T Cell Rearranging Gene $\gamma$: Diversity and mRNA Expression in Fresh Cells From T Cell Acute Lymphoblastic Leukemia

By Denis Le Paslier, Zhu Chen, Pascale Loiseau, Daniel Cohen, and François Sigaux

Rearrangement and in most cases expression of the T cell rearranging genes $\gamma$ (TRG$\gamma$) and T cell antigen receptor $\beta$ chain (TCR$\beta$) genes were studied in 19 cases of T cell acute malignancies where the surface phenotype is representative of the different stages of thymic maturation. TCR$\alpha$ gene transcription was also studied. TRG$\gamma$ and TCR$\beta$ genes were found to be rearranged in all but one case. The TRG$\gamma$ rearrangement pattern seen in most cases is compatible with biallelic rearrangement by loop excision involving the J$\gamma$2 regions. The sizes of all but two rearranged bands were identical to those of the rearranged bands seen in polyclonal T lymphocytes also studied in this work. One identical-sized band was found in 11 of the 18 rearranged cases. The expression of TRG$\gamma$ mRNA (transcripts of 1.6 kilobases [kb]) was highly variable from case to case and did not correlate with the stage of differentiation of the malignant cells. The expression of the molecules CD4 and CD8, the expression and size of the transcripts of the TCR$\beta$ genes, and the transcription of TCR$\alpha$ genes. In one CD3+$\gamma$ case, strong expression of the TRG$\gamma$ transcripts coexisted with the exclusive presence of TCR$\beta$ mRNA of 1.0 kb. The cells from this case did not react with anti-Ti antibody and exhibited no natural killer activity. These findings are suggestive of a malignancy that may express the recently isolated CD3-TRG$\gamma$ complex.

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A WIDE BODY OF EVIDENCE supports the view that the classic T cell antigen receptor (TCR) is a complex comprised of the multichain molecule CD3 (T3) and the two molecules Ti$\alpha$ and Ti$\beta$ whose genes undergo somatic rearrangements during thymic ontogeny of T cells.1-3 Transfection experiments have established that, at least in some T cells, the dimer Ti$\alpha$Ti$\beta$ determines both antigen recognition and its restriction by the major histocompatibility complex (MHC), which is characteristic of T cells.4 During attempts to isolate the genes encoding the Ti$\alpha$ chain, other genes that also undergo somatic rearrangements in T cells were brought to light in mice5-7 and then in humans.8,9 The structure of these genes, termed T cell rearranging genes $\gamma$ (TRG$\gamma$) or T cell $\gamma$ chain genes, has recently been elucidated at least in part for humans. Nine9 to 12 variable segments (V$\gamma$), including at least five pseudogenes, are located upstream of two strongly homologous regions, each comprising a junctional segment (J$\gamma$) and a constant region (C$\gamma$).8,10 The V$\gamma$ segments can recombine with both regions J$\gamma$1 and J$\gamma$2 and also with a segment called J$\gamma$P by Lefranc et al10 that is located at about 4 kilobases (kb) in S of the J$\gamma$1 region (Fig 1). The diversity generated by TRG$\gamma$ is basically of junctional origin (including N diversity). There is no evidence of D$\gamma$ segments.10,11 We and others12,13 have shown that the rearrangement of TRG$\gamma$ is not restricted to normal and neoplastic T cells. In a recent work,14 we have shown that TRG$\gamma$ are frequently rearranged but not transcribed in the malignant cells of B lineage acute lymphoblastic leukemia (ALL). Although the structure of TRG$\gamma$ has been elucidated to a large extent, the nature and function of the presumed products of TRG$\gamma$ remain a mystery.17 In mouse thymocytes,18,19 TRG$\gamma$ rearrangement and mRNA expression precede those of the genes TCR$\beta$ and TCR$\alpha$, which suggests that TRG$\gamma$ may play a crucial role in early thymic ontogeny of T cells. As shown by recent research using cross-linking experiments and immunoprecipitation by anti-CD3 monoclonal antibodies (MoAbs) and by antisera directed against synthetic V$\gamma$ and C$\gamma$ peptides, the product of TRG$\gamma$ could be a 55-kilodalton (kD) protein associated with the CD3 molecule.20 One of the more attractive hypotheses is that this TRG$\gamma$-CD3 complex could be used by certain T cells (CD3-positive, Ti-negative) as an antigen receptor.20

T cell acute lymphoblastic leukemias (T-ALL) can be considered as clonal proliferations of cells arrested at more or less early stages of thymic differentiation and are models of considerable interest.21 The present study investigated the rearrangement and expression of mRNA of TRG$\gamma$ in fresh cells from a large series of T-ALL and one patient with T blast crisis of chronic myeloid leukemia (CML). The surface phenotype of malignant cells was determined by using a panel of MoAbs directed against associated or restricted T antigens by which the stage of differentiation of the malignant cells could be determined.21,22 Rearrangement of TCR$\beta$ genes and transcription of TCR$\beta$ and TCR$\alpha$ genes were also studied in most cases. We showed that the TRG$\gamma$ rearrangement seen in all but one case most often involves the J$\gamma$2 region of both alleles and probably uses the same V$\gamma$ segments as those used by mature polyclonal T cells. It was also found that TRG$\gamma$ mRNA expression, variable from one case to another, does not correlate with surface phenotype, transcription of TCR$\alpha$ and TCR$\beta$ genes, and stage of differentiation of the malignant cells. Finally, the intense expression of TRG$\gamma$ mRNA in one CD3-positive, Ti-negative case expressing only 1.0-kb TCR$\beta$ mRNA is suggestive of a malignancy that may express the recently isolated CD3-TRG$\gamma$ complex.23

MATERIALS AND METHODS

Cells

Malignant cells from the blood or bone marrow of 18 T-ALL patients and one patient with T lymphoblastic blast crisis of CML...
were separated on a Ficoll gradient and then used as is or after conservation in liquid nitrogen. Morphological and immunologic characterization was based on the usual criteria. The surface phenotype of the malignant cells was determined by a standard method of indirect immunofluorescence and cytofluorographic reading (Ortho 50 H; Ortho Diagnostics, Westwood, MA) using a large panel of MoAbs that recognize T-associated or T-restricted antigens (anti-CD1 to -CD5, -CD7, and -CD8) and B-restricted antigens (CD19 to CD22). A number of other antibodies were also used (anti-CD1 to -CD5, -CD7, and -CD8) and B-restricted antigens (anti-CD10, OKT9, OKT10, My7, MY9, OKM1, OKM5, anti-platelet glycoprotein Ib and Iib/IIIa, and anti–MHC class II molecules DR, DP, and DQ). The specificity of these antibodies has been widely published. The WT31 antibody interacting with nonpolymorphic determinant expressed on Ti was also used in some experiments. A cell population was considered positive if more than 30% of the neoplastic cells reacted with the MoAb. Table I summarizes the surface phenotypes of the cells used. B-restricted antigens were not detected in any of the cases, nor were myeloid antigens (anti-MY9), whereas all the cases tested expressed the pan-T molecule CD7.27 The CD3 molecule was expressed in five of 18 cases of T-ALL, four of which also expressed either CD4 or CD8 (stage III of Reiner et al). Of the CD3-negative cases, six expressed at least one of the common thymocyte antigens (stage II) and seven only pan-I markers (CD5 and CD7) (stage I or I/II). OKM1 antigen was expressed in case 8. The cells of the T-cell blast crisis of CML were of a typical common cortical phenotype. Among the cases analyzed in this paper, three are described elsewhere. 3,10,11

To compare the patterns of rearrangement of TRGy in T cell malignancies and in polyclonal T cells, T cells from healthy donors were studied after E rosetting. Granulocytes, monocytes, polyclonal B cells transformed by Epstein-Barr virus (EBV) or not, blasts of acute myeloid leukemia patients and the CEM line were also used in some experiments.

**Southern Blots**

Genomic DNA was extracted according to standard methods. DNA samples were digested by the restriction endonucleases BamHI, HindIII, and EcoRI under conditions recommended by the manufacturer (Promega Biotec, Madison WI). The restriction fragments were separated in 0.6% agarose gel by electrophoresis at 40 V for 24 hours and transferred onto a hybridization membrane (Hybond-N from Amersham Corp, Arlington Heights, IL). The prehybridization and hybridization were performed at 65°C in 6 x SSC (1 x SSC is 0.15 mol/L NaCl, 0.015 mol/L sodium citrate, pH 7), 0.2% sodium dodecyl sulfate (SDS), 10% (wt/vol) dextran sulfate, and sonicated denatured salmon sperm DNA at 200 µg/mL. The filters were washed for 15 minutes in 2 x SSC and 0.2% SDS at room temperature, 15 minutes in the same solution at 65°C, 30 minutes in 0.2 x SSC and 0.2% SDS, and then 30 minutes in 0.1 x SSC and 0.2% SDS at 65°C. The blots were autoradiographed for two to four days.

**Northern Blots**

The extraction of total mRNA was performed as described by using the guanidine–cesium chloride method. The cell pellet was lysed by vortexing into 6 mol/L guanidium isothiocyanate, 5 mmol/L sodium citrate, 0.1 mol/L ß-mercaptoethanol, and 0.5% sarkosyl. The lysate was then centrifuged over a gradient of 5.7 mol/L cesium chloride at 77,000 g for 16 hours at 20°C. The RNA was precipitated in 70% ethanol and resuspended in diethylpyrocarbonate-treated water. Fifteen micrograms of total RNA were electrophore-

### Table 1. Immunophenotype, Rearrangement, and Expression of TRGy and TCR Genes

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*Abbreviations: ND, not done; H and E, rearranged bands in DNA digested by HindIII and EcoRI, respectively (for details see the text); +/−, 20% to 30% of cells positive for the surface marker (for immunophenotypes) or very weakly positive (for mRNA expression).

*4, biallelic Jy2 rearrangement; +, two rearranged bands with BamHI but only one with HindIII and EcoRI; $, two rearranged bands with HindIII and EcoRI but only one rearranged band with BamHI; J, monoclonal rearrangement involving a J segment localized 5' to the HindIII and EcoRI sites preceding the Jy2 region but included in the BamHI fragment containing Jy2 (see Fig 1); r, rearrangement not interpretable by the loop excision mechanism; " , deletion of the Jy1 — Jy2 region on one allele and Jy2 rearrangement on the other.

*Seventy-four percent of malignant cells from this case are OKM1-positive.

*T cell acute transformation of CML.
Figure 1 shows the bands seen in germ line DNA digested by the restriction enzymes BamHI, HindIII, and EcoRI. No polymorphism was observed for the BamHI sites in the control samples (granulocytes, monocytes, and B lymphocytes from four healthy donors; blasts from nine patients with typical acute myeloid leukemia; and polyclonal B cell lines obtained after transformation of the blood of 20 healthy donors by EBV). As previously described,\textsuperscript{6} polymorphism was noted for the HindIII site situated upstream to the \( J_γ_2 \) segment, which resulted in a 2.1-kb band in some individuals and both 2.1- and 5.6-kb bands in others (Fig 1). Southern blots were interpreted by assuming a loop excision mechanism without reintegration and using the following nomenclature:\textsuperscript{4} type 1, monoallelic \( J_γ_1 \) rearrangement; type 2, biallelic \( J_γ_1 \) rearrangement; type 3, monoallelic \( J_γ_2 \) rearrangement; type 4, biallelic \( J_γ_2 \) rearrangement; and type 5, \( J_γ_1 \) rearrangement on one allele and \( J_γ_2 \) on the other.

**DNA Probes**

The TCR\( β \) probe is a cDNA fragment specific for the constant region of the \( β \) chain of the TCR (a \( Bg/II/Bg/I1 \) fragment of the 4D1 cDNA\textsuperscript{13}) that was provided by J.L. Strominger. The TCR\( α \) probe is the pGA5 cDNA provided by E. Palmer.\textsuperscript{33} The \( J_γ \) probe is a subcloned EcoRI-HindIII fragment of the MH60 \( J_γ \) clone that was provided by T.H. Rabbits (Fig 1). The \( C_γ \) probe is the 0.7-kb BamHI fragment (part of exon 2 and all of exon 3) of the PH5-\( γ \) cDNA encoding for a VJC gene that was provided by J.L. Strominger.\textsuperscript{11} The entire PHT \( γ \) cDNA probe was used to detect the TRG\( γ \) transcripts. Probes were \textsuperscript{32}P-labeled by nick translation or by oligolabeling (multiprime labeling kit from Amersham).

**Interpretation of the Southern Blots**

**\( J_γ \).** Figure 1 shows the bands seen in germ line DNA digested by the restriction enzymes BamHI, HindIII, and EcoRI. No polymorphism was observed for the BamHI sites in the control samples (granulocytes, monocytes, and B lymphocytes from four healthy donors; blasts from nine patients with typical acute myeloid leukemia; and polyclonal B cell lines obtained after transformation of the blood of 20 healthy donors by EBV). As previously described,\textsuperscript{6} polymorphism was noted for the HindIII site situated upstream to the \( J_γ_2 \) segment, which resulted in a 2.1-kb band in some individuals and both 2.1- and 5.6-kb bands in others (Fig 1). Southern blots were interpreted by assuming a loop excision mechanism without reintegration and using the following nomenclature:\textsuperscript{4} type 1, monoallelic \( J_γ_1 \) rearrangement; type 2, biallelic \( J_γ_1 \) rearrangement; type 3, monoallelic \( J_γ_2 \) rearrangement; type 4, biallelic \( J_γ_2 \) rearrangement; and type 5, \( J_γ_1 \) rearrangement on one allele and \( J_γ_2 \) on the other.

**\( C_γ \).** When germ line DNA is hybridized with the \( C_γ \) probe (corresponding to a part of exon 2 and all of exon 3), two BamHI bands of 12.5 and 4.1 kb (corresponding respectively to \( C_γ_1 \) and \( C_γ_2 \)) and two HindIII bands of 13 and 4.3 kb (\( C_γ_2 \) and \( C_γ_1 \), respectively) can be seen (Fig 1).\textsuperscript{14} Monoallelic or biallelic \( J_γ_1 \) rearrangements cannot be detected with this probe. On the other hand, \( J_γ_2 \) rearrangement results in reduced intensity (monoallelic rearrangement) or disappearance (biallelic rearrangement) of the 12.5-kb BamHI band and the 4.3-kb HindIII band.

**TCR\( β \).** TCR\( β \) gene rearrangements were analyzed as previously described.\textsuperscript{14}
RESULTS

TRGγ Rearrangement in Polyclonal T Cells and T Cell Acute Malignancies

TRGγ rearrangement was first studied in polyclonal T lymphocytes after E rosetting. A representative result obtained after hybridization with the Jγ probe is shown in Fig 2. Six faint rearranged bands (termed Ha to Hf and Ea to Ef) were seen in DNA digested by HindIII and EcoRI, respectively, in addition to the germ line bands observed in the granulocytes, monocytes, and B lymphocytes of the same individual. Because the hybridization of the same filter with a TCRβ probe shows a pattern characteristic of polyclonal T cells (almost total disappearance of the 12-kb EcoRI band), the presence of germ line bands in the DNA hybridized with the Jγ and Cγ probes seems not to be due to a contamination of the E fraction (for example, by natural killer [NK] E+ cells) but is suggestive of the absence of rearrangement in some polyclonal T cells or of monoallelic rearrangement in at least a fraction of them. The CEM line, which served as a control in this experiment, exhibited type 4 rearrangement (biallelic Jγ2) with two bands of the same size of the Hc-Ee and He-Eb bands found in polyclonal T cells.

Of the 19 cases of T cell acute malignancies studied, only one instance of germ line pattern was found (case no. 8) (Table I). In nine of 18 cases of rearrangement, the pattern observed can be explained by biallelic rearrangement of region Jγ2 (type 4). In two cases (nos. 4 and 18), the pattern
obtained with the DNA digested by HindIII and EcoRI was also consistent with type 4 rearrangement, but a single rearranged band was seen in DNA digested by BamHI. In case nos. 2, 12, and 16, two rearranged bands were seen in BamHI digests of DNA but only one for the enzymes HindIII and EcoRI (Fig 3). Since the restriction patterns of certain Vγ regions were strongly homologous, these results might conceivably result from comigration of certain fragments, even though they correspond to type 4 rearrangement. In case nos. 9 and 17, the presence of a single rearranged band along with the disappearance of the germ line bands is consistent with Jγ2 rearrangement associated with the deletion of a fragment including at least the whole of the Jγ1-Jγ2 region on the other allele or possibly with a type 4 rearrangement using the same Vγ segment on both alleles (Fig 3). In case no. 5 (characterized by one rearranged band with BamHI and a germ line pattern with HindIII and EcoRI (Fig 3)), there seems to have been a monoallelic rearrangement of a joining region like the JP segment described by Lefranc et al. Finally, the type of rearrangement seen in case no. 6 remains uninterpretable on the basis of a loop excision hypothesis (Fig 3). Rearrangement patterns for the different patients are summarized in Table 1.

All the rearranged bands obtained in polyclonal T cells after DNA digestion by HindIII and EcoRI were observed in T cell acute malignancies (Fig 2). Band Hc-Ee occurred in ten of 18 cases. In polyclonal T cells, these are the bands of highest intensity (Fig 3). In certain cases of T-ALL (nos. 1, 3, 15, and 19), there were additional bands—HindIII of 5.3 and 2.6 kb (Hg and Hh, respectively) and EcoRI of 1.3 and 2.1 kb (Eg and Eh, respectively)—that were not seen in the samples of polyclonal T cells studied. The number of cases of T-ALL was sufficient to establish a correspondence between the different rearranged bands observed after digestion by the two enzymes (Fig 2, see the legend). There was no correlation between surface phenotype and the presence of any given band.

**TCRδ Gene Rearrangement**

TCRδ genes undergo very early rearrangement—before the TCRα genes—during thymic ontogeny. To establish whether a hierarchy by differentiation stage could be estab-
Germ line configuration and rearrangement of TCRβ genes. (1) DNA from normal granulocytes digested by BamHI (B), HindIII (H) and EcoRI (E). Several representative cases of T-ALL with differently rearranged TCRβ gene patterns are shown: 2. case no. 5; 3. case no. 7; 4. case no. 9. The last triplet of lanes shows the only case (patient no. 8) whose TCRβ genes remain unrearranged.

mRNA expression of the TRGγ and TCRβ and TCRα genes. B. mixture of EBV-transformed polyclonal B cells lines taken as negative control. The other lanes are samples from the T-ALL patients, their case numbers located above each lane. The size of the mRNA is established with reference to the positions of 28S and 18S ribosomal RNA.
lished with respect to rearrangement of TCRβ genes and TRGγ, the DNA previously hybridized with the TRGγ probes was dehybridized and rehybridized with the TCRβ probe (which recognizes constant regions). All of the T-ALLs showing TRGγ rearrangement also exhibited TCRβ gene rearrangement. Representative experiments are shown in Fig 4. In seven of the 17 analyzable cases, the observed pattern was consistent with biallelic Jβ2 rearrangement by loop excision. In the sole case (case no. 8) where the TRGγ were not rearranged, a germ line pattern was also found for the TCRβ genes (Table 1).

Expression of mRNA of TRGγ, TCRα, and TCRβ Genes

Since TRGγ mRNA expression seems related to the stage of differentiation,18,19 it seemed interesting to study TRGγ mRNA expression in different cases of T-ALL. In 11 of the 14 cases studied a 1.6-(kb) transcript was found (Fig 5). The expression of TRGγ mRNA seems to show considerable quantitative variation and is not connected with the surface phenotype (Table 1), nor is it correlated with the expression and size of TCRβ gene transcripts. In case nos. 3, 7, and 17, only 1.0-kb TCRβ transcripts were found, which likely correspond to incomplete DJ rearrangements. Complete transcripts of 1.3 kb (potentially functional) were found at all stages of maturation, with maximum expression in CD3+ case nos. 15 and 18. TCRα gene transcripts were observed in six cases. TCRα mRNA of about 1.6 kb tend to be observed more frequently among the CD3-positive cases (Fig 5 and Table 1). Case no. 17 was exceptional in that only 1.0-kb TCRβ transcripts were detected, whereas the CD3 molecule was clearly detected at the cell membrane. The cells did not react with the anti-Ti WT31 antibody (Fig 6). It is also interesting to note that TRGγ expression was intense (Fig 5) and that the cells exhibit no NK activity (data not shown).

DISCUSSION

Our work has demonstrated the existence of a rearrangement of the TRGγ and TCRβ genes in 18 of 19 cases of a series of acute T lymphoblastic malignancies whose surface phenotype illustrates the different stages of thymic maturation.21 In a single case (no. 8), with a common cortical phenotype, the TRGγ and TCRβ genes were seen in germ line configuration. These findings along with those obtained with cases recently reported in the literature8,11,16 and those obtained in cell lines16,34 show that these genes are rearranged in the vast majority of ALLs of T phenotype. The lineage involved in patient no. 8 remains unclear. In this case, the malignant cells were clearly peroxidase-negative and expressed the OKM1 antigen but not myeloid antigens. Recently, it was shown that CD3−, NK cells (frequently OKM1-positive) do not rearrange TRGγ or TCRβ genes.36 Indeed, we have recently found TRGγ and TCRβ genes in germin configuration in CD3−, NK cells from a patient with a chronic expansion of large granular cells.37 It is possible that the cells from case no. 8 reported in the present paper represent the neoplastic counterpart of NK precursors.

One of the objectives of this work was to discover whether there is any hierarchy in the rearrangement of the TRGγ and TCRβ genes as there is in the case of TCRβ and TCRα genes.34 Our results do not support this hypothesis. It is nevertheless possible that our T-ALL cases only imperfectly illustrate the stages of thymic maturation18 or that a hierarchy might be observed in more immature cells (prethymic?).
The demonstration of TRGγ rearrangement in most B lineage ALLs with TCRβ gene rearrangement being found in only 30% to 40% of cases is evidence in favor of this hypothesis.

One interesting finding of the present study was that TRGγ rearrangement in T-ALL very often involves the Jγ2 segment on both alleles. On the other hand, the involvement of the Jβ2 region in TCRβ gene rearrangement was observed in only six of 17 cases interpretable in the hypothesis of loop excision. These findings are comparable with what can be deduced from the data of Tawa et al. (Table 1) and with Sangster and colleagues’ data based on cell lines. If a mechanism of allelic exclusion exists for TRGγ as for the other immunoglobulin like genes, a good many of the TRGγ rearrangements must be nonfunctional in T-ALL. A large number of nonfunctional TRGγ transcripts have been demonstrated in mouse malignant T cell lines. In one series of human cell lines, only three of the nine rearranged fragments were found to be potentially functional by sequence analysis. The high rate of nonproductive rearrangements may result at least in part from N region diversity. It is interesting to note that in polyclonal T lymphocytes, as in human thymocytes, there are T cells in which TRGγ rearrangement is lacking or monoallelic. Thus this finding contrasts with what is observed in T-ALL where both alleles are usually rearranged. As already mentioned earlier, it may be that T-ALLs represent malignancies that develop preferentially from certain thymic cells.

Finding rearranged bands in the DNA of polyclonal T cells after hybridization with a Jγ probe is an observation that is consistent with the small number of Vγ segments (nine to 12) and the strong homology of the Jγ1 and Jγ2 regions. The presence of merely six bands when nine to 12 could have been expected may be accounted for by comigration of certain fragments or by the preferential use of certain Vγ segments. These findings contrast with those for the TCRβ genes (no rearranged bands are seen in polyclonal I cells) and are analogous to those recently obtained for TRGγ in human thymocytes by Lefranc et al. Using specific probes for certain Vγ segments, these authors also assigned the Vγ9 fragment to a band corresponding to band Hd, the Vγ3 fragment to band Hc, and the segment Vγ4 to band Hc. When the patterns obtained in polyclonal T cells are compared with the patterns in T-ALL, identical-sized bands are found in the vast majority of cases. Nevertheless, two additional rearranged bands were seen only in T-ALL, thereby suggesting that certain Vγ segments are little or not used in polyclonal T cells. It is also worth noting that, as in the mouse, certain Vγ segments may be more often used than others. For instance, the Hc-Ee band is found in ten of 18 cases of rearranged T-ALL. These findings are analogous to those obtained with polyclonal T cells (Fig 3) and thymocytes, where the intensity of these bands is higher than for others. Finally, the pattern observed in some ALL cells upon EcoRI and HindIII digestion is not consistent with previously published maps and may be due to atypical rearrangement or involvement of previously unreported Jγ or Vγ segments. Cloning and further analysis of these rearranged fragments are in progress in our laboratory.

As shown in our study, expression of TRGγ mRNA by fresh cells from T-ALL is highly variable from case to case and is not correlated with CD4 and CD8 antigen expression. It should be underlined that the correlation between TRGγ mRNA expression and both function and MHC restriction of T cells is not clearly defined. Our study of the transcription of the TRGγ and TCRβ genes indicates that these two groups of genes are expressed at all stages of thymic differentiation but that expression of full-length TCRβ and TCRα mRNA tends to be high only in the more mature cases (CD3+). As opposed to Furley et al., we did observe cases that, although rearranged, did not express detectable TCRβ transcripts. Case 17 seems exceptional because the CD3 molecule was detected at the cell surface whereas only TCRβ mRNA of 1.0 kb was found. Indeed, it has been shown that in T cells committed to express the CD3-Ti complex the CD3 molecule cannot be expressed at the cell surface without coexpression of the dimer Tiβ. A particular population of CD3+ T cells has recently been identified in the lymphocytes of immunodeficient patients and in human thymocytes. These Ti-negative cells coexpress the CD3 molecule with two other polypeptide chains whose molecular weights have been estimated at 55 and 40 kD in one of the reports and at 62 and 44 kD in the other. In one of these studies, the use of antiserum directed against synthetic peptides Vγ and Cγ would suggest that one of these two chains is the product of TRGγ. The TRGγ-CD3 complex could represent a second antigen receptor on T cells. As in our case no. 17, one of the groups did find strong expression of TRGγ mRNA, a CD3+/CD4-/CD8– phenotype, and the exclusive presence of nonfunctional 1.0-kb TCRβ mRNA in a clone of thymocytes. This case of T-ALL, as in the PEER cell line, could thus possibly be a malignancy developing from such cells. The relation between these cells and the CD3+/CD4−/CD8− subset of peripheral T cells and some subset of NK cells remains to be established.

NOTE ADDED IN PROOF

In addition to the Jγ3, Jγ1, and Jγ2 joining regions, two other Jγ segments were recently described (Quertermous et al: J Immunol 138:2687, 1987). In case no. 5, we have found that the rearrangement involves the J segment located between Cγ1 and Jγ2.

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