MOLECULAR ANALYSIS OF HUMAN γ/δ^+ CLONES FROM THYMUS AND PERIPHERAL BLOOD

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T lymphocytes express two different antigen receptors (TCR): α/β and γ/δ. Although the two TCRs share the same Ig-like genetic structure, they differ in the magnitude of their germline repertoire for variability (reviewed in references 1 and 2). Whereas the human α/β repertoire is based on a large number of genetic elements (50–100 Vα, 50–100 Jα, 70 Vβ, 2 Dβ, and 13 Jβ; reference 3), the TCR-γ/δ shows only eight functional V and five J segments for the γ locus (4) and a small number of V, three D, and three J for the δ locus (5–7). Thus, the variability of the γ/δ receptor appears to be concentrated at the V-J and V-D-J junctions, where nucleotides are randomly added (N region) during the recombination process (8). Such a phenomenon may play a major role, especially in the δ locus where two or even three D segments may assemble together (9–11).

Although little is known about the function in vivo of γ/δ-bearing T cells (12, 13), relatively more is known about their V gene usage. In humans, >60% of γ/δ^+ peripheral blood T lymphocytes express the Vγ9 gene product as detected by the mAb TiγA (14). It has also been shown that in these cells the Vγ9 chain is in most cases paired to the same Vδ (15), here called Vδ2, according to Hata et al. (5). Another mAb, δTCS1 (16), recognizes a Vδ1-related determinant (17), and identifies in the peripheral blood a second major population nonoverlapping with the TiγA$^+$ cells.

The reasons for this particular distribution and association of Vγ and Vδ in peripheral blood are not clearly understood. It is possible that this is due to constraints either at protein level or in the rearranging machinery, favoring particular V gene usage. Alternatively, this situation might reflect the presence of T cell subpopulations generated at different times during ontogeny or the expansion of some cells by antigen stimulation in the periphery.

It has recently been reported that in the thymus the δTCS1$^+$ cells are present at a higher frequency than in the periphery, suggesting that V gene usage in the thymus and periphery might be different (18).

In an attempt to understand the mechanisms that shape the γ/δ repertoire, we

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have performed phenotypic and genotypic analysis on γ/δ clones isolated from thymus and peripheral blood.

Our results indicate that Vγ9/Vδ2+ cells, which account for 46% in the peripheral blood clones, are rare (3%) in the thymic clones. Furthermore, most of the possible Vγ/Vδ combination can be found in the thymus, indicating that there are no major protein constraints to the formation of γ/δ heterodimers. We discuss the possible mechanisms accounting for the peripheral over representation of one particular γ/δ receptor.

Materials and Methods

T Cell Cloning. T cell clones were derived from PBMC of six healthy donors and from the thymus of five pediatric cardiac patients (age 2 mo to 7 yr). In two cases we isolated clones from both peripheral blood and thymus of the same patient. γ/δ T cells were isolated using the FACS (FACS 440; Becton Dickinson & Co., Mountain View, CA) by sorting δ1+ (19) or CD4-8- lymphocytes. The cells were immediately cloned after sorting and expanded using PHA and irradiated feeder cells as described (20). Peripheral blood clones from donor E were obtained from γ/δ+ cell lines and were not included in Table I.

Immunofluorescence. The clones were stained by standard, indirect immunofluorescence and analyzed using a FACScan analyzer (Becton Dickinson & Co.). The following mAbs were used: WT31 (21) (provided by Dr. H. Spits, The Netherlands Cancer Institute, Amsterdam); δ1 (19) and δTCS1 (16) (provided by Dr. M. Brenner, Dana-Farber Cancer Institute, Boston, MA); and TiγA (14) (provided by Dr. T. Hercend, Institut Gustave-Roussy, Villejuif, France). FITC-labeled anti-CD4 and PE-labeled anti-CD8 antibodies (Becton Dickinson & Co.) were used in double immunofluorescence.

Probes. The probes used to detect rearrangements at the TCR-γ locus are the following: JP1 (1.0-kb Hind III-Sac I), JP (0.1-kb Hind III-Eco RI), J1 (0.7 kb Hind III-Eco RI) (22), and JP2 (0.26-kb Hind III-Eco RI) (23). JP1 and JP probes were isolated by us from the Maniatis genomic library (24); J1 and JP2 were kindly provided by Dr. T. Rabbitts (N.R.C., Cambridge, UK) and P. G.Pellicci (University of Perugia, Perugia, Italy), respectively. The J1 probe, due to sequence homology, detects both J1 and J2 regions. The rearrangement and expression of Vγ9 was confirmed by hybridization to a Vγ9-specific probe (0.5-kb Pst I-Hind III) isolated from a genomic library of the T cell clone C1. Rearrangements at the TCR-δ locus were detected by the following probes: Jδ1 (1.5-kb Sac I, i.e., the JδS16 probe of reference 25); Vδ1 (0.3-kb Eco RI-Sca I) (a kind gift of Dr. M. Krangel, Dana-Farber Cancer Institute), which contains most of the variable portion of a cDNA clone (0-240/38) from the IDP2 cell line (26); and Vδ2 (1.6-kb Hind III-Nhe I) isolated from a genomic library of the clone C1. This Vδ gene is identical to the one recently reported by others and named either Vδ2 (5), Vδ3 (11), or VAB12 (15). In the present paper we have called this gene Vδ2. A 3.0-kb Bam HI-Eco RI germline fragment, mapping 5′ to Cδ, was used to detect rearrangements both at Jδ2 and Jδ3. The Cδ probe is a 1.4-kb Eco RI cDNA segment (clone 0-240; reference 26).

Southern Analysis. High molecular weight genomic DNAs from γ/δ+ clones were digested with four restriction enzymes (Bam HI, Eco RI, Bgl II, and Hind III), subjected to electrophoresis through 0.8% agarose, transferred onto a nitrocellulose filter, and hybridized to ^{32}P-nick-translated or random primed probes as described (27).

Northern Analysis. Total cellular RNA was extracted by the guanidinium thiocyanate method followed by an acidic phenolchloroform extraction and ethanol precipitation (28). RNA (10–20 μg/lane) was size fractionated by electrophoresis in a 1.5% agarose gel containing formaldehyde in MOPS buffer and blotted onto a nitrocellulose filter. Blots were prehybridized for 2–4 h in 5× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), 0.1% $NaDodSO_4$, 1× Denhardt's solution, 50% formamide at 42°C, and, using identical conditions, were hybridized overnight to random-primed labeled probes (29). Filters were then washed twice in 1× SSC, 0.1% $NaDodSO_4$ at 60°C for 15 min and twice in 0.1× SSC, 0.1% $NaDodSO_4$ at the same temperature for 20 min.

Results

Thymic and Peripheral Blood γ/δ Clones Express Different Sets of V Region Determinants. TCR-γ/δ$^+$ T cell clones were isolated from PBMC and from thymus by sorting γ/δ$^+$ cells followed by high efficiency cloning using PHA and feeder cells. All the clones reacted with the δ1 mAb, specific for a Cδ epitope (19), but not with the WT31 mAb, which recognizes a CD3 determinant present on TCR-α/β bearing cells, indicating that they express a γ/δ receptor (21) (data not shown).

To explore the diversity of the TCR repertoire, we first phenotyped these cells with two antibodies directed against V-encoded determinants: TiγA, which recognizes Vγ9 (14), and δTCS1, which recognizes a Vδ1-related epitope (16, 17). Table I shows that in clones isolated from peripheral blood these antibodies define two largely nonoverlapping subsets: TiγA$^+$δTCS1$^-$ (60/131 clones, 46%) and TiγA$^-$δTCS1$^+$ (48/131 clones, 37%), which altogether account for >80% of γ/δ cells. These subsets are represented at variable frequencies in different donors and reflect individual variability. In addition, a similar distribution was found in fresh PBMC of the same donors (data not shown). Together with the high cloning efficiency (30–80%), these findings indicate that the clones analyzed are a representative sample of peripheral γ/δ$^+$ cells.

Table I also shows that the thymus-derived clones have a different pattern of reactivity. The TiγA$^+$δTCS1$^-$ subset, which is the most highly represented in the periphery, is only a minor fraction in the thymus (16/253 clones, 6%), while TiγA$^-$δTCS1$^+$ cells represent 51%, TiγA$^-$δTCS1$^-$ represent 31%, and TiγA$^+$δTCS1$^+$ represent 12%. This distribution is similar in the clones derived from the five thymuses analyzed and reflect that of unsorted thymic populations or polyclonal γ/δ thymic cell lines (data not shown). The difference between thymus and periphery in the same individual is particularly evident in donor H.

TABLE I

Thymic and Peripheral Blood γ/δ$^+$ Clones Express Different Sets of V Region Determinants

			Number of clones reacting with mAbs				
Source	Donor	TiγA: / δTCS1:	+ / −	− / +	+ / +	− / −	Total
Peripheral blood	H		11	1	2	0	14
	A		2	9	0	1	12
	B		4	2	0	1	7
	C		4	3	0	1	8
	I		4	13	3	6	26
	D		35	20	1	8	64
			60 (46)*	48 (37)	6 (4)	17 (13)	131 (100)
Thymus	H		2	5	2	6	15
	E		9	61	7	34	111
	I		0	12	1	6	19
	F		3	43	17	28	91
	G		2	9	3	3	17
			16 (6)*	130 (51)	30 (12)	77 (31)	253 (100)

All these clones were obtained by cloning immediately after sorting; clones obtained from γ/δ$^+$ cell lines are not included.

* Total number with percent in parentheses.

In summary, these data indicate that there is a consistent difference between peripheral blood and the thymus. This difference is particularly apparent at the level of Vγ9, which in the peripheral blood is mostly expressed in the absence of Vδ1 ($p < 10^{-4}$), while in the thymus, it appears to be equally distributed on Vδ1$^-$ and Vδ1$^+$ cells ($p = 0.89$).

Vγ9$^+$ Cells Differ Extensively in Thymus and Periphery. To understand the basis for the different distribution of Vγ9, we analyzed at the molecular level Vγ9$^+$ clones from thymus and peripheral blood. Table II shows the analysis of the rearrangements at γ and δ loci in 12 TiγA$^+$δTCS1$^-$ peripheral blood clones, which represent the most frequent peripheral subset. 11 of 12 clones had rearranged Vγ9 to the

TABLE II

TCR-γ and -δ Gene Rearrangements in TiγA$^+$ Clones from PBMC

		TCR-γ*					TCR-δ‡				
Clone	CR§	JP1	JP	J1	JP2	J2	J1	J2	J3	V1	V2
TiγA$^+$δTCS1$^-$											
A1‖	2	V7	V9				V2		P	G	RG
A2	1		V9				V2,P			G	RG
B1	2	V11		V9			V2,P			G	RG
B2	1		V9				V2,P			G	RG
B3	2		V9			V2	P		V2	G	RG
B4	2		V9	V10			V2,P			G	RG
C1	2		V9			V2	V2			G	R
C2	2		V9		V10		V2,P			G	RG
C3	2		V9			V10	V2,P			G	RG
C4	2		V9	V7			R		V2	G	R
E13	2		V9	V7			R,R			G	D
I3	2		V9	(Vx)		(Vx)	P		V2	G	RG
TiγA$^+$δTCS1$^+$											
D2	2			V9		V9	V1,R			RG	G
I1	2			V9		V9	V1,R			RG	D

* The assignment of a given Vγ to a Jγ was based on the size of the rearranged Eco RI, Bam HI, and Hind III fragments (4) recognized by the J-specific probes described in Materials and Methods. Clone I3 carries an uncharacterized rearrangement (Vx) either at J1 or at J2.

‡ The Jδ1 probe used in combination with four restriction enzymes (Bam HI, Bgl II, Eco RI, and Hind III) showed that both chromosomes were rearranged in all clones. The Jδ3 probe allowed us to distinguish a Jδ3 from a Jδ2 rearrangement: a rearranged Eco RI and Bgl II and a germline Hind III pattern suggest a Jδ2 involvement, while a rearranged pattern with all the above enzymes indicates a Jδ3 involvement (see Fig. 1*b*). V1 and V2 indicate the rearranged fragments identified by the Vδ1 or Vδ2 probes. The other rearranged fragments are indicated with a P when they carry incomplete D-D-J, D-J, or D-D joinings, or with an R when the rearranged pattern is different from the above, and do not hybridize to Vδ1 and Vδ2 (see text). The results of the Vδ1 and Vδ2 probes analyses are reported as follows: G, germline at one or both alleles; RG, one chromosome rearranged and one germline; R and RR, one or two rearranged chromosomes, respectively; D, both alleles deleted. The expressed Vδ1 or Vδ2 genes are underscored. Peripheral clones from donor E are not included in Table I, since they were obtained from a cell line.

§ Number of chromosomes rearranged (CR) at the γ locus; all clones had both chromosomes rearranged at the δ locus.

‖ Clones are coded by a letter indicating the donor, followed by a number.

same Jγ segment, JP (see example in Fig. 2, clone A1), as reported by other investigators (14, 30), while only one clone (B1) carried a Vγ9-J1 rearrangement. All these 12 clones expressed Vγ9 by Northern analysis (data not shown). Since transcripts from Vγ-JP and Vγ-J1 are spliced to the first exon of the γ1 constant gene region (Cγ1) (see Fig. 1 *a*), all these clones should express the Cγ1 isotype.

When we analyzed the δ locus in the same clones by means of Jδ-specific probes and four restriction enzymes (Fig. 1 *b*), we found that 8 of 12 clones had the same pattern of rearrangement at Jδ1, suggesting that they might have rearranged the same Vδ (see Table II, and Fig. 5, clones A1 and C1). To identify this Vδ gene, we constructed a genomic library from clone C1 and isolated an 18-kb Bam HI fragment containing the rearranged Jδ1 segment. The segment 5′ to Jδ1 was sequenced and revealed a V gene, which appeared identical, except for the V-D-J junctional region, to that recently described by other authors (5, 7, 11, 15). For simplicity we have named it Vδ2, according to the Hata et al. nomenclature (5).

Using the Vδ2 gene as a probe in Northern and Southern analyses, we found that 11 of 12 peripheral blood $V\gamma9^+$ cells expressed Vδ2 mRNA (see examples in Fig. 4, clones C1, B4, B3, and B1) and rearranged Vδ2 to either Jδ1 (8/11 clones) or to Jδ3 (3/11 clones; see example in Fig. 5, clone C4).

In conclusion, the large majority of the cells from this major peripheral blood subset express a receptor of very restricted molecular composition consisting of a Vγ9-JP-Cγ1 chain, paired with a δ chain carrying the Vδ2 segment. One should note, however, that these receptors are in fact very diverse since they differ extensively for Jδ, Dδ, and the γ and δN regions.

To investigate whether the preferential Vγ9-JP/Vδ2 pairing is a consequence of constraints at the protein level or results from other mechanisms, we analyzed 18 $Ti\gamma A^+$ thymic clones (Table III).

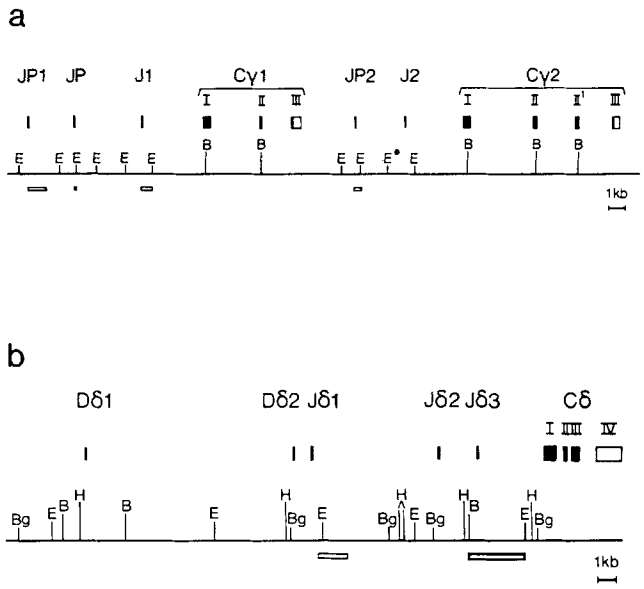

FIGURE 1. Organization and partial restriction enzyme map of the human TCR-γ and -δ gene regions (*a* and *b*, respectively). The nomenclature of Jγ segments is according to LeFranc et al. (33). The TCR-δ organization is based on published maps (34, 35) and on personal data. Roman numerals refer to the exons of the constant genes. The Cγ2 gene in this map has a duplication of exon II; Cγ2 polymorphic variants carrying a triplication of exon II are also known (23). The bars below the maps show the probes, described in Materials and Methods, used to detect rearrangements at the γ and δ regions. A polymorphic restriction site in the γ region is indicated by an asterisk. B, Bam HI; Bg, Bgl II; E, Eco RI; and H, Hind III.

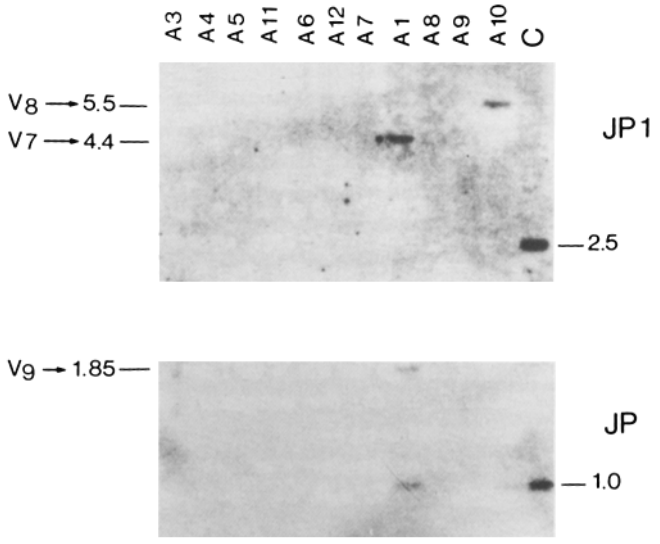

FIGURE 2. Southern blot hybridization of some peripheral blood clones with JP1 and JP probes. Genomic DNA from TiγA⁻δTCS1⁺ (A3, A4, A5, A6, A7, A8, A9, A10, and A12), TiγA⁺δTCS1⁻ (A1), TiγA⁻δTCS1⁻ (A11), and from fibroblasts as germline control (lane *C*) was digested with Eco RI. The size in kilobases of the rearranged fragments and the Vγ segment involved are indicated to the left of the panels. The size of the germline JP1- and JP-containing fragments are shown to the right.

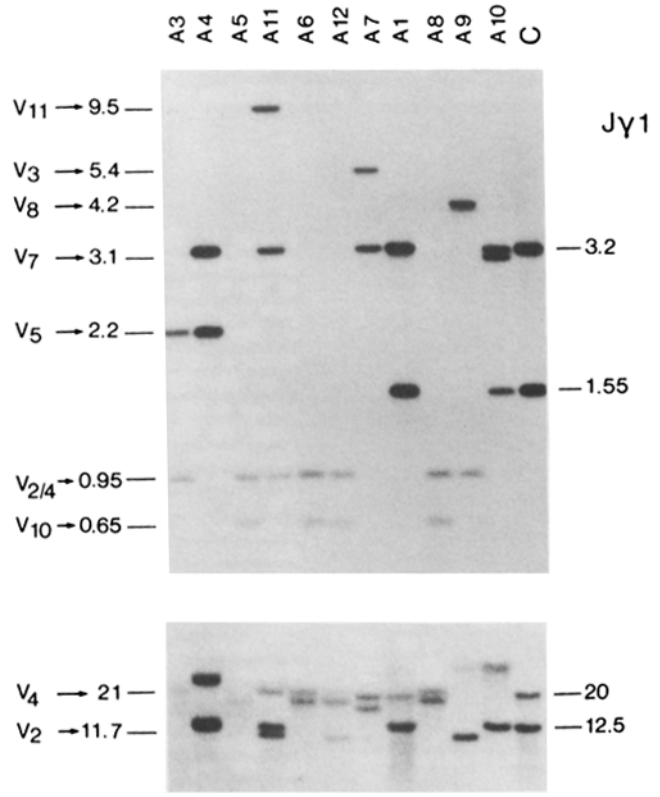

FIGURE 3. Genomic DNA from the same clones shown in Fig. 2 examined in Southern blot analysis using Jγ1 probe. DNA was digested with Eco RI in the first panel and with Bam HI in the second. The Vγ assignments (indicated to the left of the panel) are based on the size of fragments that hybridize to the Jγ1 probe. The pattern of an unrearranged control DNA from fibroblast (lane *C*) is also shown: the Eco RI 1.55- and 3.2-kb bands and the Bam HI 20- and 12.5-kb bands represent the germline Jγ1 and Jγ2 gene segments, respectively.

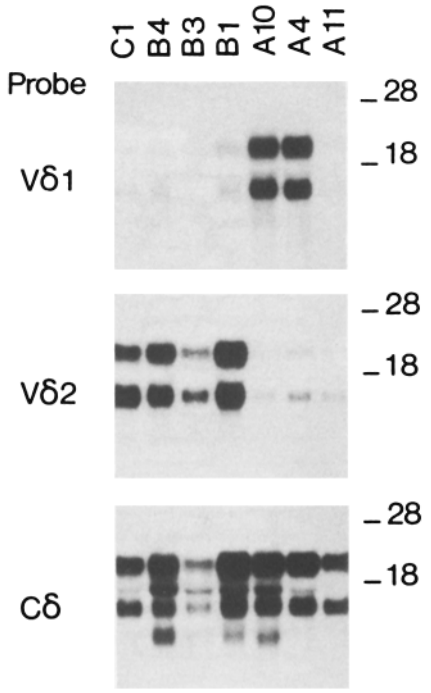

FIGURE 4. Northern blot analysis of seven representative γ/δ^+ clones from peripheral blood, hybridized to Vδ1, Vδ2, and Cδ probes. 28S and 18S ribosomal RNA serve as markers. The two stronger bands, reacting with the Cδ probe, of ~2.2 and ~1.3 kb represent differently polyadenylated transcripts from complete (VDJC) rearrangements, while the two fainter bands (~1.7 and ~0.8 kb) are different Cδ transcripts lacking Vδ segments (26).

The $Ti\gamma A^+\delta TCS1^-$ subset, which accounts for 46% of peripheral blood clones, represents only 6% of thymic clones. Six clones of this group were analyzed. Interestingly, only three of them (E1, E3, and E5) were similar to the peripheral counterpart, i.e., rearranged and expressed Vγ9-JP and Vδ2 products (see example in Fig. 6, clone E3). The other three clones either rearrange Vγ9 to J2, and therefore express the Cγ2 isotype, or use a different Vδ as indicated by Southern (see example in Fig. 6, clone F1) and Northern analyses (data not shown).

All of the 12 thymic and two peripheral $Ti\gamma A^+\delta TCS1^+$ clones (Tables II and III) expressed Vδ1 and Vγ9 as expected. Nevertheless, it is worth noting that in the 12 thymic clones, Vγ9 is never rearranged to JP, being mostly joined to J2 and occasionally to JP1, J1, and JP2 (see Table III). Thus, most of the $V\gamma9^+$ thymic cells express the Cγ2 isotype.

In summary, the data from the thymic clones show that Vγ9 can rearrange with different Jγ and is not limited in pairing with Vδ2, since Vδ1 and other Vδs (in clones E2, E6, and F1) can form functional heterodimers with Vγ9. Thus, we conclude that the overrepresentation of a particular Vγ9-JP/Vδ2 receptor in peripheral blood cannot be explained by its innate high frequency in the thymus, nor by constraints at the level of DNA rearrangements or protein pairing.

Vδ1 Gene Products Pair with Various Vγ, Preferentially Joined to Cγ2. We next analyzed 16 peripheral blood $\delta TCS1^+$ clones, which represent the second most frequent population in peripheral blood. As expected from their mAb reactivity, Northern analysis confirmed that Vδ1 (Hata et al.; 26) is expressed in all clones (see examples

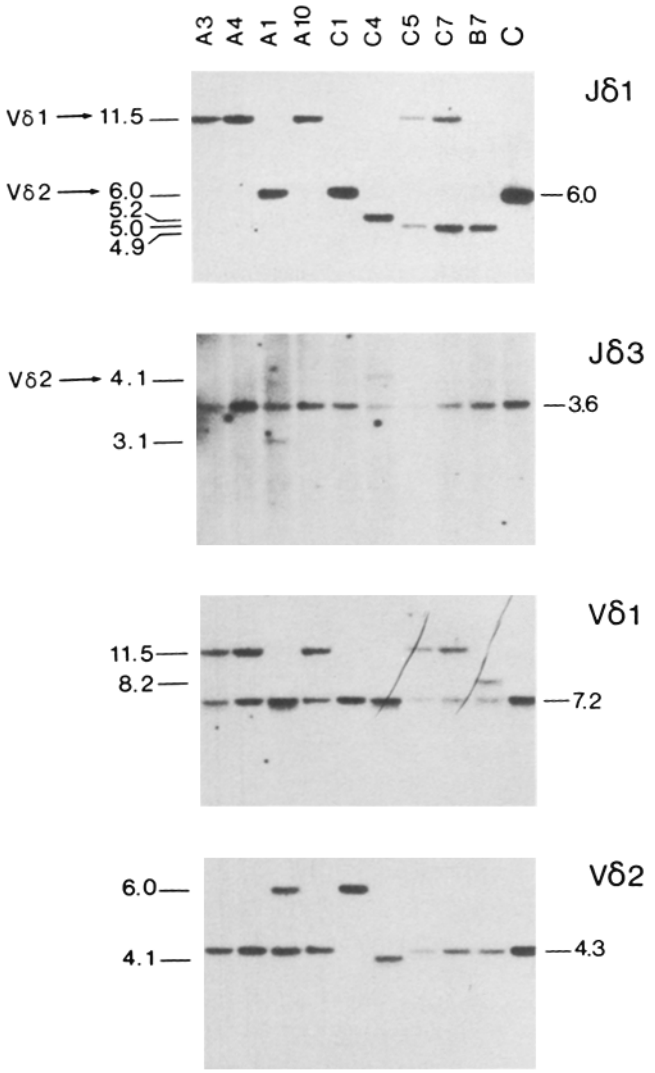

FIGURE 5. Southern blot hybridization of representative peripheral blood clones with Jδ1, Jδ3, Vδ1, and Vδ2 probes. Genomic DNA from TiγA⁻δTCS1⁺ (A3, A4, A10, C5, and C7), TiγA⁺δTCS1⁻ (A1, C1, and C4), TiγA⁻δTCS1⁻ (B7) clones, and from fibroblasts as germline control (lane *C*) was digested with Hind III. Note that the Hind III fragment containing the Vδ2-Jδ1 rearrangement (*top*, clones A1 and C1) has the same size of the germline Jδ1-containing band. Indeed, in all clones carrying a Vδ2-Jδ1 rearrangement, no germline Jδ1-containing band was found with Bam HI, Bgl II, and Eco RI restriction enzymes (data not shown). The presence of only one rearranged and no germline band after Jδ1 hybridization and multiple restriction enzyme analyses might be due to: (*a*) the same type of rearrangement on both chromosomes; (*b*) the involvement of a different Jδ (Jδ2 or Jδ3) on the second chromosome; and/or (*c*) a deletion of the Jδ1-containing region from the other chromosome.

in Fig. 4, clones A10 and A4). Indeed, all these clones have a common type of rearrangement in Jδ1 (Fig. 5, clones A3, A4, A10, C5 and C7; and see Table IV).

The γ locus analysis revealed that in this subset there is no clear bias for any particular Vγ gene (see Table IV and examples in Fig. 3, clones A3, A4, A5, A6, A12, A7, A8, A9, and A10). Remarkably, Vγ9 is never rearranged in the nonproductive chromosome. There is, however, a clear preference for the Cγ2 isotype, since 10 of 16 clones have deleted the Cγ1-coding region from both chromosomes, as shown by the absence of Jγ1 and Jγ2 germline segments, (Fig. 3, clones A3, A5, A6, A12, A8, and A9), and 23 of 29 fully typed, rearranged chromosomes have involved J2 or JP2.

TABLE III

TCR-γ and -δ Gene Rearrangements in TiγA⁺ Clones from Thymus

		TCR-γ					TCR-δ				
Clone	CR	JP1	JP	J1	JP2	J2	J1	J2	J3	V1	V2
TiγA⁺δTCS1⁻											
E1	2		V9,V9				V2,P			G	RG
E2	2			V9/10		V10/9	RR			G	D
E3	2		V9			V4	V2*		V2	G	RR
E5	1		V9				V2		R	G	R
E6	2					V5,V9	R		R	G	G
F1	2					V4,V9	R,R			G	D
TiγA⁺δTCS1⁺											
E4	2					V8,V9	V1,R			R	D
E7	2					V4,V9	V1,P			RG	G
E8	1					V9	V1,P			RG	G
E9	2					V8,V9	V1			R	D
F2	2				Vx	V9	V1,P			RG	G
F3	2	V9,V10					V1,V2*			RG	R
F4	2	V10		(V9)		(V9)	V1,P			RG	G
F5	2				V11	V9	V1,P			RG	G
F6	2			V9/10		V10/9	V1	P		RG	G
F7	2					V9,V9	V1,P			RG	G
G1	2			V9	V7		V1,P			RG	G
G2	1				V9		V1,P			RG	G

See footnotes to Table II. In clone E2 and F6 (as well as in clones B6, Table IV, and A11 and E11, Table V) both J1 and J2 at the γ locus are rearranged, but we could not establish precisely which of them is associated with which of the two indicated Vγ. Clone E1 and F7 (as clone A4, Table IV, and E15, Table V) has been considered to carry an identical Vγ-Jγ rearrangement on both alleles, since they have lost any germline Jγ-containing fragment 5′ to the J involved and the only band rearranged showed a signal twice as strong. In clone F4 it is not clear whether Vγ9 is rearranged to Jγ1 or Jγ2.

* A partial VDD rearrangement.

Characterization of TiγA⁻δTCS1⁻ Clones. TiγA⁻δTCS1⁻ clones (three from thymus and six from peripheral blood) were analyzed. The results shown in Table V can be summarized as follows. (*a*) Clone B7, although δTCS1⁻, expressed Vδ1 rearranged to Jδ2 (Fig. 5). This was demonstrated by the fact that both Jδ3 and Vδ1 probes hybridize to a Bgl II–rearranged segment of identical size, while the unproductive chromosome carries only a partial DDJ rearrangement (data not shown). Furthermore, a band of the predicted size was amplified by a polymerase chain reaction using Vδ1- and Jδ2-specific primers (data not shown). Since the δTCS1 mAb recognizes clones with Vδ1-Jδ1 rearrangements, but does not bind to clone B7 (Vδ1-Jδ2), we conclude that the epitope recognized is likely to be encoded by the Vδ1-Jδ1 combination. (*b*) Two clones in this group demonstrate that Vδ2 can form heterodimers with Vγ different from Vγ9 and with Cγ2. Clone E11 has a productive rearrangement involving Vδ2, while the other chromosome carries a deletion spanning all three Jδ segments. Therefore, in clone E11, the Vδ2-Jδ3 product is paired with Vγ2 or Vγ4. Clone E15 has a partial DDJ rearrangement at one Jδ1 allele and expresses a Vδ2-Jδ3 product (confirmed by Southern and Northern analyses, data not shown)

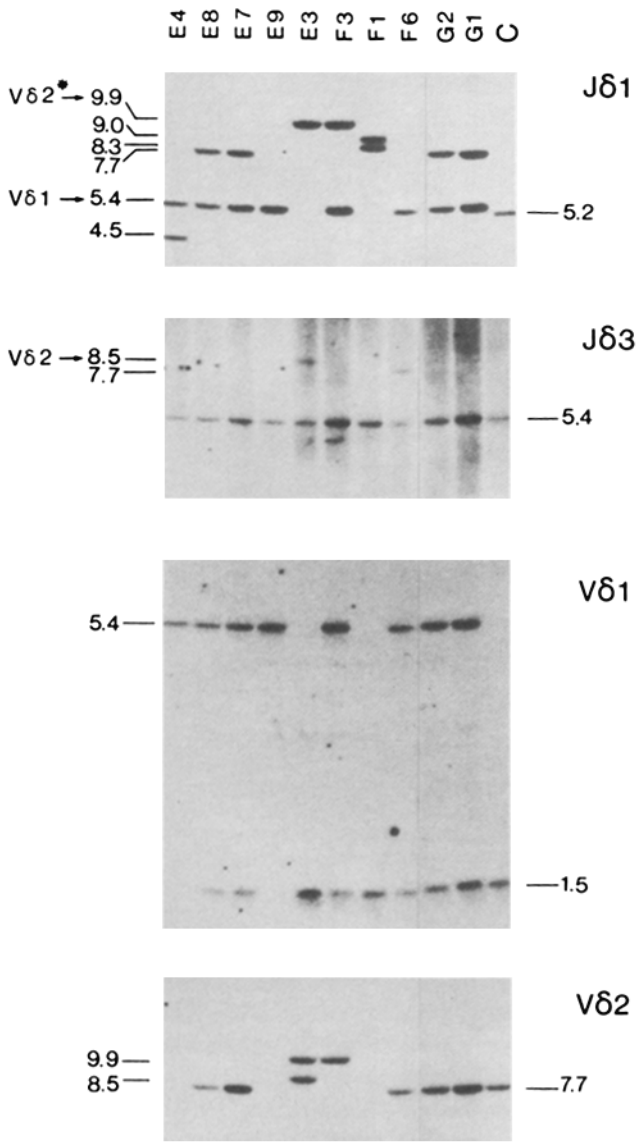

FIGURE 6. Southern blot hybridization of representative thymus-derived clones with Jδ1, Jδ3, Vδ1, and Vδ2 probes. Genomic DNA from TiγA+δTCS1+ (E4, E7, E8, E9, F3, F6, G1, and G2), TiγA+ δTCS1- (E3 and F1) clones, and from fibroblasts, as germline control (lane *C*), was digested with Bgl II. The size in kilobases of the rearranged segments are indicated to the left of the panel. The fragments that contain Vδ1 or Vδ2 segments rearranged to Jδ1 or Jδ3 are also shown. Vδ2* denotes a partial VDD rearrangement.

associated with Vγ4 and Cγ2. (*c*) Six of the nine clones (A11, C8, E10, E12, E16, and E17) should express Vδ genes that differ from Vδ1 and Vδ2 (see below).

The Repertoire of Rearrangeable Vδ Genes. The study of the rearrangements at the δ locus in a large number of γ/δ clones allowed us to collect data that may reveal the extent of the Vδ gene pool.

By means of a four-restriction enzyme analysis and the Jδ1 and Jδ3 probes shown in Fig. 1, we identified 15 different patterns of rearrangements at Jδ1 or Dδ2, five at Jδ3, and three at Jδ2. At least four different types of incomplete joining, e.g., DDJ, DD, DJ, or VDD, were identified at Jδ1. One example of the latter type (VDD)

TABLE IV

TCR-γ and -δ Gene Rearrangements in TiγA⁻δTCS1⁺ Clones from PBMC

		TCR-γ					TCR-δ				
Clone	CR	JP1	JP	J1	JP2	J2	J1	J2	J3	V1	V2
A3	2					V4,V5	V1			RG	G
A4	2			V5,V5			V1	P		RG	G
A5	2					V4,V10	V1,R			RG	D
A6	2					V4,V10	V1	P		nd	G
A7	2				V3	V3	V1			R	D
A8	2					V4,V10	V1,R			RG	D
A9	2					V2,V8	V1	P		RG	G
A10	2	V8				V7	V1			RG	G
A12	2					V2,V10	V1	P		RG	G
B5	2			V4	V8		V1,V2*			RG	R
B6	2			V8/10		V10/8	V1,P			RG	G
C5	2				Vx	V7	V1,P			RG	G
C6	2					V3,V10	V1,P			RG	G
C7	2	V4			V8		V1,P			RG	G
D1	2					V2,V8	V1,P			RG	G
I4	2	V10		(V3)		(V3)	V1,P			RG	G

See footnote to Tables II and III. In clone I4, it is not clear whether Vγ3 is rearranged to J1 or J2.

TABLE V

TCR-γ and -δ Gene Rearrangements in TiγA⁻δTCS1⁻ Clones from PBMC and Thymus

		TCR-γ					TCR-δ				
Clone	CR	JP1	JP	J1	JP2	J2	J1	J2	J3	V1	V2
Peripheral blood											
A11	2			V4/11		V11/4	R			G	G
B7	2					V2,V8	P	V1		RG	G
C8	2		V9			V4	R,R			G	G
E10	2					V3,V4	R		P	G	G
E11	2			V2/4		V4/2	D	D	V2	G	RG
E12	2					V3,V4	R,V1			RG	D
Thymus											
E15	2					V4,V4	P		V2	G	RG
E16	2					V4,V7	P		R	G	G
E17	2				Vx	V3	RP			G	G

See footnotes to Tables II and III.

was found in clones E3 and F3 (Table III) and B5 (Table IV). The V involved was identified as the Vδ2, by means of Southern analysis (see examples in Fig. 6, clones E3 and F3) and gene amplification through the polymerase chain reaction (Migone, N., manuscript in preparation).

Limiting our analysis to those clones that do not express either Vδ1 or Vδ2, we found that three of them (two from the thymus, E2 and E17, and one from periphery, E13) showed different patterns of rearrangements at Jδ1 on both chromosomes. Therefore, we conclude that three different Vδ genes should be expressed in these clones.

Finally, since in all clones we determined the presence or absence of Vδ1 and Vδ2 germline segments, we propose the relative 5'-3' order of the five putative Vδ genes in the chromosome to be as follows: Vδ1, $V\delta x_1$, $V\delta x_2$, Vδ2, $V\delta x_3$.

At least five other types of rearrangements, although carried by the nonproductive chromosome, are possible candidates for additional Vδ genes. Indeed, in all these rearranged chromosomes the Vδ2 is lost, ruling out incomplete joinings. However, we can not exclude that some of them might represent recombinations to non-V-containing segments like the REC element recently described (31).

Discussion

Our analysis shows a different Vγ and Vδ gene usage and pairing in human thymus and peripheral blood. In agreement with recent reports (14, 15), our data show that the majority of γ/δ cells in peripheral blood express either Vδ2 (45% of the clones) or Vδ1 (35% of the clones). The pairing of these Vδ with γ chains is not random, since Vδ2 is almost always associated with a unique γ chain, i.e., Vγ9-JP-Cγ1, while Vδ1 appears to be associated mostly with Vγ other than Vγ9, linked to the Cγ2 isotype. Thus, the peripheral γ/δ repertoire appears to be nonrandom at four levels: (*a*) V gene usage; (*b*) V-J combination; (*c*) pairing of Vδ chains to the Cγ1 or Cγ2 isotype; and (*d*) pairing of Vγ and Vδ. This situation could be due to constraints either at the level of gene rearrangements, or at the level of pairing of γ and δ chains.

In an attempt to clarify the possible role of these mechanisms, we have analyzed γ/δ^+ clones isolated from postnatal thymus. The differences in V gene usage and pairing between peripheral blood and thymus are striking. The Vγ9-JP/Vδ2-bearing cells, which are predominant in peripheral blood (46% in our sample), are rare in the thymus, since they account for only 3% of the clones (when the data from Table I are corrected on the basis of the DNA findings in Table III). Furthermore, in the thymus, in contrast to peripheral blood, the Vγ9 gene can be found rearranged to all Jγ elements, and thus expressed on both Cγ1 and Cγ2 isotype, and can pair to Vδ chains different from Vδ2. In addition, Vδ2 can pair with Vγ other than Vγ9 and with both Cγ1 and Cγ2. In summary, our data show that there is a less restricted γ/δ chain pairing in the thymus than in peripheral blood. Therefore, the overrepresentation of a particular Vγ9-JP/Vδ2 heterodimer in peripheral blood cannot be due to physical constraints nor to its innate high frequency in the thymus, and must be explained by other mechanisms.

A first possibility is that Vγ9-JP/Vδ2 cells represent a "fetal" population that seeds to peripheral lymphoid organs and is almost absent in postnatal thymus. Indeed, it has been shown in the mouse that two waves of γ/δ cells populate the mouse fetal thymus, the earliest of which expresses a unique Vγ3/Vδ1 receptor (10, 32). Remarkably, the mouse Vδ1 shows the highest homology to the human Vδ2 (32/95 amino acid identities). Thus, it is tempting to speculate that the Vγ9-JP-Vδ2 heterodimer might be present on a fetal cell subset that leaves the thymus early to colonize the periphery.

Another possibility is that the Vγ9-JP/Vδ2 cells are attracted and expanded in the periphery because of their specificity. Since these cells bear receptors with identical Vγ and Vδ, which, however, differ extensively at the junctional region, it is tempting to speculate, according to the current view of MHC-restricted antigen recognition (2), that they might recognize foreign antigens in association with non-

polymorphic, not yet identified restriction elements with tissue-specific distribution. If this is the case, then the peripheral overrepresentation of Vγ9/Vδ2 cells might simply reflect their expansion by antigen in peripheral lymphoid organs.

The analysis of γ/δ cells from fetal thymuses and the identification of the antigen(s) recognized by Vγ9-JP/Vδ2 cells will help the understanding of the function of this common peripheral blood γ/δ^+ population.

Summary

We analyzed the Vγ and Vδ gene usage in TCR-γ/δ-bearing T cell clones isolated from human peripheral blood and postnatal thymus using V-specific mAbs and Southern and Northern analyses. In peripheral blood most of the γ/δ cells express the Vγ9-JP-Cγ1 chain paired with a δ chain bearing the Vδ2 gene product. This heterodimer is very rare in the postnatal thymus, where a different and less restricted pairing of Vγ9 and Vδ2 chains is found. These findings indicate that physical constraints cannot explain the overrepresentation of a particular Vγ9-JP/Vδ2 heterodimer in the peripheral blood, and we discuss alternative mechanisms that may account for this differential distribution. In addition, this analysis allowed us to map the specificity of the δTCS1 mAb to Vδ1-Jδ1 and to identify at least five different expressed Vδ genes.

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