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Human T-cell-receptor δ chain: Genomic organization, diversity, and expression in populations of cells

(thymic T cells/junctional diversity/polymerase chain reaction)

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ABSTRACT **The locus of the δ chain of the human T-cell receptor has been isolated and examined. Three D (diversity) regions and two J (joining) regions are present on the 5′ side of the C (constant) region. The closest V (variable) region to the constant region is $V_δ2$, which in the germ line is found on the 3′ side of the constant region in an inverted direction. The genomic structure of the human locus closely parallels its mouse counterpart. Several cDNA sequences and a series of rearranged genomic sequences are compared which demonstrate an enormous potential diversity in the junctional region, between the variable region and the joining region. We find the predominant utilization of the PEER variable region in thymic polyclonal γδ cell lines and in some peripheral blood γδ cell lines. Thus, the δ chain may have relatively limited variable-region diversity but a large junctional-region diversity. The implications of this observation are discussed.**

The T-cell antigen receptor (TCR) expressed on most thymocytes and mature T lymphocytes is a CD3 associated disulfide-linked heterodimer composed of α and β glycoprotein subunits (for review, see ref. 1). A minor population of $CD3^+$ thymocytes and peripheral blood T lymphocytes do not express the αβ heterodimer but instead express a heterodimer composed of γ and δ glycoprotein subunits (2–7). Chien *et al.* (8, 9) identified TCR gene located within the α chain of the TCR (TCRα) locus that encodes the murine δ chain of the TCR (TCRδ) glycoprotein (10, 11). The murine TCRδ gene is rearranged and transcribed early during thymic development (8, 9), and fetal thymocytes predominately express γδ-TCR heterodimers on the plasma membrane (12). The human homologue has been isolated, cloned, and sequenced (13, 14). Antisera generated against a synthetic peptide based on the constant (C) region of the δ chain ($C_δ$) sequence specifically reacted with the TCRδ glycoprotein expressed on the plasma membrane (13, 15). Like the murine TCRδ, the human TCRδ is composed of variable (V), diversity (D), joining (J), and C regions. In the present studies, we have isolated the genomic region coding for three D regions, two J regions, the C region, and a contiguous V region. By using probes from this region, we have examined the transcription of human TCRδ genes in normal and leukemic thymic and peripheral blood lymphocyte (PBL) lines.

MATERIALS AND METHODS

Cells. Mononuclear cells from normal peripheral blood (Stanford Blood Center, Stanford, CA) were isolated using Ficoll/Hypaque (Pharmacia). After plastic adherence and passage through nylon wool to remove monocytes and B cells, respectively, lymphocytes were fractionated by centrifugation on discontinuous Percoll gradients (16). Natural killer (NK) cells were isolated from the low-buoyant-density fraction, while the high-buoyant-density fraction was small resting T lymphocytes (>95% expressing αβ TCR). Normal thymocytes were obtained from pediatric cardiac patients. $CD3^-$,$CD16^+$ NK cell lines, γδ-TCR PBL cell lines (16), γδ-TCR thymic cell lines (17), and αβ-TCR cell lines (17) were cultured in RPMI 1640 (M.A. Bioproducts, Walkersville, MD) containing 10% (vol/vol) fetal bovine serum (Kansas City Biological, Lexena, KS), 1 mM glutamine (GIBCO), gentamycin at 100 μg/ml (GIBCO), and recombinant interleukin 2 (IL-2) at 800 international units/ml (Cetus, Emeryville, CA).

Libraries. A λgtll library (18) was constructed from poly(A)-enriched RNA isolated from a γδ-TCR thymic cell line (16). The human genomic library was a gift from Anne Bowcock (Stanford, CA) and was prepared in Lambda FIX (Stratagene, San Diego, CA) from leukocytes of a pygmy individual. Libraries were also constructed from MOLT-13 and thymic DNA by using the polymerase chain reaction method (19). The primers used were TACTCGAGCCCAGTCATCAGTATCC for $V_δ1$ and GGGTCGACTTACTTGGTTCCACAGTCAC for $J_δ1$. Two micrograms of genomic DNA was amplified 25 times and digested with *Sal* I and *Xho* I. The right-size fragment was purified on a low-melt agarose gel and cloned into Bluescript vectors (Stratagene). The plasmids were screened to identify clones homologous to $V_δ1$ and the appropriate clones were sequenced in both directions.

Probes. The 8A2 $C_γ$ cDNA probe for the C region of the γ chain ($C_γ$) (5) was generously provided by Jim Allison (University of California, Berkeley). Other probes are as described.

Northern Blot Analysis. Total cellular RNA was isolated by using the guanidine isothiocyanate method (20). Northern analysis of RNA gel blots was performed as described (21). Probe inserts were isolated and labeled with [^{32}P]dCTP (Amersham) (>10^9 cpm/μg of DNA) (22). Membranes were hybridized at 65°C for 24 hr with ≈2 × 10^7 cpm of labeled probe in 35 ml of hybridization buffer containing 5× SSPE, 1× Denhardt's solution, 0.3% $NaDodSO_4$, 10% (wt/vol) dextran sulfate, and denatured salmon sperm DNA at 100 μg/ml (1× SSPE = 0.18 M NaCl/10 mM sodium phosphate, pH 7.4/1 mM EDTA; 1× Denhardt's solution = 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin). Membranes were washed twice for 15 min and twice for 30 min (room temperature) in a solution of 10 mM sodium phosphate, 1 mM disodium EDTA, and 0.2% $NaDodSO_4$. After a final wash for 45 min at 65°C, the membranes were autoradiographed.

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Abbreviations: TCR, T-cell antigen receptor; C, constant; D, diversity; J, joining; V, variable; TCRα, TCRδ, etc., TCR α chain, TCR δ chain, etc., respectively; $C_δ$, $V_δ$, $C_γ$, etc., δ chain C region, δ chain V region, γ chain C region, etc., respectively; PBL, peripheral blood lymphocyte; NK, natural killer; IL-2, interleukin 2.

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DNA Sequencing. DNA fragments were subcloned into Bluescript vectors and sequenced by the double-stranded method (23) with the modified T7 DNA polymerase (24) (United States Biochemical, Cleveland, OH).

RESULTS

Sequences of cDNA Clones from a γδ-TCR Thymocyte Library. A λgt11 library was screened with a C_δ-region probe (13). Five phage were selected which were positive for both the 5′ and 3′ ends of the C_δ region. The *Eco*RI fragments were subcloned and sequenced. The C regions were not sequenced entirely, but partial sequences and restriction digests were identical to the published sequence (13, 14). The 5′ fragments were sequenced entirely and fell into several categories. Two were J-C fragments containing a J-C splice but had germ-line unrearranged sequence on the 5′ side of the J region. One of these utilized the $J_\delta1$ sequence and one utilized the $J_\delta2$ sequence, which will be described subsequently. [A similar transcript was found by Hata *et al.* (25).] Two cDNAs included complete VDJC structures that were in-frame. One of these, T3, had a V region identical to PEER with a different sequence between the V and J regions. The second cDNA, T7, differs throughout. We will refer to the PEER V region as $V_\delta1$ and the T7 sequence as $V_\delta2$. A fifth sequence contained a truncated molecule that ends just on the 5′ side of a J region in what is probably an N sequence.

Germ-Line Sequences Homologous to the cDNA Sequences. A λ library prepared from human leukocyte DNA was screened with a J-C_δ probe. Three phage were selected from 750,000 phage screened. Three phage with homology to the J, C, and $V_\delta2$ regions were characterized in detail and a partial restriction map is shown in Fig. 1. Portions of the phage were subcloned and sequenced in both directions as shown in Fig. 2. The regions sequenced include the germ-line regions around three D regions, two J regions, and one V region on the 3′ side of the C region (Fig. 2). The exon–intron boundaries of the C regions were also sequenced (E.Y.L., unpublished data), and the positions of the junctions were identical to those of the mouse (27).

Sequences of Junctional Regions of $V_\delta1$-Bearing δ Chains. Diversity of TCRs can be examined on many levels. The potential diversity is the largest set, including all possible rearrangements, independent of selection for a functional receptor. This level of diversity is difficult to directly study since it would require the isolation of pre-T cells that have rearranged receptors but have not yet expressed them. In preliminary experiments, we found that thymic $CD3^+$,$CD4^+$ cells, of which >95% bear the αβ TCR, have a large proportion of cells that had a $J_\delta1$ rearrangement of $V_\delta1$. Thus these cells have a nonfunctional δ-chain rearrangement. The examination of these sequences, added to the diversity of functional δ chains, would give an estimate of potential δ-chain diversity. The diversity of mouse δ-chain junctional region (28) illustrated the importance of this region so we chose oligonucleotide primers bracketing it. A library of these sequences was produced and sequenced. Fig. 3 shows 19 junctional regions from thymic $CD3^+$,$CD4^+$ cells, as well as several functional δ sequences. [Additional functional δ sequences have been published since the preparation of this figure (25).] The inclusion of known V-region and J-region sequences between the primers provides for an internal control for the accuracy of the amplification method. We found two substitution errors [out of ≈3000 base pairs (bp)] and no other errors, which is in keeping with the expected error frequency (19).

Expression of TCRδ Genes in Polyclonal Cell Lines. Transcription of TCRδ genes was analyzed by Northern blot analysis. By using a C_δ probe, abundant transcripts of 2.2 and 1.5 kilobases (kb) were detected in the PEER leukemia cell line, in the IL-2-dependent thymic-derived γδ-TCR cell line 22, and in the IL-2-dependent PBL-derived γδ-TCR cell line 67 (Fig. 4*A*). Prior studies have indicated that both the 2.2- and 1.5-kb TCRδ transcripts may be functional, but differ in polyadenylylation sites (13, 14). The IL-2-dependent αβ-TCR thymic cell line 23, phytohemagglutinin-activated T lymphoblasts (>95% αβ TCR), and the HPB-ALL leukemia cell line (expressing αβ TCR) did not contain C_δ transcripts. Northern blots were rehybridized with the C_γ probe (Fig. 4*C*). PEER and all thymic and PBL γδ-TCR cell lines demonstrated TCR γ-chain (TCRγ) transcripts, while phytohemagglutinin-activated T lymphocytes and NK cells did not. Although TCRγ RNA was detected in HPB-ALL, there is no evidence that this transcript encodes functional protein.

In preliminary studies, we noted that the $V_\delta1$ probe isolated from PEER hybridized with RNA isolated from a thymic γδ-TCR cell line (13). Use of this V region was further investigated by rehybridizing the Northern blots shown in Fig. 4*A* with the $V_\delta1$ probe. As expected, the $V_\delta1$ probe hybridized with PEER RNA, but also hybridized strongly with RNA from γδ-TCR thymic cell line 22 (Fig. 4*B*). Quantitative densitometry was performed to estimate use of this V region in this polyclonal thymic cell line. This technique has previously been used to estimate relative use of immunoglobulin V region genes by a polyclonal population of pre-B cells (29). By comparing the ratio of the $V_\delta1$ and C_δ bands in the clonal PEER cell line with the V_δ/C_δ ratio of the γδ-TCR thymic cell lines, it was possible to estimate the relative use of the PEER $V_\delta1$ segment. Most of the TCR_δ transcripts of the polyclonal γδ-TCR thymic cell line 22 express the PEER $V_\delta1$. Similar results were obtained by

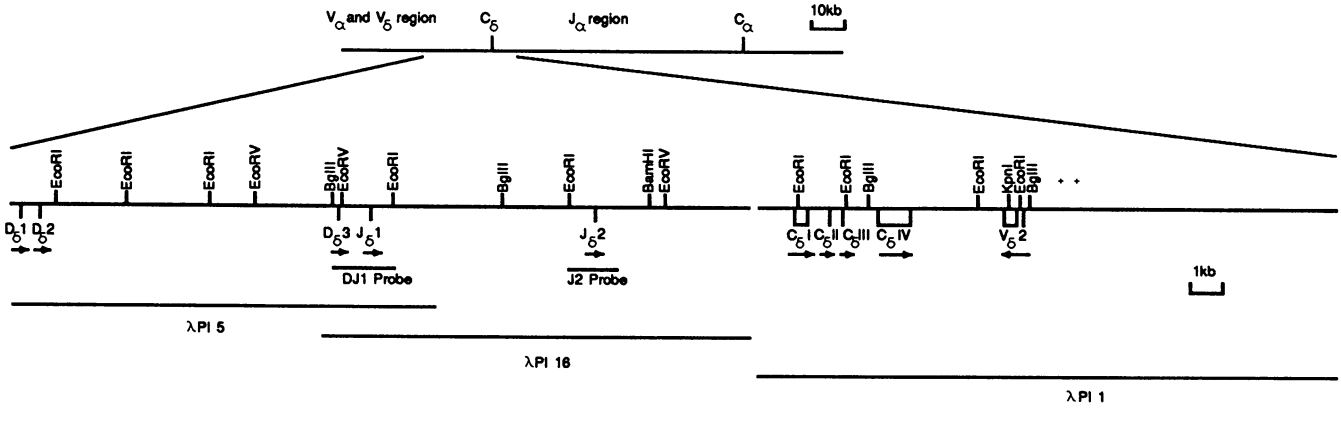

FIG. 1. Genomic organization of the human δ locus. The arrows indicate the direction of transcription. The + indicates a region in which the restriction sites have not been mapped in detail. C_δ I, C_δ II, C_δ III, and C_δ IV refer to the C-region exons. The regions included in the λ clones are drawn below the map. The position of the C regions relative to the J regions is according to ref. 26.

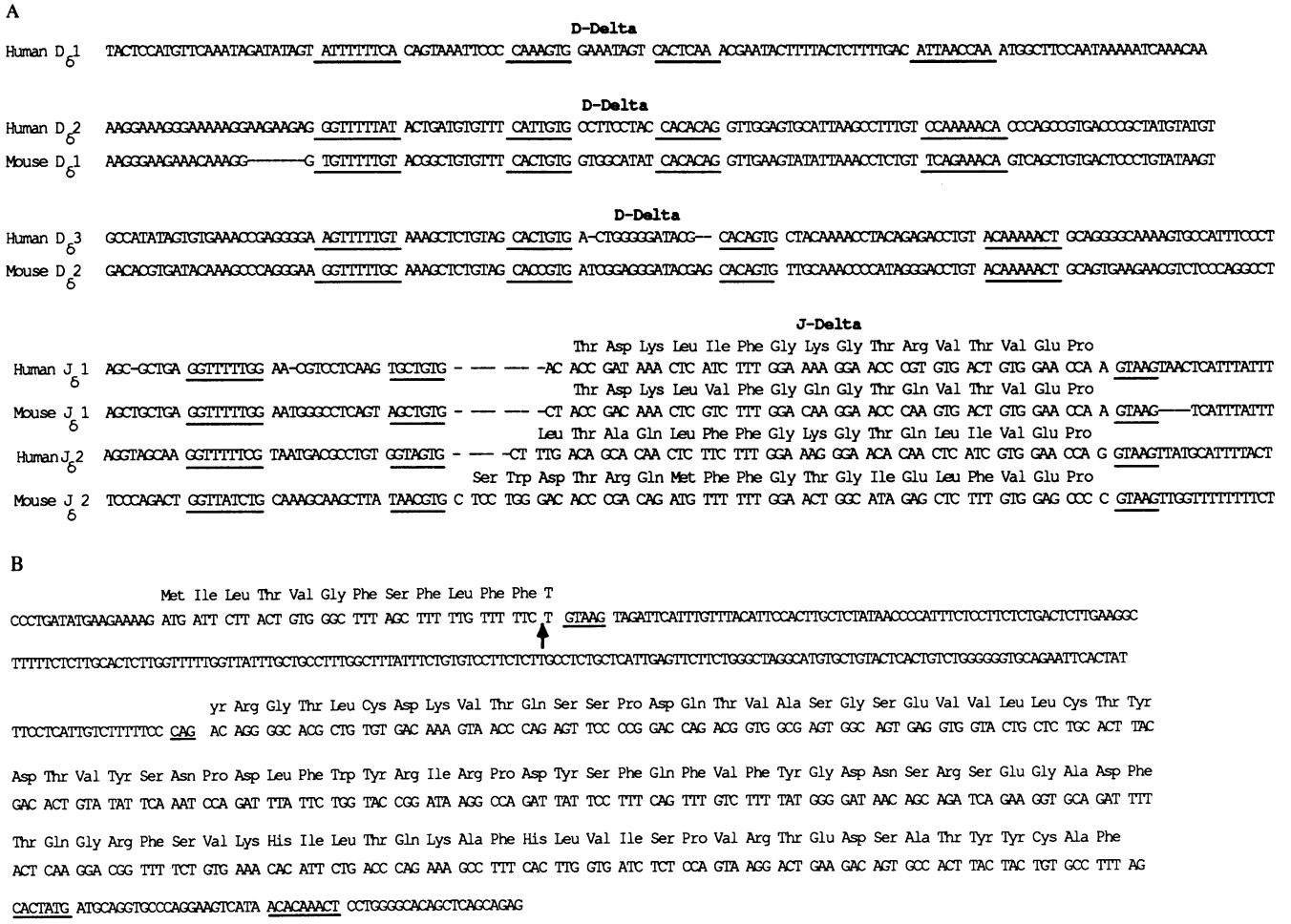

FIG. 2. (*A*) Sequences of D regions and J regions of the δ chain, compared to homologous regions of the mouse (8, 9). The signal sequences are underlined. (*B*) Sequence of $V_\delta 2$. The genomic sequence of the cDNA T7 is shown. The leader exon is hypothetical since the cDNA sequence began with the sequence TACAG as marked by the arrow, 1 bp into the proposed leader sequence.

using two other thymic cell lines, demonstrating that the PEER $V_\delta 1$ is predominantly used by some IL-2-dependent γδ-TCR thymic cell lines (data not shown).

In contrast to results with thymic γδ-TCR cell lines, the PEER $V_\delta 1$ probe failed to hybridize to RNA from PBL γδ-TCR cell line 67 (Fig. 4*B*). Analysis of RNA from polyclonal PBL γδ-TCR cell lines established from four donors revealed that two cell lines completely lacked expression of the PEER $V_\delta 1$, lines 81 and 67, whereas in two cell lines, 61 and 88, the PEER $V_\delta 1$ gene was used (Fig. 4). This difference could not be accounted for by selective culture conditions, since all thymic and PBL γδ-TCR cell lines were established and maintained using identical culture conditions. However, we cannot exclude polymorphism, age-related differences, or other individual differences.

It should be noted that none of the cell lines examined, including the PBL γδ-TCR cell lines 61 and 81, hybridized with the second V-region probe ($V_\delta 2$) isolated from the thymic library (data not shown). We were also unable to detect visible bands when the $V_\delta 2$ probe was hybridized to mRNA isolated from the same thymic CD3, γδ-TCR cell line from which the probe was originally isolated. Therefore, $V_\delta 2$ is apparently infrequently expressed in these polyclonal thymic and PBL γδ-TCR cell lines.

Interestingly, NK cell line 1 expressed a 1.9-kb C_δ transcript, but not the two usual ones. NK cell lines established from three donors all failed to hybridize with the PEER $V_\delta 1$ (Fig. 4 *B* and *D*), although a 1.9-kb C_δ transcript was detected in all lines (data not shown). A similar 1.9-kb transcript is present in MOLT-13, (a γδ-TCR leukemia cell line) and in low amounts in thymic γδ-TCR cell lines (16). Since NK cells do not transcribe TCRγ mRNA (Fig. 4*C*) and since NK cells do not rearrange the TCRγ genes (30) or TCRδ genes (data not shown), NK cells cannot express a functional γδ TCR. Therefore, it is likely that this 1.9-kb TCRδ transcript is nonfunctional, similar to the presence of nonproductive 1.0-kb TCRβ mRNA in these cells (31).

DISCUSSION

The Genomic Structure of the Human TCRδ Locus. The presence of the human δ locus within the α locus on chromosome 14 has been described (26, 32). Here we present detailed sequence structure of several components within that locus including three D regions, two J regions, four-C region exons, and one V region, all within 30 kb. It is quite striking that the genomic structure of the region is highly conserved between the mouse and the human on several levels. (For details of the mouse genomic structure, see ref. 27.) (*i*) The organization of the locus within the α locus is conserved. (*ii*) The existence of two D regions (the homologous region in the mouse of the human $D_\delta 1$ has not been sequenced) and two J regions is conserved although the sequence of the D regions and the second J region are not highly homologous (see Fig. 2*A*). (*iii*) The spacings between the structures are quite conserved. (*iv*) Both species have a highly homologous 3′ V region. (*v*) The exon–intron structure of the C region is tightly conserved. The functional or evolutionary constraints that are responsible for these similarities remain unknown.

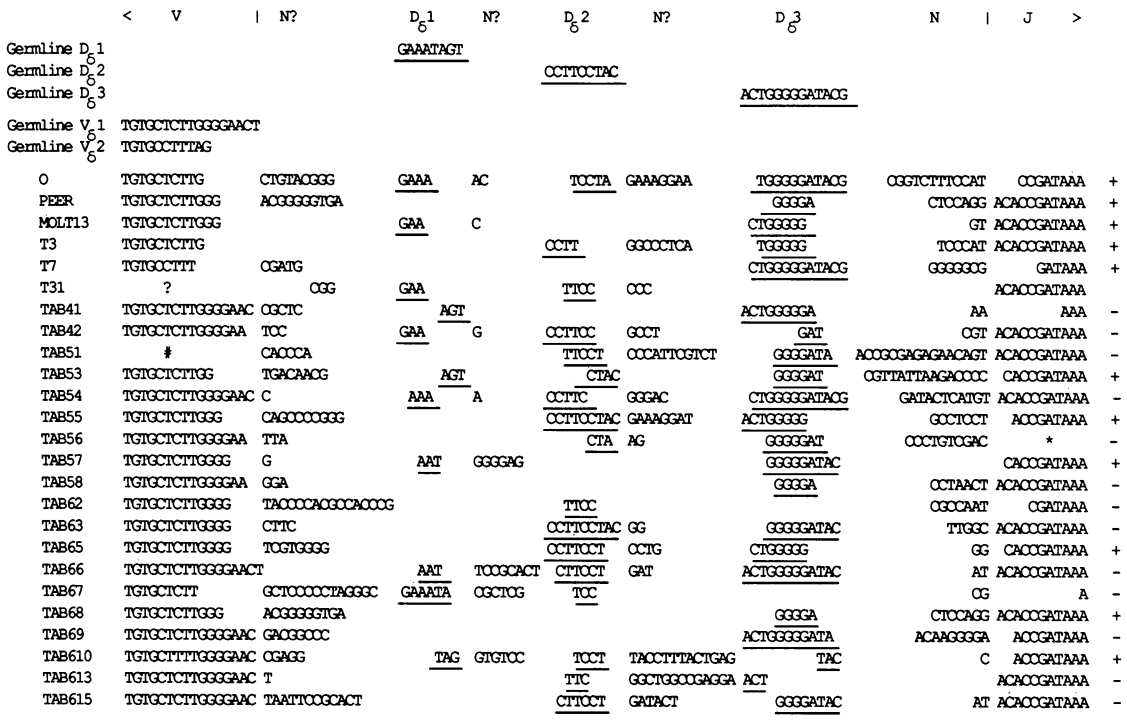

FIG. 3. J-region comparison. The O sequence is from ref. 14 and the PEER sequence is from ref. 13. The ends of the V and J regions are included to show alignment. The 3′ end of the germ-line $V_\delta 1$ is from E.Y.L. (unpublished results). The T31 sequence is only a fragment lacking a 5′ end. The sequences with names beginning with TAB are from a $CD3^+,CD4^+$ thymocyte library. The underlined sequences show homology to the germ-line D regions. Only three or more matches are included. The + and − indicate potential in-frame or out-of-frame peptides, respectively. The # indicates a deletion of 23 bp from the V region and the * indicates a 28-bp deletion of the J region.

A Second J Region of the δ Chain. We have found a cDNA which bears a second J region, but which does not have a rearranged D region. To date, we have yet to find this J region rearranged on a cDNA or as a discrete rearranged band on a Southern blot. Thus, in the populations of cells that we have examined, the predominant J region used is the most 5′ one, which we call $J_\delta 1$. In the mouse, the predominant J region used in the adult δ chain is also the most 5′ one, $J_\delta 1$ (28), whereas the second J region is expressed in fetal thymocytes (9) and in dendritic γδ-TCR cells. It is of great interest to see if $J_\delta 2$ is used in similar human cells.

The V-Region Diversity of the δ Chain May Be Limited. Several lines of evidence suggest that the PEER V region is preferentially utilized. (*i*) The PEER V region is expressed at an RNA level in all four of the T-cell tumor lines which are known to express the γδ TCR (13, 14). (*ii*) The thymic γδ cell lines shown in Fig. 4 also preferentially express this V region. In fact, a rough quantitation suggests that >80% of the δ mRNA of the expected size bears this V region. A similar V-region usage appears to be true for some peripheral cultured γδ-TCR cells (Fig. 4*D*). Thus, some populations of T cells appear to use predominantly $V_\delta 1$. In the mouse, a related situation exists where different populations of T cells utilize different V regions (9, 28).

We found a second V region which is expressed as an in-frame VDJC mRNA. However, it is used infrequently in the population of T cells that we have examined by either Northern or Southern analysis. As with $V_\delta 1$, it does not belong to any of the defined V_α-region subfamilies (33, 34). Comparison of the two human V_δ sequences with those of the mouse show that $V_\delta 1$ is closest to the murine $V_\delta 7$ and that T7 is closest to $V_\delta 6$. These are the predominant V regions used in the adult mouse thymus, and they account for 16 of 21 total adult mouse sequences examined (28). In two polyclonal IL-2-dependent γδ-TCR cell lines established from normal PBLs, we failed to detect expression of either $V_\delta 1$ or $V_\delta 2$, indicating the existence of one or more additional V_δ genes.

Multiple D Regions and N Regions Generate Enormous Junctional Diversity of δ Chains. In Fig. 2*A*, we show the germ-line sequences of three possible D_δ regions, based on the presence of heptomer–nonamer signals. Two of these have known homologous partners in the mouse. The actual coding portions of these D regions diverge greatly between species while the heptamer nonamer signals are conserved. It is striking that the 12-bp spacer within the first heptamer nonamer of the 3′ D region is completely conserved. This suggests that this spacer may have an important sequence-dependent function.

The junctional regions from 24 human δ chains from both γδ-TCR- and αβ-TCR-bearing cells are shown in Fig. 3. These sequences show the enormous potential diversity in this portion of the molecule. Note that the sequences suggest that one junctional region can utilize all three D regions thus necessitating four possible N regions. Additional D regions may exist on the 5′ side of $D_\delta 1$, or between $D_\delta 2$ and $D_\delta 3$, but not between $D_\delta 1$ and $D_\delta 2$ or between $D_\delta 3$ and $J_\delta 1$, since these regions have been completely sequenced (E.Y.L., unpublished data) and they contain no possible D regions. The N regions can be quite long; for example, TAB51 has an N region 16 bp long. Thus, the δ chain has a stretch of roughly 5–15 amino acids, which may confer enormous diversity.

Implications on the Possible Functions of the δ Chain. A major unresolved question regarding the γδ TCR is the nature of the ligand that it recognizes. The similarities and differences between the αβ TCR and γδ TCR give some clues to that question. Davis and Bjorkman (35) have proposed that the junctional regions that comprise the CDR3 (complementarity determining region 3) of the TCR are the main contact points between the antigenic peptide and the receptor, whereas the CDR1 and CDR2 of the V region form contact points with the major histocompatibility complex (MHC) molecule. This idea postulates that the V region and the junctional region have separate, though not independent, recognition functions. The overall V_δ regions as a group are

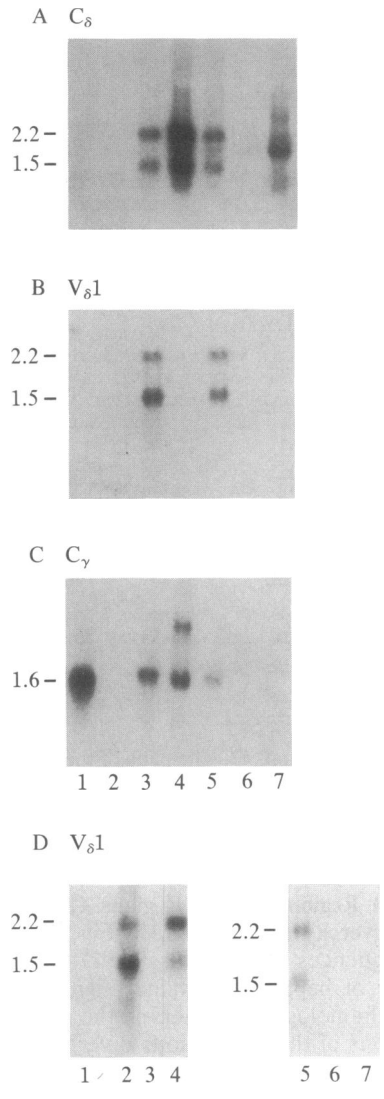

FIG. 4. Expression of TCRδ. RNAs were isolated from the indicated cell lines. Equal amounts of each RNA (10 μg per lane) were analyzed by the Northern blot technique using ^{32}P-labeled C_δ (*A*), $V_\delta 1$ (*B* and *D*), and C_γ (*C*) probes. (*D*) Membranes were rehybridized with ^{32}P-labeled C_δ probe and PEER, PBL γδ cell lines 81, 61, and 88 all demonstrated abundant amounts of 2.2- and 1.5-kb C_δ transcript (data not shown). Lanes for *A–C*: 1, HPB-ALL cells; 2, phytohemagglutinin-activated T lymphocytes; 3, PEER cells; 4, γδ PBL 67; 5, γδ thymic-derived cell line 22; 6, αβ thymic-derived cell line 23; 7, NK cell line 1. Lanes for *D*: 1, phytohemagglutinin-activated T cells; 2, PEER cells; 3, γδ PBL line 81; 4, γδ PBL line 61; 5, γδ PBL line 88; 6, NK line 2; 7, NK line 3.

indistinguishable from V_α as a group and one would not expect a radical change in their recognition properties. The striking features of the γδ TCR that differ from the αβ TCR are the relative lack of V-region diversity—i.e., fewer V regions and thus fewer CDR1 and CDR2 possibilities—and vastly greater diversity of the junctional region. Furthermore, it has been observed in the mouse that different V regions are utilized by different populations of γδ T cells. Consequently, we propose the following model. Each V_δ region has a preferred V_γ which together recognize a presenting molecule which is major histocompatibility complex-related (36), nonpolymorphic, and possibly tissue-specific. For example, skin γδ V regions might recognize a tissue-specific molecule on Langerhans cells or epidermal cells. The junctional region would then provide recognition of foreign or altered self peptides. Janeway *et al.* (37) have proposed a related model where the γδ T cells may mediate immune surveillance.

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1. Allison, J. P. & Lanier, L. L. (1987) *Annu. Rev. Immunol.* **5,** 503–540.
2. Brenner, M. B., McLean, J., Dialynas, D. P., Strominger, J. L., Smith, J. A., Owen, F. L., Seidman, J. G., Ip, S., Rosen, F. & Krangel, M. S. (1986) *Nature (London)* **322,** 145–149.
3. Weiss, A., Newton, M. & Crommie, D. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 6998–7002.
4. Lanier, L. L. & Weiss, A. (1986) *Nature (London)* **324,** 268–270.
5. Lanier, L. L., Federspiel, N. A., Ruitenberg, J. J., Phillips, J. H., Allison, J. P., Littman, D. & Weiss, A. (1987) *J. Exp. Med.* **165,** 1076–1094.
6. Bank, I., Depinho, R. A., Brenner, M. B., Cassimeris, J., Alt, F. W. & Chess, L. (1986) *Nature (London)* **322,** 179–181.
7. Brenner, M. B., McLean, J., Scheft, H., Riberdy, J., Ang, S.-L., Seidman, J. G., Devlin, P. & Krangel, M. S. (1987) *Nature (London)* **325,** 689–694.
8. Chien, Y.-h., Iwashima, M., Kaplan, K. B., Elliott, J. F. & Davis, M. M. (1987) *Nature (London)* **327,** 677–682.
9. Chien, Y.-h., Iwashima, M., Wettstein, D. A., Kaplan, K. B., Elliott, J. F., Born, W. & Davis, M. M. (1987) *Nature (London)* **330,** 722–727.
10. Bonyhadi, M., Weiss, A., Tucker, P. W., Tigelaar, R. E. & Allison, J. P. (1987) *Nature (London)* **330,** 574–576.
11. Born, W., Miles, C., White, J., O'Brien, R., Freed, J. H., Marrack, P., Kappler, J. & Kubo, R. T. (1987) *Nature (London)* **330,** 572–574.
12. Pardoll, D. M., Fowlkes, B. J., Bluestone, J. A., Kruisbeek, A., Maloy, W. L., Coligan, J. E. & Schwartz, R. H. (1987) *Nature (London)* **326,** 79–81.
13. Loh, E. Y., Lanier, L. L., Turck, C. W., Littman, D. R., Davis, M. M., Chien, Y.-h. & Weiss, A. (1987) *Nature (London)* **330,** 569–572.
14. Hata, S., Brenner, M. B. & Krangel, M. S. (1987) *Science* **238,** 678–682.
15. Band, H., Hochstenbach, F., McLean, J., Hata, S., Krangel, M. S. & Brenner, M. B. (1987) *Science* **238,** 682–684.
16. Phillips, J. H. & Lanier, L. L. (1987) *J. Exp. Med.* **164,** 814–825.
17. Phillips, J. H., Weiss, A., Gemlo, B. T., Rayner, A. A. & Lanier, L. L. (1987) *J. Exp. Med.* **166,** 1579–1584.
18. Huynh, T. V., Young, R. A. & Davis, R. W. (1985) in *DNA Cloning, A Practical Approach,* ed. Glover, D. M. (IRL, Washington, DC), Vol. 1, pp. 49–78.
19. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1987) *Science* **239,** 487–491.
20. Chirgwin, J. M., Przybyla, A. E., MacDonald, J. R. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
21. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 382–389.
22. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
23. Chen, E. Y. & Seeburg, P. H. (1985) *DNA* **4,** 165–170.
24. Tabor, S. & Richardson, C. C. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1074–1078.
25. Hata, S., Satyanarayana, K., Devlin, P., Band, H., McLean, J., Strominger, J., Brenner, M. B. & Krangel S. (1988) *Science* **240,** 1541–1544.
26. Boehm, T., Baer, R., Lavenir, I., Forster, A., Waters, J. J., Nacheva, E. & Rabbitts, T. H. (1988) *EMBO J.* **7,** 385–394.
27. Iwashima, M., Green, A., Davis, M. M. & Chien, Y.-h. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8161–8165.
28. Elliott, J. F., Rock, E. P., Davis, M. M. & Chien, Y.-h. (1988) *Nature (London)* **331,** 627–631.
29. Coffman, R. L. & Weissman, I. L. (1983) *J. Mol. Cell. Immunol.* **1,** 31–38.
30. Lanier, L. L., Cwirla, S. & Phillips, J. H. (1986) *J. Immunol.* **137,** 3375–3377.
31. Young, H. A., Ortaldo, J. R., Heberman, R. B. & Reynolds, C. W. (1986) *J. Immunol.* **136,** 2701–2704.
32. Griesser, H., Champagne, E., Tkachuk, D., Takihara, Y., Lalande, M., Baille, E., Minden, M. & Mak, T. W. (1988) *Eur. J. Immunol.* **18,** 641–644.
33. Yoshikai, Y., Kimura, N., Toyonaga, B. & Mak, T. W. (1986) *J. Exp. Med.* **164,** 90–103.
34. Klein, M. H., Concannon, P., Everett, M., Kim, L. D. H., Hunkapiller, T. & Hood, L. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6884–6888.
35. Davis, M. M. & Bjorkman, P. J. (1988) *Nature (London)* **334,** 395–402.
36. Matis, L. A., Cron, R. & Bluestone, J. A. (1987) *Nature (London)* **330,** 262–264.
37. Janeway, C. A., Jones, B. & Hayday, A. (1988) *Immunol. Today* **9,** 73–76.