γ/δ Lineage Relationship Within a Consecutive Series of Human Precursor T-Cell Neoplasms


We analyzed the gene rearrangements associated with the newly described δ T-cell receptor (TCR) gene from a series of 19 consecutive precursor T-cell (lymphoblastic) neoplasms that represent discrete stages surrounding the TCR gene rearrangement process. Significantly, the δ TCR gene showed rearrangement in most (13 of 19) of these T cells, and in addition it was rearranged in two cells displaying no rearrangement for any other TCR gene. Our survey showed three types of δ gene rearrangements associated with cell-surface TCR expression that presumably represent usage of three Vγ genes. This analysis demonstrates (1) a major subclass of human precursor T-cell neoplasms belonging to the γ/δ T-cell receptor-rearranging subtype; (2) a narrow repertoire of human Vγ gene usage; and (3) the utility of δ gene rearrangements as a diagnostic clonal marker in precursor T lymphoblastic neoplasms.

This is a US government work. There are no restrictions on its use.

MATERIALS AND METHODS

Cells. Nineteen cases of T-cell lymphoblastic neoplasms involving lymph nodes (cases 1 through 10, 14 through 19) or pleural effusions (cases 11 through 13) were selected as described by Pitaluga et al.26 for the properties of typical morphology and intranuclear terminal deoxynucleotidyl transferase. Greater than 90% of the cells were neoplastic in all instances except for cases 16 and 19, which were focally involved lymph nodes. T-cell lineage was defined as reactivity with both the earliest T-cell-specific marker, CD7, and at least one of the pan–T-cell markers, CD2 or CD5. None of these neoplasms expressed the B-cell markers CD19 or CD20. In addition, these lymphomas are typically characterized by aggressive growth and by mediatinal involvement. The cell lines 702 and ID7 were established in our laboratory and are human T-cell lymphotropic virus (HTLV)-I transformants bearing the γ/δ TCR. MV is
an IL-2-dependent cell line bearing the \( \gamma/\delta \) TCR and described by Ciccone et al.\(^{27} \)

**DNA.** DNA was prepared from biopsy specimens as described. Restriction digestion with the enzymes EcoRI, BamHI, or HindIII was performed according to the manufacturers’ recommendations. Agarose gel electrophoresis, blotting onto Genescreen membranes, hybridization, and autoradiography were performed according to previously published protocols.\(^{13,26} \)

**Genomic clones.** DNA from acute T-lymphocyte leukemia (T-ALL) cases 5 and 10 was digested to completion with the restriction enzyme EcoRI, and a size corresponding to the J\(_{\delta}\) rearrangement was obtained by size-fractionation using agarose gel electrophoresis. The DNA was ligated into EcoRI-cut λZAP arms (Stratagene, La Jolla, CA), packaged, and plated on Escherichia coli strain BB4 (Stratagene). Sequencing of subcloned double-stranded plasmid DNA was performed according to Sanger et al.\(^{28} \)

**Probes.** Rearrangements of the \( \gamma/\delta \) TCR gene were detected using a \( J_{\delta1} \) gene probe that, based on known EcoRI restriction fragment size upon rearrangement to \( J_{\delta} \), demonstrates the predominantly used \( V_{\delta} \) genes.\(^{29} \) \( V_{\gamma} \) could be resolved from \( V_{\delta} \) on the basis of BamHI digests. Certain rearrangements involving the seldom utilized regions \( (J_{\delta3}, J_{\delta12}, \) and \( J_{\delta11} ) \) may not necessarily be detected with this approach.\(^{30} \) The \( \beta \) TCR probe is the \( \text{AVa}_{1-}	ext{Pst}1 \) constant region fragment from the clone YT35.\(^{29} \) The \( J_{\gamma} \) probe is the \( Xba1-\text{Sac}1 \) region derived from the GTH311 rearrangement, as shown in Fig 1A. The \( V_{\gamma} \) probe was the \( \text{HincII-EcoRI} \) fragment from a \( V_{\gamma} \) cDNA clone (Fig 1B), or its genomic \( \text{Pst}1-\text{Pst}1 \) equivalent isolated as previously described.\(^{30} \) The \( C_{\gamma} \) probe was the \( \text{EcoRI} \) fragment subcloned from a full-length \( \delta \) cDNA clone isolated from the cell line PEER (Fig 1B). The \( V_{\delta} \) probe was generated in this report from case 10. Probes were \(^{30} \) labeled using random hexamers as primers, as described by Pittaluga et al.\(^{12} \)

**Cell staining and monoclonal antibodies.** Surface expression of T-cell markers was determined using the monoclonal antibodies (MoAbs) Leu-5 (CD2), Leu-1 (CD5), 3A1 (CD7), Leu-4 (CD3), Leu-3 (CD4), and Leu-2 (CD8).\(^{13} \) The MoAb WT31 and the second anti-\( \delta \) antibody, TCS-1, was purchased from Becton-Dickinson Monoclonal Antibodies (Mountain View, CA). Binding of antibodies was assessed by indirect immunofluorescence using fluorescein-conjugated goat anti-mouse immunoglobulin and flow cytometry or by immunoperoxidase immunohistology of frozen tissue sections, as previously described.\(^{13,26} \)

**RESULTS**

**Immunophenotype of the tumors.** The earliest-appearing human thymocytes lack the CD4 and CD8 accessory molecules, which are thought to augment major histocompatibility complex (MHC)-restricted cellular interactions.\(^{34,35} \) Consistent with the immature status of these tumors, 11 of 18 cases (61\%) express neither the CD4 or CD8 determinants (Table 1). These "double-negative" cells have been highly correlated with cell surface \( \gamma/\delta \) TCR expression in both humans\(^{2} \) and mice.\(^{3} \) Only one case (case 11) expressed the CD4 antigen in the absence of CD8, a phenotype that has never been associated with \( \gamma/\delta \) TCR expression.\(^{3} \) Four cases expressed only the CD8 determinant, while another two cases expressed both CD4 and CD8 accessory molecules.

**TCR gene rearrangements.** In order to explore the possibility that these consecutive neoplasms derived from a lineage of \( \delta \) TCR rearranging cells, Southern blot analysis was performed on the entire panel of cases with the genomic \( J_{\delta} \) probe. This analysis showed TCR \( \delta \) gene rearrangement on at least one chromosome in 13 of 19 specimens (Fig 2A, summarized in Table 1). These \( \delta \) gene rearrangements were confirmed using \( \text{BamHI-} \) and \( \text{HindIII} \)-restricted DNA. No additional rearrangements were detected by this strategy, and no rearrangements were seen using a constant (C) \( \delta \) probe on the identical blots. Accordingly, while we did not directly analyze these tumors for rearrangements to the rarely used \( J_{\delta3} \) or \( J_{\delta13} \) loci, no rearrangement to at least the \( J_{\delta3} \) locus was apparent in these cases, because such a rearrangement should be detected on the \( \text{BamHI} \) digest with the \( C_{\delta} \) probe (data not shown). In three cases (cases 2, 7, and 9), the \( \delta \) locus was germline. In contrast to the \( \delta \) gene rearrangements observed here, most mature T-cell neoplasms and mature \( \alpha/\beta \) TCR-expressing T cells delete the \( \delta \) gene on both chromosomes.\(^{30,36} \)

Southern blot analysis using \( C_{\delta} \) and \( J_{\delta} \) probes and three different restriction enzymes (noted previously) showed rearrangement of both the \( \beta \) and \( \gamma \) genes in 14 of 19 cases. The remaining five cases retained germline \( \beta \) and \( \gamma \) genes.

![Fig 1](https://example.com/fig1.png)

**Fig 1.** Restriction maps of the germline \( J_{\delta} \) locus and of an assembled \( \delta \) cDNA clone. (A) Genomic restriction sites surrounding the predominantly used human \( J_{\delta} \). B indicates BglII; P, PstI; RV, EcoRV; X, XbaI; S, SacI. (B) Restriction map of the productive \( V_{\delta} \) cDNA used by the \( \gamma/\delta \) cell line 702, whose protein sequence is given in Fig 7B. The \( V_{\delta} \) cDNA probe was the \( \text{HincII} \)-EcoRI fragment; the EcoRI site is generated during the cDNA cloning. RI indicates EcoRI; H2, HindIII; HI, HindIII.
including two cases that displayed rearrangement of the \( \delta \) TCR gene (Table 1). Coincident rearrangement of the \( \beta \) and \( \gamma \) genes is a consistent feature of normal \( \text{CD3}^+ \) and neoplastic immature human T lymphocytes,\(^{11-13} \) whereas retention of germline configuration \( \beta, \gamma, \) and \( \delta \) genes is associated with the most immature forms of precursor T-cell neoplasm. As expected, none of the five neoplasms with germline \( \beta \) and \( \gamma \) genes (cases 2, 4, 6, 7, and 9) expressed the TCR-associated cell surface \( \text{CD3} \) complex as a consequence of their inability to produce either \( \alpha/\beta \) or \( \gamma/\delta \) TCRs.

We conclude that this panel of precursor T-cell neoplasms overwhelmingly rearranges the \( \delta \) TCR gene. Excluding case 18, which could not be definitively classified for TCR usage (Table 1), 16 of 18 of these phenotypically grouped T lymphoblastic neoplasms either belong within the \( \delta \) TCR-utilizing lineage (13 of 18 specimens) or are precursor cells expanded before lineage selection (3 of 18 specimens) (summarized in Table 1).

\[ \text{Table 1. Survey of } \text{CD3}^+ \text{ Tumors} \]

<table>
<thead>
<tr>
<th>Case</th>
<th>CD3</th>
<th>CD4</th>
<th>CD8</th>
<th>( \delta )</th>
<th>( \gamma )</th>
<th>( \beta )</th>
<th>( \alpha )</th>
<th>( \gamma )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>3.6</td>
<td>1</td>
<td>3.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>G</td>
<td>-</td>
<td>G</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>+</td>
<td>RD</td>
<td>1</td>
<td>-</td>
<td>2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.6</td>
<td>-</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>-</td>
<td>RR</td>
<td>3.0</td>
<td>?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.6</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.6</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.6</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.6</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>5.9</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>-</td>
<td>D</td>
<td></td>
<td>RR</td>
<td>5.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>+</td>
<td>NA</td>
<td></td>
<td>RR</td>
<td>5.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>+   +</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>-</td>
<td>-</td>
<td>RG</td>
<td></td>
<td>RR</td>
<td>2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>-</td>
<td>RR</td>
<td>3.6</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>+</td>
<td>+</td>
<td>RR</td>
<td>3.6</td>
<td>1</td>
<td>4.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>+</td>
<td>+</td>
<td>RG</td>
<td>3.6</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>+</td>
<td>+</td>
<td>(G or D)</td>
<td></td>
<td>(G or D)</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>RRR</td>
<td>RRR</td>
<td>3.6</td>
<td>1</td>
<td>RRR</td>
<td>2.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Nineteen cases were evaluated for cell surface phenotype and TCR gene rearrangements. The \( \gamma \) nomenclature is derived from Forster et al.\(^{48} \) A second \( J_y \) rearrangement in case 5 may involve \( J_y \) and an unknown \( V_y \) region, as described in the text and denoted here by (?). The presence of germline DNA in cases 16 and 18 may be due to contaminating normal tissue, as indicated by the parentheses.

Abbreviations: R. rearranged; D. deleted; G. germline.

*An indeterminant rearrangement not previously observed.

occurred within the DNA spanned by the probe, which would be expected to simultaneously create two rearrangements. Other possibilities consistent with these additional restriction fragments might include the contamination of the tumor samples with normal DNA, gene duplication, trisomy of chromosome 14, or polyclonality of the tumors, but no particular evidence obtained from the other TCR probes favors these other explanations in these four cases (Table 1).\(^{38} \) Additional rearrangements not easily ascribable to either \( V_y \) gene usage or allelically excluded rearrangements seen in the other cases studied here might also be due to chromosomal translocations or aberrant attempts at rearranging the \( \delta \) TCR gene (Fig 2A).

\( \gamma \) gene usage by precursor T-cell neoplasms and normal thymocytes. Six cases are candidates for expression of the \( \gamma/\delta \) TCR, since they expressed the cell surface \( \text{CD3} \) determinant that is obligatorily co-expressed with a surface TCR,\(^{38} \) and they have rearranged the \( \delta \) TCR gene. Two other surface \( \text{CD3} \)-positive cases (cases 11 and 13) have deleted the \( \delta \) TCR on both chromosomes and, lacking the \( \gamma \) gene, cannot be using the \( \gamma/\delta \) TCR and must be \( \alpha/\beta \) TCR-expressing neoplasms. These six \( \text{CD3}^+ \), \( \text{TCR}^\delta \)-rearranged cases are associated with only three patterns of rearrangement (Table 1). Consistent with this limited pattern of rearrangement in neoplasms were data derived from normal human thymic DNA. Analysis of \( \text{EcoRI} \)-digested human thymic DNA with a \( J_y \) probe shows only two rearrangements at 3.6 and 3.0 kb, both of which are observed among the thymic tumors (Fig 3A, thymus). Similarly, when \( Xba \) I-digested human thymic DNA was analyzed with the genomic \( J_y \) probe, three major rearrangements and one minor rearrangement were detected (Fig 3B, thymus, arrows). We proceeded to analyze the significance of each of these rearrangements and to compare them with rearrangements observed in the \( \text{T-precursor neoplasms} \).

A single, predominantly used \( V_{\text{D}3} \), rearrangement has been described in humans, as demonstrated by Southern blot analysis of PEER (Fig 3A), IDP2, PBL C1, PBL L1, and MOLT-13, and confirmed by sequence analysis of cDNAs.\(^{22} \) In addition, sequence analysis established that patient ID (pt ID)\(^{31} \) and cell line 702 use this same \( V_y \) gene (\( V_y \)) that creates a 3.6-kb \( \text{EcoRI} \) band upon rearranging to \( J_y \) (Fig 3A, lanes 3 and 5). This identical 3.6-kb rearrangement is carried by five of the cases in the series reported here (Fig 2A and B, arrow), four of which are also surface \( \text{CD3}^+ \) (cases 1, 6, 17, and 19).

To confirm use of the common \( V_y \) gene, blots were stripped of \( J_y \) probe and rehybridized with the \( V_y \) probe. As shown in Fig 2B, the only rearranged band that hybridized with the \( V_y \) probe was the 3.6-kb \( \text{EcoRI} \) fragment. In two cases (cases 1 and 17) we further documented the presence of a cell surface \( \delta \) TCR, using flow cytometry with the MoAbs TCS-1 and anti-\( \delta \), both of which react with these cells (Fig 4B). In order to better understand the 3.6- and 3.0-kb rearrangements to \( J_y \) seen in \( \text{EcoRI} \)-digested DNA from cases 10 and 5, respectively, we obtained genomic clones corresponding to these rearrangements. The restriction map of the genomic rearrangement from case 10 is shown in Fig 5A. The \( J_y \) hybridizing DNA is contained on a 750-bp \( \text{BamHI-}

From www.bloodjournal.org by guest on August 30, 2017. For personal use only.
Fig 2. Southern analysis of 19 T-ALL neoplasms with the J4 and V4 probes. EcoRI-digested high-molecular-weight DNA was separated in 0.9% agarose gels and transferred to nylon filters. (A) The J4 probe is the 1.1-kb Xba I-Sac I fragment indicated in Fig 1A. Size markers are λ HindIII-digested DNA. (B) The same blot was stripped and rehybridized with a V4 cDNA probe3' (left) or a genomic Pst I-Pst I V4 fragment (right). The arrow points to the rearrangement recognized by the V4 probe.

Sac I fragment as shown (Fig 5A). Consequently, an upstream probe containing the V4 sequences (Fig 5A) was utilized to probe DNA from case 10 and the γ/δ cell line MV.27 The expected 5.9-kb EcoRI rearrangement was obtained in both cells (Fig 5B, lanes 2 and 3). In addition, the cell line ID7 (Fig 3B, lane 4) can be shown to have the identical rearrangement as MV and case 10 on Sac I and EcoRI restriction digestion (data not shown), represented by the larger Xba I band observed in Fig 3B. We conclude that case 10, MV, and ID7 have all rearranged the same V4 gene (termed V4δ), which is distinct from the previously defined V4 (termed V4α) and which, while clearly detectable as a rearrangement in thymic DNA (Fig 3B), is less abundantly used by these thymic lymphomas than is V4α. Interestingly, when ID7 (Fig 4A) or MV (data not shown) was stained for the presence of cell surface δ TCR protein, MoAb anti-δ stained both cells, but MoAb TCS-1 stained neither. This observation contrasts with γ/δ cells using V4α, which are consistently stained by both antibodies (Fig 4B). The most likely explanation for this finding is that antibody TCS-1 is specific and perhaps idiotypic for V4α, while anti-δ appears to recognize a shared region of δ TCRs used by either V4 gene, such as a C region determinant. An alternative explanation proposed by Moretta et al,39 which suggests that TCS-1 recognizes δ in association with a particular (nondisulfide-linked) form of the γ TCR, is not excluded by these data. Surprisingly, despite the rearrangement of V4δ to J4δ in case 10, these cells were CD3+, WT3−, TCS-1−, anti-δ−, and this result is most consistent with expression of the α/β surface membrane TCR. Thus, a T cell may manufacture and express the α/β TCR in the face of a rearranged δ TCR.40

To show that case 5 belongs to the γ/δ TCR-expressing lineage, we stained tissue sections with TCR-related antibodies βF1, recognizing the β chain of the TCR2 and Leu-4
A. Fig 3. Southern analysis of selected cell lines and human tissue with the J_{41} probe. (A) Human thymic, placental, and cell line DNA was digested with EcoRI and analyzed. Pt ID is from a neoplasm reported previously, which is known by sequence analysis to have a DDJ rearrangement on one chromosome and a productive V_{41} rearrangement on the other. Markers are λ Bst X-digested DNA. (B) Xba I-digested human DNA. Arrows indicate the location of V_{41}, V_{42}, and DDJ rearrangements as verified by sequence analysis of these rearrangements and as described in the text. A fourth arrow points to a minor thymic rearrangement that has not been characterized. Markers are λ HindIII-digested DNA. γ/δ T-cell lines are PEER, 702, and ID7; SG1 is an EBV-transformed B-cell line; and HUT78 is a mature T-cell line.

(B) These sections confirmed the flow cytometry data that the tumor is surface CD3^{+} (Fig 6B) and demonstrated additionally that it is not βF1^{+} (Fig 6A). In contrast, the normal cells in case 5 (Fig 6A) stain with the βF1 antibody, as do most normal thymocytes (Fig 6C). By this criteria, case 5 cannot be using the α/β TCR because it synthesizes no detectable β protein; instead, this analysis is consistent with the neoplasm using a γ/δ TCR. Rearrangement analysis of case 5 DNA with the J_{33}, V_{41}, and V_{42} probes established that the cell is using a novel V gene but could not conclusively delineate this gene. Consistent with the absence of V_{41} use, the V_{42} gene remains germline in case 5 DNA (Fig 2, lane 5). Similarly, the V_{42} gene remains germline, proving that it is not used (Fig 5B, lane 4). Cloning of the 3.0-kb EcoRI J_{33} associated rearrangement did not identify the V gene being used, because this 3.0-kb EcoRI rearrangement represented the nonproductive DDJ rearrangement previously described. We conclude that case 5 employs a distinctive V gene differing from V_{41} or V_{42}, in conjunction with TCR γ/δ, but that the rearrangement of this V gene was not detected by the J_{33} probe used here, consistent with the possibility that this cell uses a J_{33} segment, such as J_{34}, distinct from those analyzed here.

To summarize the data derived from this consecutive panel of precursor neoplasms, only three V genes were used in association with cell surface CD3 expression and with rearrangement of the J_{33} segment, and V_{41} was employed in four of those six cases (66%). Similarly, we were able to define within human thymic DNA, the presumed source tissue for these tumors, four J_{41}-related rearrangements (Fig 3B, thymus, arrows). The V_{41} rearrangement, also seen in T-cell lines 702 and PEER, and the V_{42} rearrangement, seen in cell line ID7 (Fig 3), are also the predominant rearrangements in the lymphoblastic neoplasms. We have previously described the 2.8-kb Xba I band (equivalent to the 3.0-kb EcoRI band observed in case 5) as a DDJ allelic exclusion rearrangement. A fourth minor rearrangement (Fig 3B, unlabeled arrow) occurring in human thymic DNA remains to be defined. We conclude that the V_{41} repertoire employed by thymocytes or lymphoblastic neoplasms is quite narrow.

Sequence analysis of V_{42}. The sequence of the V_{41} gene cloned from case 10 (V_{42}) is depicted in Fig 7A. A separate mini-exon encoding the protein leader sequence is located just upstream of the V gene exon and spliced to it in the productive message. Interestingly, two splice acceptor sites are noted at the beginning of the V_{42} gene (Fig 7A), which determines the initiation of the cell surface V_{42} protein. Depending on whether the 5' or 3' acceptor is actually used, the conserved cysteines required for proper V gene folding are observed at residues 26 and 95 or 23 and 92, respectively (Fig 7A). The latter splice assignment is more consistent with the cysteine arrangement found among other murine V_{41} TCR genes and with the protein alignment with V_{41} (Fig 7B).
Fig 4. Cell sorter profiles of γ/δ TCR-bearing T cells using Vα or Vδ. Approximately 10^7 cells either from the Vα-rearranged, continuous T-cell line ID7 (A) or from the Vδ-rearranged neoplasm case 1 (B) were stained with anti-Leu4 (CD3), anti-δ, TCS-1 anti-δ antibody, or WT31, which ordinarily recognizes CD3+ cells of the α/β phenotype. A second developing antibody of fluoresceinated anti-mouse antibodies was then used. The background staining of each cell with the fluoresceinated antibody alone is indicated as (---). The small shoulder of WT31+ cells seen in case 1 is presumed to be due to normal α/β cells in the specimen.

Computer-assisted comparison of the predicted protein sequence of V42 with that of V4 shows 20 conserved residues among 95 that can be clearly assigned to the V genes, for an overall homology of 21% (Fig 7B). This rather distant homology has also been seen among the murine family of Vδ genes where the most distant members show 20% overall homology. While there is no easily identifiable murine homolog to V42, it conserves 11 of the 14 residues characteristic of murine Vδ genes as described by Elliot et al23 (Fig 7B, asterisks). Finally, one open reading frame extends through this sequence into the Jδ, coding frame, indicating that this rearrangement can encode a functional δ TCR gene. While we have not documented a membrane-associated γ/δ TCR for case 10, if it makes a cell surface α/β TCR, it must do so in the face of a functionally rearranged δ gene.

TCR δ versus TCR α gene selection. Finally, further data on δ TCR gene rearrangements emerging from our study of these human neoplasms added insight into δ versus α TCR gene selection. One model proposed in murine systems provides that exhaustive VDJ δ rearrangement precedes α TCR gene utilization.24 In contrast, none of these tumors have rearranged Vδ on both chromosomes, and in fact, rearrangement of Vδ is associated with a productive TCR in four of five cases. Interestingly, of the 13 cases revealing Jδ rearrangements, 7 retained a germline Jδ allele as well, presumably derived from the other chromosome, indicating that δ gene rearrangement was frequently occurring on only one chromosome in δ lineage cells. Finally, few of these cells showed the DDJ nonproductive chromosomal rearrangement characteristic of an allelic exclusion event. In addition to case 5, case 8 showed an apparent DDJδ rearrangement upon HindIII (data not shown) and EcoRI digests, while case 15 perhaps showed this rearrangement as assessed by EcoRI digest (Fig 2A). These findings indicated that completed Vδ
that have rearranged γ in the absence of β gene rearrangement, suggesting that γ and β gene rearrangements proceed as relatively synchronous events, at least among human T-cell neoplasms. Among these tumors, it appears that δ gene rearrangement is a very early event, which can precede the rearrangement of any other TCR gene. These tumor data are certainly most consistent with the interpretation that the γ/δ-rearranging subset of T cells may constitute a distinct lineage that is phenotypically, and possibly functionally, distinct from α/β TCR-bearing T cells, and almost all of these phenotypically classified tumors are candidates for this proposed γ/δ TCR lineage.

The T-cell accessory molecules, CD4 and CD8, are postulated to enhance class II and class I MHC-restricted recognition, respectively. As previously reported, none of the precursor T-cell neoplasms within the δ-rearranging lineage solely displayed the CD4 accessory molecule. The majority of these tumors (11 of 18) showed the “double negative” phenotype for CD4 and CD8, which has been strongly associated with expression of the γ/δ TCR in humans and mice. Six of the neoplasms showed the CD8 determinant either alone or in association with the CD4 determinant, and CD8 has been suggested to be present on roughly one-third of normal γ/δ TCR-bearing T lymphocytes. The absence of CD4 accessory molecule usage may imply that the typical ligand for the γ/δ TCR is unlikely to be a class II MHC molecule or a peptide that obligatorily interacts with a class II molecule.

The limited number of V genes displayed by these tumors suggests that the entire Vγ repertoire is quite small. In fact, only three V genes are used by this panel, and the principally rearranged Vγ11 is used (cases 1, 16, 17, and 19) in 66% of the tumors that potentially express surface γ/δ TCR protein (cases 1, 5, 12, 16, 17, and 19). The predominance of Vγ11 is consistent with previous observations based on immature T-cell lines derived from immunodeficiency patients. The second Vγ gene described in this report is identical to a previously published Vγ24 but is quite different from Vγ11, bearing only 21% homology at the protein level. This level of divergence is slightly more than what has been reported among the murine Vγ genes. Further, there appears to be no obvious homolog to this new Vγ gene among the previously reported murine series. In addition, a recent publication describes a third Vγ gene located 3' to Cγ1 and bearing no close sequence relationship to the second Vγ gene described. An explanation for the profound divergence of the Vγ genes while the total number appears to be so limited remains to be elucidated once the ligand of the γ/δ TCR is better understood.

One question raised by these results is whether CD3- Jγ-rearranged neoplasms were destined for γ/δ- or α/β-expressing lineages. Although a site-specific deletional mechanism deletes human Jγ-Cγ, it is not known if some α/β T cells may pass through a Jγ rearrangement before α TCR rearrangement on the productive chromosome, as suggested by the finding of rare δ TCR rearrangement in α/β cells. However, many (7 of 13) of the Jγ-rearranged neoplasms in this study also appear to have retained a germline Jγ fragment, suggesting that they recombined a δ gene on only one chromosome. While seven of these cases (cases 2, 4, 6, 7,
Fig 6. Immunohistology of case 5 compared with normal tissues. Tissue sections from case 5 were stained with 9F1 (A), recognizing the \( \beta \) chain of the TCR or Leu4 (CD3) (B), in contrast to the staining of normal human thymus (C) with 9F1 antibody.

8, 9, and 18) retained germline \( J_\alpha \) genes, they were germline for all three TCR genes analyzed, thereby raising the possibility that contaminating DNA from normal cells contributed the germline bands. We favor the interpretation that at least five of these cases (cases 2, 4, 6, 7, and 9) are instead immature precursor T cells arrested before gene rearrangement. Furthermore, completed VDJ recombination on both chromosomes or DDJ recombination on the excluded chromosome was an unusual event among the 13 candidate \( \gamma/\delta \) T-precursor neoplasms, suggesting that a mechanism other than exhausted \( V_\alpha \) and DDJ rearrangements may be used to select between \( \gamma/\delta \) TCR gene usage and \( \alpha/\beta \) TCR gene.
usage in precursor T-cell neoplasms. The association of δ rearrangement and deletion with α/β versus γ/δ lineage will require further careful analysis of both the Jδ region of precursor T neoplasms and the δ region of mature α/β T cells.

δ TCR gene rearrangements are readily assayed. There further appears to be a striking correlation between the phenotype of these immature tumors and δ gene rearrangement. We conclude that assaying similarly phenotyped tumors for δ gene rearrangement should provide an easy diagnostic method for classifying these tumors and a simple way in which to assess clonal tumor recurrence. When using Tδ rearrangement analysis to distinguish between a minor clone and normal polyclonal T cells, it is recommended that caution be exercised since the limited rearrangement possibilities may produce non-germline bands among normal cells, as is the case for the Tγ gene.29 The frequent and early rearrangement of the δ TCR gene in lymphoblastic neo-
ACKNOWLEDGMENT
We thank Drs I. Rabbitts (Medical Research Council) and T. Mak (Ontario Cancer Institute) for Tγ and Tδ probes, respectively.

REFERENCES


Gamma/delta lineage relationship within a consecutive series of human precursor T-cell neoplasms

JP de Villartay, AB Pullman, R Andrade, E Tschachler, O Colamenici, L Neckers, DI Cohen and J Cossman