization and CD8 accessory molecule expression of T γδ cells in humans. J Immunol 1989; **142**:3045–9.

3 Falini B, Flenghi L, Pileri S, Fagioli M, Martelli MF, Moretta L, Ciccone E. Distribution of T cells bearing different forms of the T cell receptor γδ in normal and pathological human tissue. J Immunol 1989; **143**:2480–8.

4 Treibel F, Faure F, Graziani M, Jitsukawa S, Lefranc MP, Hercend T. A unique V-J-C rearranged gene encodes a gamma protein expressed on the majority of $CD3^+$ T cell receptor α/β^- circulating lymphocytes. J Exp Med 1988; **167**:694–9.

5 Bottino C, Tambussi G, Ferrini S *et al.* Two subsets of human lymphocytes expressing γδ antigen receptors are identifiable by monoclonal antibodies directed to two distinct molecular forms of the receptor. J Exp Med 1988; **168**:491–505.

6 Jitsukawa S, Faure F, Lipinski M, Triebel F, Hercend T. A novel subset of human lymphocytes with a T cell receptor-γ complex. J Exp Med 1987; **166**:1192–7.

7 Ciccone E, Ferrini S, Bottino C *et al.* A monoclonal antibody specific for a common determinant of the human T cell receptor γ/δ directly activates $CD3^+WT31^-$ lymphocytes to express their functional program (s). J Exp Med 1988; **169**:1–11.

8 Porcelli S, Brenner MB, Band H. Biology of the human γδ T-cell receptor. Immunol Rev 1991; **120**:137–83.

9 Parker CM, Groh V, Band H *et al.* Evidence for extrathymic changes in the T cell receptor γ/δ repertoire. J Exp Med 1990; **171**:1597–624.

10 Wu YJ, Tian WT, Snider RM, Rittershaus C, Rogers P, LaManna L, Ip SH. Signal transduction of γ/δ T cell antigen receptor with a novel mitogenic anti-δ antibody. J Immunol 1988; **141**:1476–9.

11 Casorati G, De Libero G, Lanzavecchia A, Migone N. Molecular analysis of human $\gamma\delta^+$ clones from thymus and peripheral blood. J Exp Med 1989; **170**:1521–35.

12 Miyawaki T, Kasahara Y, Taga K, Yachie A, Taniguchi N. Differential expression of CD45RO (UCHL1) and its functional relevance in two subpopulations of circulating TCR-γ/δ^+ lymphocytes. J Exp Med 1990; **171**:1833–8.
13 Braakman E, Sturm E, Vijverberg K, Van Krimpen A, Gratama JW, Bolhuis RLH. Expression of CD45-isoforms by fresh and activated human $\gamma\delta$ T lymphocytes and natural killer cells. Int Immunol 1991; **3**:691–7.
14 Akbar AN, Terry L, Timms A, Beverly PCL, Janossy G. Loss of CD45RA and gain of UCHL1 reactivity is a feature of primed T cells. J Immunol 1988; **140**:2171–5.
15 Ho M, Webster HK, Tongtawe P, Pattanpanyasat K, Weidanz WP. Increased $\gamma\delta$ T cells in acute *Plasmodium falciparum* malaria. Immunol Letters 1990; **25**:139–44.
16 Scalise F, Gerli R, Castellucci G *et al.* Lymphocytes bearing the gamma delta T-cell receptor in acute toxoplasmosis. Immunology 1992; **76**:668–70.
17 De Paoli P, Gennari D, Martelli P, Cavarzerani V, Comoretto R, Santini G. $\gamma\delta$ T cell receptor-bearing lymphocytes during Epstein–Barr virus infection. J Infect Dis 1990; **161**:1013–6.
18 Brennan FM, Plater-Zyberk C, Maine RN, Feldmann M. Coordinate expansion of 'fetal type' lymphocytes (TCR $\gamma\delta^+$ T and $CD5^+$ B) in rheumatoid arthritis and primary Sjörgren's syndrome. Clin Exp Immunol 1989; **77**:175–8.
19 Smith MD, Broker B, Moretta L *et al.* T $\gamma\delta$ cells and their subsets in blood and synovial tissue from rheumatoid arthritis patients. Scand J Immunol 1990; **32**:585–93.
20 Meliconi R, Pitzalis C, Kingsley GH, Panayi GS. $\gamma\delta$ T cells and their subpopulations in the blood and synovial fluid from rheumatoid arthritis and spondyloarthritis. Clin Immunol Immunopathol 1991; **59**:165–72.
21 Keystone EC, Ritterhause C, Wood N *et al.* Elevation of a $\gamma\delta$ T cell subset in the peripheral blood and synovial fluid of patients with rheumatoid arthritis. Clin Exp Immunol 1991; **84**:78–82.
22 Olive C, Gatenby PA, Serjeantson SW. Evidence for oligoclonality for T cell receptor δ chain transcripts expressed in rheumatoid arthritis patients. Eur J Immunol 1992; **10**:2587–93.
23 Lunardi C, Marguerie C, Bowness P, Walport MJ, So AK. Reduction in T $\gamma\delta$ cell numbers and alteration in subset distribution in systemic lupus erythematosus. Clin Exp Immunol 1991; **86**:203–6.
24 Halstensen TS, Scott H, Brandtzaeg P. Intraepithelial T cells of the TCR γ/δ^+CD8^- and $V\delta1/J\delta1^+$ phenotypes are increased in coeliac disease. Scand J Immunol 1989; **30**:665–72.
25 Wucherpfennig KW, Newcombe J, Li H, Kedy C, Cuzner ML, Hafler DA. T cell repertoire in acute multiple sclerosis lesions. Proc Natl Acad Sci USA 1992; **89**:4588–92.
26 Shimonkevitz R, Colburn C, Burnham JA, Murray RS, Kotzin BL. Clonal expansion of activated $\gamma\delta$ T cells in recent onset multiple sclerosis. Proc Natl Acad Sci USA 1993; **90**:923–7.
27 Wen L, Peakman M, Mieli-Vergani G, Vergani D. Elevation of activated $\gamma\delta$ T cell receptor bearing T lymphocytes in patients with autoimmune chronic liver disease. Clin Exp Immunol 1992; **89**:78–82.
28 Autran B, Triebel F, Katlama W, Hercend T, Debre P. T cell receptor γ/δ^+ lymphocyte subsets during HIV infection. Clin Exp Immunol 1989; **75**:206–10.
29 Vaessen LMB, Ouwehand AJ, Baan CC, Jutte NHPM, Balk AHMM, Claas FHJ, Weimar W. Phenotypic and functional analysis of T cell receptor $\gamma\delta$-bearing cells isolated from human heart allografts. J Immunol 1991; **147**:846–50.
30 Moliterno R, Woan M, Bentlejewski C *et al.* Heat shock protein-induced T-lymphocyte propagation from endomyocardial biopsies in heart transplantation. J Heart Lung Transplant 1995; **14**:329–37.
31 Borst J, van Dongen JJ, Bolhuis RL, Peters PJ, Hafler DA, de Vries E, van de Griend RJ. Distinct molecular forms of human T cell receptor $\gamma\delta$ detected on viable T cells by a monoclonal antibody. J Exp Med 1988; **167**:1625–44.
32 Hohlfeld R, Engel AG, Ii K, Harper MC. Polymyositis mediated by lymphocytes that express the $\gamma\delta$ receptor. N Eng J Med 1991; **324**:877–81.
33 Lamour A, Jouen-Beades F, Lees O, Gilbert D, Le Loet X, Tron F. Analysis of T cell receptors in rheumatoid arthritis: the increased expression of HLA-DR antigens on circulating $\gamma\delta^+$ T cells is correlated with disease activity. Clin Exp Immunol 1992; **89**:217–22.
34 Reme T, Portier M, Frayssinoux F *et al.* T cell receptor expression and activation of synovial lymphocyte subsets in rheumatoid arthritis. Arthritis Rheum 1990; **33**:485–92.
35 Kjeldsen-Kragh J, Quayle A, Kalvenes C *et al.* T $\gamma\delta$ cells in juvenile rheumatoid arthritis and rheumatoid arthritis. Scan J Immunol 1990; **32**:651–60.
36 Orsini DLM, Kooy YMC, Van der Tol M, Struyk L, Van den Elsen PJ, Koning F. T cell receptor usage of interleukin-2-responsive peripheral blood $\gamma\delta$ T cells. Immunology 1995; **86**:(in press).
37 Kjeldsen-Kragh J, Quayle AJ, Skalhegg BJ, Sioud M, Oystein F. Selective activation of resting human $\gamma\delta$ T lymphocytes by interleukin-2. Eur J Immunol 1993; **23**:2092–9.
38 Jutte NHPM, Vandekerckhove BAE, Vaessen LMB *et al.* $\gamma\delta$ T-cell receptor-positive T-cell clones derived from human heart transplants do not show donor-specific cytotoxicity. Human Immunol 1990; **28**:170–4.
39 Harboe M, Quayle AJ. Heat shock proteins: friend and foe? Clin Exp Immunol 1991; **86**:2–5.
40 Van Besouw NM, Daane CR, Vaessen LMB *et al.* Different patterns in donor specific production of T-helper 1 and T-helper 2 cytokines by cells infiltrating the rejecting heart allograft. J Heart Lung Transplant 1995; **14**:in press.