Peripheral Selection of Vδ1+ Cells with Restricted T Cell Receptor δ Gene Junctional Repertoire in the Peripheral Blood of Healthy Donors

By Kheïra Beldjord,*‡ Cherif Beldjord,§ Elisabeth Macintyre,* Philippe Even,‡ and François Sigaux*

*From the *Laboratory of Molecular Hematology, Centre Hayem, Hôpital Saint Louis, 75475 Paris; the ‡Laboratoire Universitaire d'Immunologie, Laennec Hospital, 75007 Paris; and §Institut National de la Santé et de la Recherche Médicale, Unit 129, Institut Cochin de Génétique Moléculaire, 75014 Paris, France*

Summary

To characterize the T cell receptor (TCR) repertoire expressed by the Vδ1+ γ/δ T cell population, we have studied the Vδ1-Jδ1 junctional sequences from peripheral blood samples of healthy donors. We show that, surprisingly, this repertoire is restricted in most healthy adults, with a donor-specific and relatively stable pattern, whereas this repertoire remains unrestricted in infants, and is similar to that of thymocytes. These data contrast with the general assumption that the junctional repertoire of Vδ1+ γ/δ T cells is extensive, and strongly suggest that peripheral recruitment of Vδ1+ cells bearing particular TCR occurs in humans during the postnatal stage.

The majority of peripheral blood (PB)[1] T cells express the TCR-α/β and use this receptor to recognize peptides presented by the products of the MHC polymorphic genes. A minor population of T lymphocytes express another TCR composed of γ and δ chains (TCR-γ/δ). The diversity of this latter TCR is mainly due to random and clone-specific nucleotide sequence deletions/additions that occur at the V(D)J junctions or CDR3, based on analogy with Ig genes. It is generally assumed that this diversity is fundamental to the establishment of the antigen repertoire of these cells (1). Circulating human γ/δ T cells fall into two distinct subsets expressing reciprocal profiles. The major one (>70% of γ/δ T cells in most adult individuals) (2) coexpresses the Vγ9 and Vδ2 variable gene segments, whereas the minor one uses a Vδ1 gene that is usually rearranged to the Jδ1 junctional segment and is mainly paired with a Vγ gene other than Vγ9 (3). The latter is the predominant γ/δ subset found in the human thymus (4) and large intestine (5). To date, very little is known about the biology of these cells (6).

To define the TCR-δ repertoire of Vδ1+ cells, we have characterized the Vδ1-Jδ1 junctional diversity in the PB of healthy donors. We show that the junctional repertoire of normal infants displays extensive diversity, whereas this repertoire is restricted in most healthy adults with a donor-specific and relatively stable pattern. These data strongly suggest that peripheral selection occurs in the postnatal period.

[1] *Abbreviations used in this paper:* AJO, antijunctional oligonucleotide; PB, peripheral blood; PBL, peripheral blood leukocyte; SSCP, single-strand conformation polymorphism.

Materials and Methods

Donors and Cells. Blood samples were obtained from 29 healthy adult blood donors. Cells from two pediatric thymuses (obtained from children undergoing heart surgery) and PBL from healthy infants (n = 8; 5-d–24-mo-old) and children (n = 6; 25–72-mo-old) were studied. Four umbilical cord blood samples were also used in some experiments. Immunophenotypes of fresh or frozen PBMC were determined by direct fluorescence analysis on a FACScan® flow cytometer (Becton Dickinson & Co., Mountain View, CA) using the FITC-δTCS1 (Vδ1-Jδ1/Jδ2 specific) mAb (3) from T Cell Sciences (Cambridge, MA). The Vδ1+ cell count was found to be normal in all cases (n = 20).

PCR Analysis of Vδ1(D)Jδ1 Junctional Sequences. Genomic DNA from PBL was phenol-chloroform extracted. Total cellular RNA was isolated by the Chomczynski and Sacchi method (7) using RNAzol (Bioprobe System, Montreuil Sous Bois, France). cDNA were synthesized from 1–5 μg of total RNA using random hexanucleotide primers and Moloney murine leukemia virus reverse transcriptase in the presence of RNAsin (Promega Corp., Madison, WI). RNA were primed at 42°C for 30 min in a final vol of 20 μl. PCR reactions (8) were performed in a thermocycler (Perkin-Elmer Corp., Norwalk, CT) using either whole cDNA or 500 ng of genomic DNA, 2.5 U of Taq DNA Polymerase (Perkin-Elmer Corp.), and 25 pmoles of each primer. Amplifications were carried out in a total vol of 100 μl containing 67 mM Tris-HCl (pH, 8.8), 16.6 mM $(NH_4)_2SO_4$, 6.7 mM $MgCl_2$, 10% DMSO (vol/vol), and

J. Exp. Med. © The Rockefeller University Press • 0022-1007/93/07/0121/07 $2.00
Volume 178 July 1993 121–127

1 mM of all four deoxynucleotides. Amplifications before single-strand conformation polymorphisms (SSCP) analysis (9) were performed with α-[^{32}P]dCTP (Amersham International, Amersham, Bucks, UK). The reaction mixture was heated at 94°C for 7 min and 57°C for 2 min in the first round of denaturation and annealing. Afterward, 35 cycles were performed (50 s at 94°C, 50 s at 57°C, and 50 s at 72°C). The last cycle was followed by an incubation at 72°C for 7 min. The sequences of the specific primers used are as follows: Vδ1, 5′-AAAGTGGTCGCTATTCTGTC and Jδ1 5′-GGAAAAGGAACCCGTGTGACT (10).

Extensive precautions were taken to minimize carry-over of amplified DNA (11).

PCR Analysis by PAGE and SSCP. PCR products were analyzed by 6% PAGE, visualized by ethidium bromide staining, and then transferred to a nylon membrane (Hybond N; Amersham) in 20× SSC. For SSCP analysis, after ethanol precipitation, products were applied on a nondenaturating 6% polyacrylamide gel and run at 4 W at room temperature (9).

Cloning and Sequencing. The amplified products were purified from 6% PAGE and the appropriate sized bands were cut out and extracted overnight in Maxam-Gilbert buffer (12). After two ethanol precipitations, the purified product was filled-in with Kleenow followed by kinasing with T4 polynucleotide kinase and ATP. After phenol and chloroform extraction, the blunt-ended and kinased inserts were ligated into the SmaI site of M13mp18. The plaques were screened with a ^{32}P end-labeled oligoprobe recognizing Vδ1 sequences located 3′ to those specific for the Vδ1 amplimer (5′-AAAGCAGCGAAATCCGTCGCCTTA). Filters (Hybond N; Amersham) were prehybridized for 4 h at 55°C in 2× SSC, 5× Denhart's solution, 0.1% SDS, and then hybridized overnight in the same solution with addition of the oligoprobe. The filters were washed in 2× SSC for 15 min at room temperature, and twice in 2× SSC, 0.1% SDS for 15 min at room temperature, and then again at 55°C for 20 min. The washed filters were exposed for 4–16 h to Kodak XAR-5 film at −80°C with intensifying screens. Clones containing appropriate inserts were picked out at random. Single-stranded DNA clones were prepared and sequencing was done by the dideoxy chain termination method using Sequenase version 2.0-kit and universal M13 primer (United States Biochemical Corp., Cleveland, OH).

Results

Restriction of the Vδ1-Jδ1 Junctional Diversity in Adults. To define the CDR3 repertoire of normal Vδ1-Jδ1 rearrangements, the junctional sequences from two pediatric thymocyte samples and 29 unselected healthy adult donor peripheral blood leukocytes (PBL) were amplified by PCR. Sizes of amplified products were analyzed by PAGE. Representative experiments are shown in Fig. 1.

As expected, in the case of postnatal thymocytes, major variations of PCR product size were observed, leading to a smearlike electrophoresis pattern due to the extensive junctional diversity of TCR-δ rearrangements (13).

In marked contrast, very different results were obtained with healthy donor PBL (Fig. 1). A smearlike pattern similar to that observed in thymus DNA was found in only 8 of 29 cases. Predominant rearrangements were present in the other cases. Each pattern appeared to be specific for a given individual and was reproducible in independent PCR performed at least twice in two different laboratories (data not shown). The PAGE pattern did not correlate with the Vδ1$^+$ cell count (data not shown).

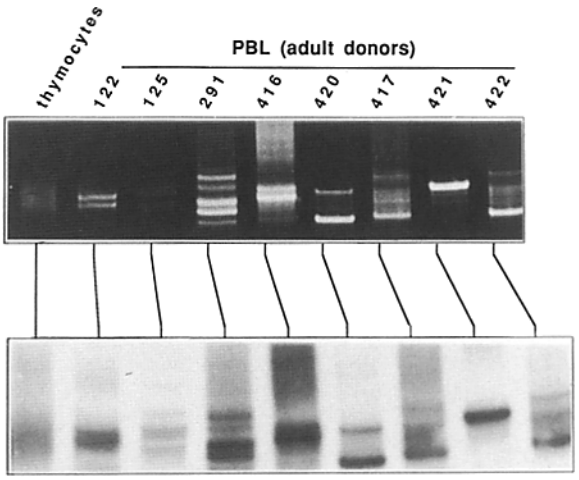

Figure 1. PCR analysis of the peripheral blood Vδ1-Jδ1 CDR3 repertoire from healthy adult donors. PAGE of PCR-amplified Vδ1-Jδ1 junctions. (*Top*) Ethidium bromide staining. Highly restricted patterns are observed in cases 122, 291, 416, 420, and 421. The sample 422 is characterized by a smearlike pattern associated with a predominant band. A predominantly unrestricted pattern is observed in cases 125 and 417. (*Bottom*) Hybridization of the blot with a Vδ1 probe.

To assess whether the predominantly amplified band(s) correspond(s) to repeated junctional sequences and not merely to junctions of identical size, genomic DNA PCR products obtained from eight adult donors, selected to be representative of restricted and unrestricted PAGE patterns, were cloned and sequenced (Fig. 2). Diverse and nonrepeated Vδ1-Jδ1 sequences were found in the two samples displaying a predominantly nonrestricted PAGE pattern (samples 125 and 134). Conversely, repeated junctions were observed in all six cases demonstrating a more or less discrete Vδ1-Jδ1 PAGE pattern (Figs. 1 and 3, and data not shown). None of these sequences were shared by different donors. The predominant rearrangement in most cases includes extensive random nucleotide deletions/additions and the use of D regions. In all but one case (sample 127), repeated sequences were in-frame and could therefore encode a functional δ chain. In one case (sample 119), cloning procedures were used on both DNA- and cDNA-derived Vδ1-Jδ1 PCR products. The same predominant Vδ1-Jδ1 junction was observed in both experiments (of 14 cDNA PCR-derived clones hybridizing to an internal Vδ1 probe, 8 also hybridized to an antijunctional oligonucleotide (AJO)–specific for the predominant DNA junctional sequence), suggesting that the restricted sequence may be expressed at the cell surface of at least a part of the Vδ1$^+$ γ/δ T cells in this donor.

An alternative strategy based on SSCP was used to confirm the CDR3 restriction in selected samples, including three samples also studied by sequence analysis. PCR carried out with α-[^{32}P]dCTP were performed on genomic DNA and the resulting products fully denatured. One aliquot was loaded as a control on a high resolution denaturing acrylamide gel and another aliquot on a nondenaturing one to analyze single-

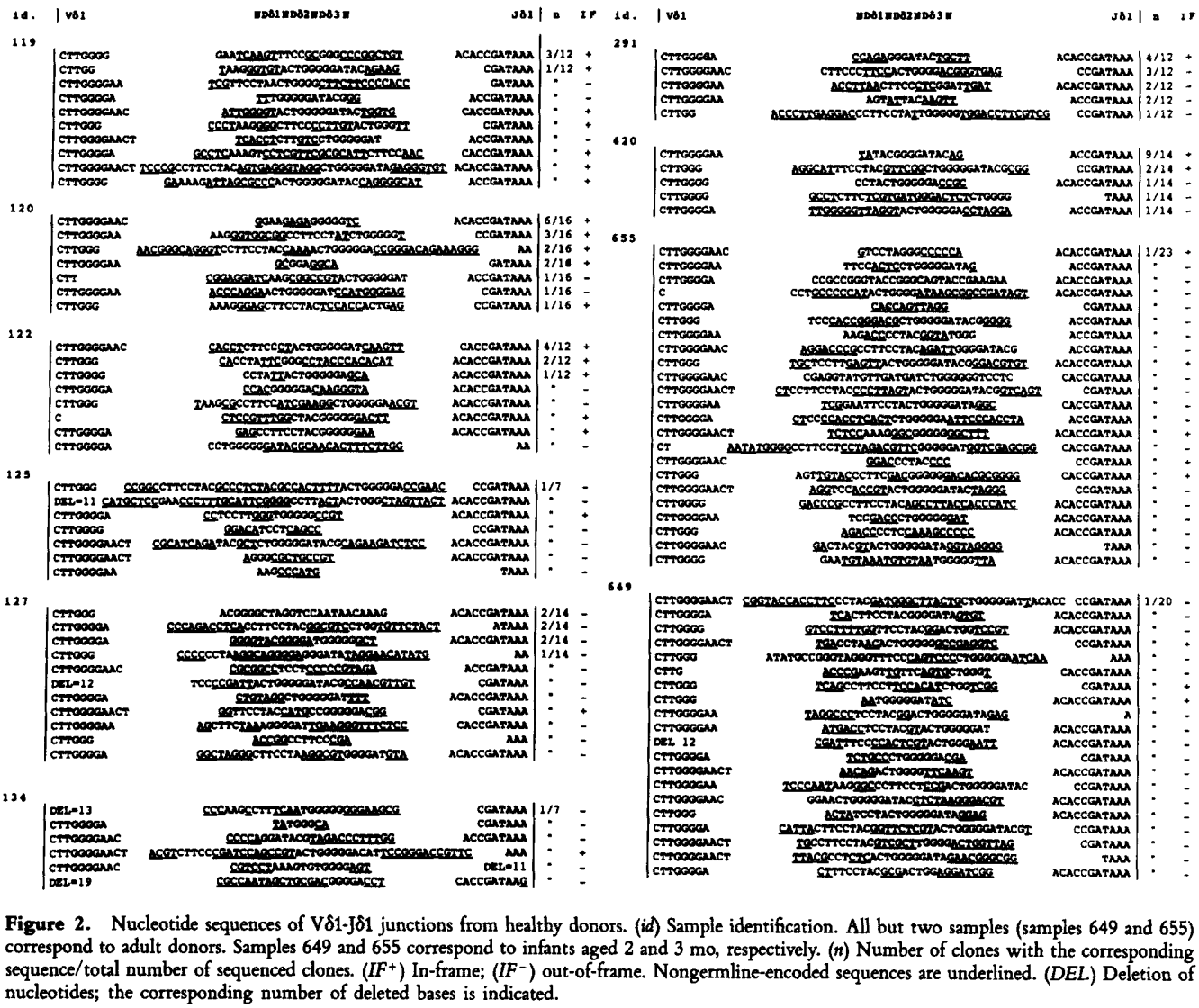

Figure 2. Nucleotide sequences of Vδ1-Jδ1 junctions from healthy donors. (*id*) Sample identification. All but two samples (samples 649 and 655) correspond to adult donors. Samples 649 and 655 correspond to infants aged 2 and 3 mo, respectively. (*n*) Number of clones with the corresponding sequence/total number of sequenced clones. (IF^+) In-frame; (IF^-) out-of-frame. Nongermline-encoded sequences are underlined. (*DEL*) Deletion of nucleotides; the corresponding number of deleted bases is indicated.

stranded PCR product conformations (Fig. 3). This strategy theoretically discriminates distinct CDR3 junctions of identical size from CDR3 junctions displaying restricted sequences. In both cases, discrete bands are expected to be demonstrated by electrophoresis on denaturing gels. In contrast, discrete bands are expected to be observed by SSCP analysis only if repeated junctional sequences are present in the analyzed sample. Results obtained on denaturing acrylamide gels are shown in Fig. 3, confirming the patterns obtained by standard PAGE (thymocytes, samples 122, 125, 420, 421, 422, and data not shown) as well as from sequencing data (samples 120, 127, and 420). As expected from the assumption that fragments of restricted size correspond to restricted sequences, discrete bands were observed in the SSCP assay in the relevant cases (Fig. 3).

Although the previously mentioned reproducibility of the PAGE pattern in independent experiments performed on a given sample suggests that the observed CDR3 restriction is not due to a sampling effect, it was important to demonstrate this point more directly. One sample (number 119) obtained from a donor with a normal $V\delta1^+$ cell count (i.e., lymphocytes, 1,269/mm^3; $V\delta1^+$ cells, 9/mm^3) was further studied by dilution analysis. It is to be expected that the PAGE-specific pattern would be lost or would vary in increasing dilutions if the CDR3 restriction is only due to a sampling effect. The characteristic oligoclonal-specific pattern was observed unchanged from 580 ng (roughly equivalent to 87,000 PBL and to 610 $V\delta1^+$ cells, as expected from the percentage of δTCS1$^+$ cells in the PB of this particular donor) to 15 ng (equivalent to 16 $V\delta1^+$ cells) dilutions and lost only at 7 ng (equivalent to eight $V\delta1^+$ cells) (Fig. 4). Moreover, the AJO (14) recognizing the overrepresented junctional sequence was used as a probe in this experiment showing the corresponding clone-specific sequence to be detectable in experiments performed with as little as 15 ng of DNA (Fig. 4). This experiment shows that the observed CDR3 restriction is not due to a sampling effect.

Stability of the Vδ1-Jδ1 Junctional Repertoire in Adults. To

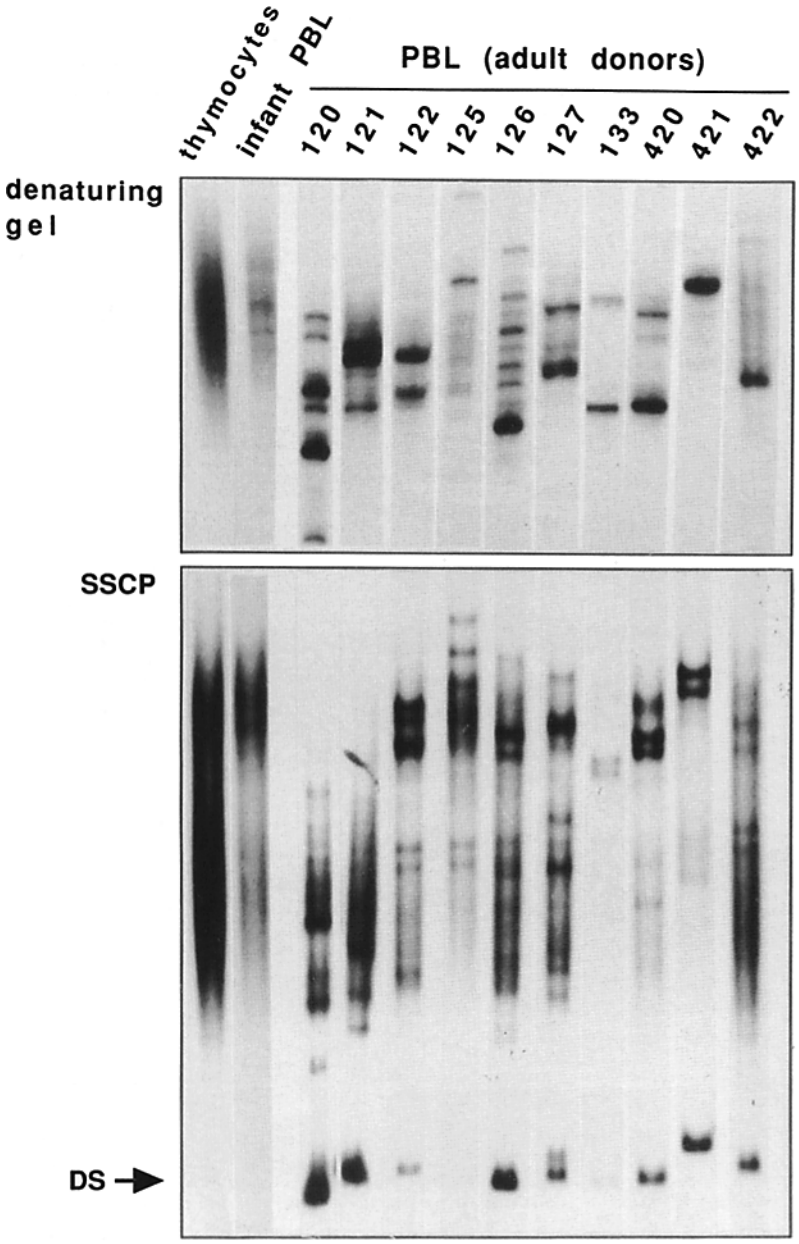

Figure 3. Demonstration by SSCP analysis of the presence of restricted junctional sequences. (*Top*) Electrophoresis on denaturing gels used as a control for the presence of junctions with identical sequences and/or sizes. (*Bottom*) SSCP analysis. (*DS*) double-stranded DNA. Single-stranded DNA predominantly migrates at the top of the gel and the smear of heteroduplexes is predominantly found in the middle part of the gel. Note the lack of discrete bands in lanes corresponding to thymocyte and 655 (3-mo-old infant) PBL DNA.

analyze the stability of the Vδ1-Jδ1 CDR3 repertoire, sequential studies were performed in six healthy adult donors (Fig. 5).

In one of these (sample 813), a complex unrestricted PAGE pattern was evident, that remained unchanged 15 mo later.

In the five other donors (samples 119, 120, 127, 811, and 815), a more or less restricted repertoire was documented. In three cases (samples 119, 120, and 127), the PAGE pattern was again found to be identical 4 yr later. Vδ1-Jδ1 fragments amplified from two 119 samples taken at 2-yr intervals were hybridized with the AJO specific for the predominant and formerly identified sequence. As shown in Fig. 5, the same dominant junctional sequence was found. In the sample 811, the PAGE pattern was roughly similar at 3-yr intervals, although some alterations could be observed. Contrasting with these findings, significant alterations were documented in the last case (sample 815).

Unrestricted Vδ1-Jδ1 Junctional Repertoire in Infants. We wondered whether the nonrandom presence of certain Vδ1 junctions might be related to the donor's age. To answer this question, PB from 5-d–24-mo-old infants (n = 8) and from 25–72-mo-old children (n = 6) were evaluated. Four umbilical cord blood samples were also studied. Representative experiments are shown in Fig. 6 and all data are summarized in Table 1.

Samples from infants aged <2 yr and umbilical cord blood samples display a smearlike PAGE pattern, comparable with

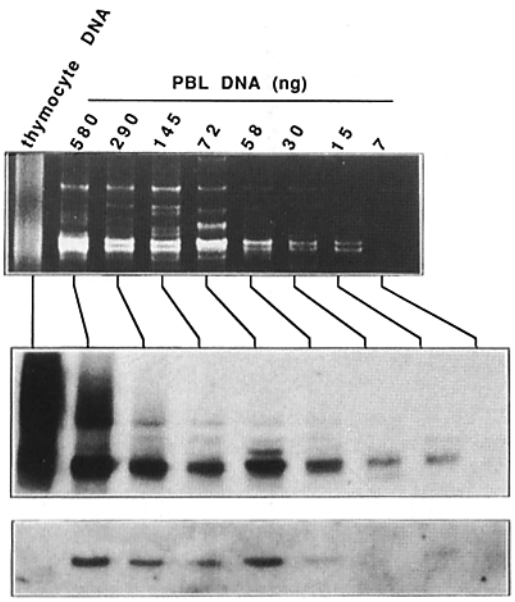

Figure 4. Dilution analysis of the Vδ1-Jδ1 predominant sequence in the 119 healthy donor. (*Top*) Ethidium bromide staining. (*Middle*) Hybridization with a Vδ1 probe. (*Bottom*) Hybridization with a clone-specific AJO recognizing the predominant sequence AATCAAGTTTCCGCG-GGCCC.

that found in thymic cells (Fig. 6). Vδ1-Jδ1 PCR products from two samples, 649 (2-mo-old) and 655 (3-mo-old) were sequenced. As expected from the PAGE pattern, no repeated sequences were found (Fig. 2). One sample (sample 655) was also studied by SSCP analysis. As expected, a smearlike pattern was observed (Fig. 3).

In four children (36–60-mo-old), an unrestricted pattern was found. A discrete band associated with a predominantly

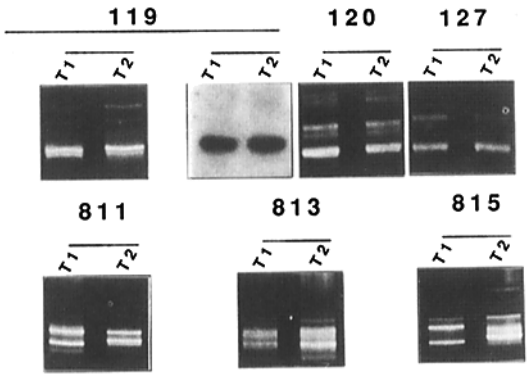

Figure 5. Sequential analysis of the Vδ1-Jδ1 CDR3 repertoire in healthy adult donors. PBL DNAs from donors 119, 120, 127, 811, 813, and 815 were analyzed on two separate occasions (*T1*, *T2*). Delays between T1 and T2: 119, 24 mo; 120, 44 mo; 127, 44 mo; 811, 36 mo; 813, 15 mo; and 815, 44 mo. Ethidium bromide stained gels are shown in all cases including 119 (119, *left*). (119, *right*) Southern analysis of PCR products with a clone-specific probe corresponding to the predominant junctional sequence characterized in sample T1 after cloning and sequencing (see Fig. 2 and the legend to Fig. 4 for the relevant sequence). An identical PAGE pattern was documented in a sample from the donor 119 taken 43 mo after T1 (data not shown).

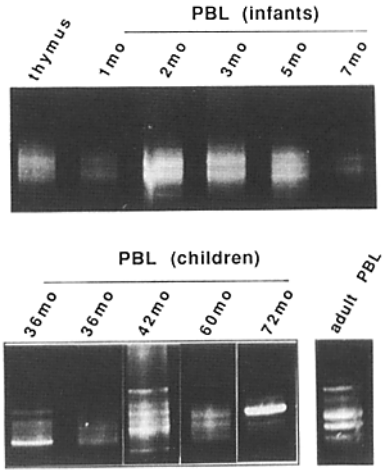

Figure 6. Vδ1-Jδ1 CDR3 repertoire in PBL from infants and children. Ages are indicated in months. Thymic DNA (*3-mo*-old child) and one PBL DNA samples from an adult donor (sample 291) are included as controls.

unrestricted pattern was documented in two donors (25- and 36-mo-old). Finally, a clearly restricted repertoire is evident in the sample from the oldest child (72-mo-old).

As is shown in Table 1, there is an evident increase in the number of individuals demonstrating a restricted repertoire with increasing age.

Collectively, these data clearly demonstrate that a restriction of the PB lymphocyte Vδ1-Jδ1 junctional repertoire is frequent among healthy donors, that this restriction is acquired during the postnatal period, and finally, that this repertoire is generally a stable feature. These results are strongly suggestive of a postnatal positive selection process involving Vδ1$^+$ γ/δ T cells expressing specific CDR3 sequences.

Discussion

In this work, we have characterized the TCR-δ gene diversity in the PBL of healthy donors. We have demonstrated that the Vδ1-Jδ1 junctional repertoire is restricted in a majority

Table 1. *Correlation between the Vδ1-Jδ1 Junctional Repertoire and the Donor Age*

	Total number of cases	Number of cases with a restricted PAGE pattern
Umbilical cord blood	4	0
Infants (5 d–24 mo)	8	0
Children (25–72 mo)	6	3*
Adults	29	21

* Includes two cases with a discrete band associated with a predominantly unrestricted pattern.

of healthy adult donors, leading to a relatively stable and donor-specific pattern. Moreover, we have also shown that this repertoire is not restricted in the PBL of infants, and is therefore similar to that observed in postnatal thymus.

It is generally assumed that γ/δ T cells function as a first line of defense against infectious pathogens. The nature of presenting molecules that may be potentially involved in antigen recognition is unclear, at least for the majority of γ/δ T cells, although evidence for allorecognition of MHC class I (15) and II (16), and of other less polymorphic molecules (17–19) has been documented. A few examples of clones recognizing nominal antigens in the context of MHC molecules have also been described (20, 21).

Little is known about the function of the Vδ1 subset. It has been shown, however, that the binding to Vδ1$^+$ cells of a Vδ1-specific mAb elicits Ca^{2+} signaling and cell proliferation (22), and that activated Vδ1$^+$-expressing clones are cytolytic and can secrete IL-5, IFN-γ, and GM-CSF (23). Moreover, TCR-dependent recognition of CD1c (17) and CD48 (TCT.1) (18, 19) molecules by Vδ1$^+$ cells has been documented. More recently, it has been shown that the Vδ1$^+$ subset of γ/δ T cells may be triggered to proliferate by cell–cell interaction with EBV-infected Burkitt's lymphoma cells or EBV-transformed B cells (24).

The new molecular data we have obtained regarding Vδ1$^+$ cells in healthy donors are consistent with the involvement of Vδ1$^+$ cells in the recognition of processed peptides. Peptide plus MHC recognition by α/β T cells is mainly mediated by the V(D)J junctional CDR3 (25, 26), and the role of TCR-γ/δ junctional sequences in the specificity of antigen recognition has been recently stressed (27). In the present work, we have shown that Vδ1-Jδ1 junctions, while displaying the extensive alterations of germline sequences that are characteristic of post fetal stages (14), are not randomly represented among PBL Vδ1$^+$ cells in a large fraction of healthy donors. The description of such repeated sequences in the PBL of healthy donors is unprecedented since only a few Vδ1-Jδ1 sequences have been published to date (28–31). Two studies showed an unrestricted repertoire in PBL from patients and normal controls, but predominant sequences in acute multiple sclerosis (29) and leprosy lesions (28), respectively, suggesting in situ selection of particular Vδ1$^+$ cells by so far undefined antigens. One further study demonstrated a restricted Vδ1 repertoire in the peripheral blood of certain patients with rheumatoid arthritis but not in four healthy controls (30). However, it is worth noting that a recent report of preferential Vδ1-Jδ1 junctional repertoires in sarcoidosis, demonstrated a repetitive sequence in cDNA from the PBL of one of the normal control (31).

The occurrence of dominant receptors in PBL demonstrated here may be due to thymic selection, since it has been suggested that negative thymic selection of a limited part of the γ/δ T cell population can occur in appropriate transgenic mice (32). In addition, it is generally assumed that Vδ1$^+$ PBL derive from the predominant Vδ1$^+$ thymic γ/δ T cell subset. On the other hand, the hypothesis of thymic selection of defined Vδ1-Jδ1 junctions is not substantially supported by our demonstration that these junctions are not restricted in infant PBL, in whom the pattern is roughly similar to the one observed in thymus. The age corresponding to the switch between an unrestricted and a restricted repertoire remain to be determined. However, it is worth noting that a clearly restricted repertoire was documented in a 72-mo-old child in our series. We therefore favor the hypothesis that the restricted repertoire we have found in the majority of healthy donors is due to a recruitment of γ/δ T cells that express particular Vδ1-Jδ1 junctions. A similar mechanism has been speculated to occur in the case of murine pulmonary γ/δ T cells with canonical invariant TCR-δ sequence (33). Since the deduced CDR3 amino acid sequences vary from one donor to another, the results suggest that these cells recognize a limited number of nominal antigens, distinct from one donor to another, and presented by nonclassical proteins like the CD1 family. Alternatively, these antigens may be similar in all donors, but could be presented by highly polymorphic molecules. Studies of identical twins should be performed to further clarify this point.

We thank N. Duparc for the synthesis of oligonucleotides and all GBBM members for stimulating discussions.

This work is supported by the Direction de la Recherche et des Etudes Doctorales, the Comité de Paris de la Ligue Nationale contre le Cancer, and by Association de Recherche contre le Cancer.

Address correspondence to Dr. F. Sigaux, Laboratory of Molecular Hematology, Centre Hayem, Saint Louis Hospital, 2 Place du Docteur Fournier, 75475 Paris, France.

Received for publication 22 September 1992 and in revised form 22 March 1993.

References

1. Davis, M.M., and P.J. Bjorkman. 1988. T-cell antigen receptor genes and T-cell recognition. *Nature (Lond.).* 334:395.
2. Parker, C.M., V. Groh, H. Band, S.A. Porcelli, C. Morita, M. Fabbi, D. Glass, J.L. Strominger, and M.B. Brenner. 1990. Evidence for extrathymic changes in the T cell receptor γ/δ repertoire. *J. Exp. Med.* 171:1597.
3. Triebel, F., and T. Hercend. 1989. Subpopulations of human peripheral T gamma delta lymphocytes. *Immunol. Today.* 10:186.

4. Casorati, G., G. De Libero, A. Lanzavecchia, and N. Migone. 1989. Molecular analysis of human γ/δ^+ clones from thymus and peripheral blood. *J. Exp. Med.* 170:1521.
5. Deusch, K., F. Lüling, K. Reich, M. Classen, H. Wagner, and K. Pfeffer. 1991. A major fraction of human intraepithelial lymphocytes simultaneously expresses the γ/δ T cell receptor, the CD8 accessory molecule and preferentially uses the Vδ1 gene segment. *Eur. J. Immunol.* 21:1053.
6. Porcelli, S., M.B. Brenner, and H. Band. 1991. Biology of the human gamma/delta T-cell receptor. *Immunol. Rev.* 120:137.
7. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate phenol chloroform extraction. *Anal. Biochem.* 162:156.
8. Saiki, R.K., D.H. Gelfand, S. Stoffel, S.J. Scharf, R. Higuchi, G.T. Horn, K.B. Mullis, and H.A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science (Wash. DC).* 239:487.
9. Orita, M., H. Iwahana, H. Kanazawa, K. Hayashi, and T. Sekiya. 1989. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc. Natl. Acad. Sci. USA.* 84:2766.
10. Macintyre, E.A., L. d'Auriol, F. Amesland, P. Loiseau, Z. Chen, L. Boumsell, F. Galibert and F. Sigaux. 1989. Analysis of junctional diversity in the preferential Vδ1-Jδ1 rearrangement of fresh T-acute lymphoblastic leukemia cells by in vitro gene amplification and direct sequencing. *Blood.* 74:2053.
11. Kwok, S., and R. Higuchi. 1989. Avoiding false positives with PCR. *Nature (Lond.).* 339:237.
12. Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. Molecular Cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
13. Mcvay, L.D., S.R. Carding, K. Bottomly, and A.C. Hayday. 1991. Regulated expression and structure of T-cell receptor gammadelta-transcripts in human thymic ontogeny. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:83.
14. Macintyre, E.A., L. d'Auriol, N. Duparc, G. Leverger, F. Galibert, and F. Sigaux. 1990. Use of oligonucleotide probes directed against T-cell antigen receptor gamma-delta variable-(diversity)-joining junctional sequences as a general method for detecting minimal residual disease in acute lymphoblastic leukemias. *J. Clin. Invest.* 86:2125.
15. Ciccone, E., O. Viale, D. Pende, M. Malnati, G.B. Ferrera, S. Barocci, A. Moretta, and L. Moretta. 1989. Specificity of human lymphocytes T expressing a gamma/delta T cell antigen receptor. Recognition of a polymorphic determinant of HLA class I molecules by a gamma/delta clone. *Eur. J. Immunol.* 19:1267.
16. Bosnes, V., E. Quigstad, K.E. Lundin, and E. Thorsby. 1990. Recognition of a particular HLA-DQ heterodimer by a human gamma/delta T cell clone. *Eur. J. Immunol.* 20:1429.
17. Porcelli, S., M.B. Brenner, J.L. Greestein, S.P. Balk, C. Terhorst, and P.A. Bleischer. 1989. Recognition of cluster of differentiation 1 antigens by human $CD4^-CD8^-$ cytolytic T lymphocytes. *Nature (Lond.).* 341:447.
18. Mami-Chouaib, F., C. Miossec, P. Del Porto, C. Flament, F. Triebel, and T. Hercend. 1990. T cell target 1 (TCT.1): a novel target molecule for human non-major histocompatibility complex–restricted T lymphocytes. *J. Exp. Med.* 172:1071.
19. Mamichouaib, F., P. Del Porto, D. Delorme, and T. Hercend. 1991. Further evidence for a gamma/delta T-cell receptor mediated TCT.1/CD48 recognition. *J. Immunol.* 147:2864.
20. Kozbor, D., G. Trinchieri, D.S. Monos, M. Isobe, G. Russo, J.A. Haney, C. Zmijewski, and C.M. Croce. 1989. Human TCR-γ^+/δ^+ $CD8^+$ T lymphocytes recognize tetanus toxoid in an MHC-restricted fashion. *J. Exp. Med.* 169:1847.
21. Holoshitz, J., L.M. Vila, B.J. Keroack, D.R. McKinley, and N.K. Bayne. 1992. Dual recognition by cloned human γ/δ T cells. *J. Clin. Invest.* 89:308.
22. Wu, Y.J., W.T. Tian, R.M. Snider, C. Rittershaus, P. Rogers, L. LaManna, and S.H. Ip. 1988. Signal transduction of gamma/delta T cell antigen receptor with a novel mitogenic anti-delta antibody. *J. Immunol.* 141:1476.
23. Krangel, M.S., H. Yssel, C. Brocklehurst, and H. Spits. 1990. A distinct wave of human T cell receptor γ/δ lymphocytes in the early fetal thymus: evidence for controlled gene rearrangement and cytokine production. *J. Exp. Med.* 172:847.
24. Hacker, G., S. Kromer, A. Falk, K. Heeg, H. Wagner, and K. Pfeffer. 1992. V$\delta1^+$ subset of human γ/δ T cells responds to ligands expressed by EBV infected Burkitt's lymphoma cells and transformed B lymphocytes. *J. Immunol.* 149:3984.
25. Jorgensen, J.L., U. Esser, B. Fazekas de St-Groth, P.A. Reay, and M.M. Davis. 1992. Mapping of T-cell receptor-peptide contacts by variant peptide immunization of single-chain transgenics. *Nature (Lond.).* 355:224.
26. Hong, S.C., A. Chelouche, R.H. Lin, D. Shaywitz, N.S. Braunstein, L. Glimcher, and C.A. Janeway, Jr. 1992. An MHC interaction site maps to the amino-terminal half of the T cell receptor α chain variable domain. *Cell.* 69:999.
27. Rellahan, B.L., J.A. Bluestone, B.A. Houlden, M.M. Cotterman, and L.A. Matis. 1991. Junctional sequences influence the specificity of γ/δ T cell receptors. *J. Exp. Med.* 173:503.
28. Uyemura, K., R.J. Deans, H. Band, J. Ohmen, G. Panchamoorthy, C.T. Morita, T.H. Rea, and R.L. Modlin. 1991. Evidence for clonal selection of γ/δ T-cells in response to a human pathogen. *J. Exp. Med.* 174:683.
29. Wucherpfennig, K.W., J. Newcombe, H. Li, C. Keddy, M.L. Cuzner, and D.A. Hafler. 1992. T-cell receptor repertoire in acute multiple sclerosis lesions. *Proc. Natl. Acad. Sci. USA.* 89:4588.
30. Olive, C., P.A. Gatenby, and S.W. Serjeantson. 1992. Evidence for oligoclonality of T cell receptor δ chain transcripts expressed in rheumatoid arthritis patients. *Eur. J. Immunol.* 10:2587.
31. Forrester, J.M., L.S. Newman, Y. Wang, T.E. King, and B.L. Kotzin. 1993. Clonal expansion of lung V$\delta1^+$ T cells in pulmonary sarcoidosis. *J. Clin. Invest.* 91:292.
32. Dent, A.L., L.A. Matis, F. Hooshmand, S.M. Widacki, J.A. Bluestone, and S.M. Hedrick. 1990. Self-reactive γ/δ T cells are eliminated in the thymus. *Nature (Lond.).* 343:714.
33. Sim, G.K., and A. Augustin. 1990. Dominantly inherited expression of BID, an invariant undiversified T cell receptor δ chain. *Cell.* 61:397.