A γ8+ T-Cell Leukemia Bearing a Novel t(8;14)(q24;q11) Translocation Demonstrates Spontaneous In Vitro Natural Killer-Like Activity

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A highly malignant human T-cell leukemia was identified by cell surface analysis as a member of the T-cell receptor (TCR) γ8 lineage. Cytogenetic and molecular analysis showed a novel t(8;14)(q24;q11) rearrangement involving the Jc gene segment on chromosome 14 and the distal end of chromosome 8 near the c-myc proto-oncogene locus. The γ8 TCR of the leukemia blasts was functionally intact and could be activated to generate intracellular calcium flux and to target Fc receptor-mediated redirected tumor cell lysis. In addition, non-major histocompatibility complex restricted lysis of a limited target cell panel was shown by fresh leukemic blasts and by the in vitro-maintained leukemia cells that was comparable to known T-cell lines with natural killer-like activity. These data suggest that the T-cell leukemia potentially had in vivo functional cytolytic activity. However, whether this activity did contribute to the patient’s clinical condition could not be determined.

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MACROMOLECULAR chromosomal rearrangements have been the hallmark finding in many malignant proliferations of hematopoietic origin. Recently, an increasing number of T-cell leukemias have been identified that have associated specific, recurrent, nonrandom chromosome rearrangements. These cytogenetic abnormalities often involve components of the genomic T-cell receptor (TCR) regions and, in some situations, have been shown to activate nearby cellular proto-oncogenes. Chromosomal in situ hybridization studies have shown unregulated oncogene expression associated with the fusion chromosome. In addition, abnormal proto-oncogene messenger RNA (mRNA) transcripts that are the result of the chromosomal fusion have been reported. It has been hypothesized that the unregulated expression of the cellular proto-oncogene product confers the malignant phenotype upon the cell.

The c-myc proto-oncogene, a nuclear protein intimately involved with normal cell growth, was shown to be commonly associated with cytogenetic alterations in Burkitt’s lymphoma. These abnormalities include (1) point mutations in the promoter region; (2) exon 1 deletions that allow the expression of the c-myc gene in the absence of regulatory sequences; and (3) rearrangements that place the c-myc gene in close proximity to Ig enhancer sequences. In addition, a number of variants have breakpoints downstream of the c-myc oncogene on chromosome 8. Several of these Burkitt’s lymphoma variants have been identified in which the chromosomal breakpoint was found approximately 140 kb downstream from the c-myc gene locus in a region designated bvr-1. Interestingly, the human pvt1 locus, another potential cellular proto-oncogene, had also been identified 260 kb downstream from c-myc. Recent analysis of these particular B-cell Burkitt lymphomas has shown that interruption of the PVT transcription unit can generate aberrant transcripts. However, this did not correlate with an abnormal PVT protein product; rather, transformation due to deregulation of the c-myc locus at a distance far upstream remained a possibility. Despite this unresolved issue of the precise molecular events that result in neoplastic transformation in human Burkitt’s lymphoma variants, it is clear that deregulation of c-myc can lead to lymphoid transformation because transgenic mice that express the Ig μ heavy chain enhancer in close proximity to the c-myc proto-oncogene develop multiple lymphoid neoplasms directly attributable to the activated transferred c-myc proto-oncogene.

In this report, a TCRγ8 T-cell leukemia is described that bears a novel translocation involving the Jc locus of the TCR γ chain on chromosome 14, which was translocated to the vicinity of the c-myc locus on chromosome 8 as defined by cytogenetics. Further analysis shows that it is a considerable distance far from the structural locus of the c-myc oncogene, reminiscent of the Burkitt lymphoma variants that translocate to either the pvt1 or the bvr locus. Isolated leukemic blasts had a functional TCR as shown by transmembrane signalling and redirected cytolysis, and also had functional natural killer (NK)-like activity as determined by in vitro tumor cell lysis. Thus, this novel translocation appeared to be associated with malignant transformation but did not abrogate the cellular function normally associated with the differentiated activated state of the TCRγ8 T cell.

MATERIALS AND METHODS

Patient material and clinical history: The patient, DOC, was a 10-month-old white male who presented with evidence of sepsis. On evaluation, he was found to have marked hepatosplenomegaly and a circulating white blood cell count (WBC) of 8 × 10^9/mm^3, nearly 95% blast forms. No thymic masses were noted. A diagnosis

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1523
of T-acute lymphoblastic leukemia (T-ALL) was made and he was treated per protocol for high-risk T-ALL patients. Cytoreduction was obtained, but reinduction was needed to achieve remission. However, the patient relapsed rapidly and died on day 131.

**Cell lines.** Leukemic cells were isolated from the peripheral blood of the patient. The mononuclear cells were separated on a Ficoll-hypaque gradient and used directly or were maintained in tissue culture (RPMI-1640 with 10% fetal calf serum, supplemented with L-glutamine, HEPES-buffer; GIBCO Laboratories, Grand Island, NY), and in the presence of 10% interleukin-2 (IL-2)-enriched T-cell factors (Electro-nucleonics, Silver Springs, MD). Long-term culture of the leukemia was not successful in this medium due to inability to survive crisis.

Nonadherent tumor cell lines used were maintained in the same media without T-cell factors and included P815 (murine mastocytoma), K562 (human chronic myelogenous leukemia line with erythroleukemic characteristics), H69 (small cell lung carcinoma), Jurkat (TCRβ T-cell leukemia), JY and Daudi (Epstein-Barr virus [EBV]-transformed B-lymphoblastoid cells), E95A-BL (Burkitt's lymphoma), and PEER (TCRγδ T-cell leukemia). The adherent melanoma tumor line, Hs294T, and the cervical carcinoma, HELA, were maintained in Dulbecco's modified Eagle's medium (DMEM), 10% fetal calf serum, and supplemental HEPES buffer and L-glutamine.

The generation and maintenance of the CD4+CD8+ TCRβ and TCRγδ T-cell lines have been previously described.7

**Monoclonal antibodies (MoAbs).** MoAbs used in this study include: WT31, anti-TCRβ (T Cell Sciences, Cambridge, MA); 8TCS-1, anti-TCR γ chain, Vγ68, specific (T Cell Sciences); δTCR-1, anti-framework δ chain (gift of Dr M. Brenner); Leu9 (anti-CD7) (Becton Dickinson, Mountain View, CA); Leu11 (anti-CD16; Becton Dickinson); TS1/18, anti-CD188; TS2/7, anti-LFA-3A; Leu1, anti-CD5 (Becton Dickinson); 9.3, anti-CD28 (gift of Dr V. Groh); Leu2, anti-CD8 (Becton Dickinson); Leu3, anti-CD4 (Becton Dickinson); Leu4, anti-CD3 (Becton Dickinson); W6/32, anti-HEMA class I (ATCC, Rockville, MD); LI 3.1, anti-HEMA class II (ATCC); OKM1, anti-CD11b (Ortho, Raritan, NJ); L-10, anti-CD43 (gift of Dr J. Park, Dana-Farber Cancer Institute, Boston, MA); anti-transferrin receptor (Becton Dickinson); Leu6, anti-CD1a (Becton Dickinson).

**Histochemistry.** Cells were stained with Wright-Giemsa stains, as well as with specific leukemic stains—periodic acid schiff, nonspecific esterase, specific esterase, and myeloperoxidase—according to standard procedure.9

**Flow cytometry.** Surface antigen expression was assessed by flow cytometry. Antigen-specific MoAbs were incubated at 4°C for 45 minutes with 2 × 10^5 leukemic cells. The cells were extensively washed with cold RPMI-1640, 1% fetal calf serum, and 0.02% sodium azide, and then incubated with fluorescein isothiocyanate-conjugated F(ab’)2, sheep antimouse IgG (Cappel, Malvern, PA) at a dilution of 1:100 at 4°C for 45 minutes. Cells were then washed three times and resuspended in RPMI + 1% paraformaldehyde. Levels of cell surface expression were then determined on a Becton Dickinson FACSscan.

**Cytogenetics.** Bone marrow aspirates and the peripheral blood specimens were cultured in RPMI 1640 with 16% (vol/vol) fetal bovine serum at 37°C in 5% CO2 atmosphere and humidity. Standard air-dried slides were used for Q- and R-bandings.10

DNA analysis. DNA was isolated from circulating mononuclear cells when the patient was in leukemic phase. HELA cells were used as a source of germline DNA. Purification of DNA was by alkaline:detertant lysis. DNA was cut with specific restriction endonucleases (BRL, Gaithersburg, MD) under appropriate salt conditions and was electrophoresed through a 0.9% agarose gel. The DNA was then transferred to a polymer filter (Genescreen, Stratagene, La Jolla, CA) where crosslinking of the DNA to the filter was performed. Gene rearrangements were determined by hybridizing with specific probes radioactively labeled by the method of Southern. Specific DNA probes for the TCR locus included J6,11 and Vδ22 (gift of M. Krangel, Duke University). DNA probes used for the analysis of chromosome 8 include exon 2 (Amersham, Buckinghamshire, UK) and the set of 3′ myc probes pCA1.7S, pFA1.3S, and pA1.2S2 (gift of L. Showe, Wistar Institute, Philadelphia, PA).

**Determination of changes in free cytoplasmic calcium.** Cells were loaded with 3 μmol/L of the acetoxyethyl ester of Indo-1 (Molecular Probes, Eugene, OR) for 60 minutes at 37°C, washed twice, resuspended in calcium containing RPMI-1640 without phenol 2 red, and analyzed on a FACScan Analyzer (Becton Dickinson). Indo-1 fluorescence was excited by the mercury arc lamp with a 535 nm band pass filter, emission split using a 450 nm dichroic mirror (Corion, Holliston, MA), and defined with a 480 nm band pass filter (Ditrich, Hudson, MA) and a 405 nm line filter (Corion). Relative intracellular calcium concentration was expressed as the ratio of fluorescence emission 405 nm:480 nm. A shift in fluorescence for any cell greater than two standard deviations above the mean ratio fluorescence of unactivated cells was considered a significant increase in intracellular calcium. Cells were maintained at 37°C during all analyses, agonist MoAbs (8TCS-1, Leu-1, OKT3, WT31) were added in saturating concentrations, and percent cells that had significantly increased cytoplasmic Ca2+ concentration was determined at time points 3 minutes and 5 minutes after addition of agonist. In some experiments, anti-CD5 MoAb was added to the cells for 10 minutes, change in cytoplasmic Ca2+ determined, and then a second agonist MoAb added. In no case did Leu1 MoAb alone lead to a change in [Ca2+].

**Cytosis assays.** Fresh leukemic cells or cells carried in complete media with 10% IL-2-enriched T-cell factor (Electro-nucleonics) were placed in a 4-hour cytolysis assay with a number of standard human cytolytic T lymphocytes (CTL) and NK cell chromium51 labeled targets.8 Percent specific release was calculated as:

\[
\text{Total Release (cpm) — Spontaneous Release (cpm)} / \text{Experimental Release (cpm)} 
\times 100
\]

Total release was determined by the incubation of the target cells with 2.5% Triton X-100.

Redirected lysis51 was shown by using the NK-resistant target cell, P815 (a murine mastocytoma cell line), that is rich in Ig Fc receptors. MoAbs specific for the TCR and controls were added at the time of the assay and TCR stimulation was recorded.

**RESULTS**

**Determination of cellular characteristics of leukemic blasts.** Evaluation of leukemic cells (DOC) was performed on initial presentation and at time of relapse. Bone marrow aspirates showed diffuse replacement with small and medium-sized immature blast forms, some with basophilic cytoplasm and rare cytoplasmic granules (Fig 1). Mitotic figures were frequently noted. Histochemistry showed slight positivity for myeloperoxidase and specific esterase, but no staining was noted for nonspecific esterase or periodic acid Schiff reagents. This initial evaluation was felt to be consistent with an immature acute myelogenous leukemia, French-American-British (FAB) classification M1.

However, despite the myeloid morphology, flow cytometric analysis confirmed the diagnosis of a T-cell leukemia.
Fig 1. Morphologic characteristics of the patient’s T-cell acute lymphoblastic leukemia. Standard Wright-Giemsa stain was performed on bone marrow at time of presentation. Representative histology is shown. Blast forms had diffuse chromatin, large nuclei with distinct nucleoli and slight grayish-blue cytoplasm. Frequent mitotic figures were noted.

Molecular characterization of DOC leukemic blasts. Because the t(8;14)(q24;q11) translocation had previously been associated with rearrangements involving TCRα gene segments, Southern blot analysis was performed to identify whether the chromosomal translocation was associated with unique rearrangements of the TCR. Limited patient germline DNA was available due to the brevity of the patient’s remission. Therefore, germline DNA from the human tumor line, Hela, was used.

Genomic rearrangements of the δ locus were identified on comparing germline and tumor DNA cut with the restriction endonuclease Xba I (Fig 3). When probed with a Vδ1 probe, a rearranged band of approximately 7 kb (in addition to the 9-kb germline band) was found in the leukemic DNA that was not present in germline DNA (Fig 3A). This rearranged band must represent a functional rearrangement of the Vδ1 gene segment on one chromosome that allowed surface expression of the TCRγδ complex, because GTCS-1 surface expression correlates with Vδ1 usage. However, when the same DNA was probed with a Jδ1 probe, two rearrangements were noted when compared with germline DNA (Fig 3B). The 7.0-kb band correlated with the band identified by the Vδ1 probe (Fig 3A), which identified the functional Vδ1-Jδ1 rearrangement. A novel 1.4-kb band was also shown by hybridization with the Jδ1 probe as compared with the germline 1.7-kb band. Because the Jδ1 1.4-kb band had not been identified in total normal human thymus DNA when probed with Jδ1, this finding suggested a novel rearrangement involving the transfer of Jδ1 on the nonexpressed chromosome to chromosome 8.

The human c-myc locus was next evaluated for similar rearrangements in DOC cells. Four probes were used that span the human c-myc locus from approximately 10 kb 5’ of exon 1 to a site approximately 43 kb 3’ of exon 3 within the c-myc gene. In all cases, the DOC DNA fragments were
identical to those found in germline DNA (Fig 4). Thus, over this region of the c-myc locus, no detectable rearrangement had occurred. Therefore, the transforming rearrangement involving the J, fragment, unlike all previous t(8; 14)(q24;q11) rearrangements involving the TCRα locus, occurred in the c-myc locus either at a variant location, as noted recently in some Burkitt lymphoma variants, or in a previously unidentified region. That this rearrangement involves chromosome 8 has been confirmed recently by the molecular cloning of the 1.4-kb J, breakpoint fragment and the demonstration of positive hybridization to chromosome 8 sequences defined within a human-hamster somatic cell hybrid library (Kasai, Maziarz, and Strominger, unpublished data; see Discussion).

Functional characterization of DOC leukemic blasts. Immunologic analyses were performed to assess whether the highly malignant DOC TCRγδ leukemia maintained T-cell functions associated with the mature T cell. The capacity to generate cellular activation signals in a T lymphocyte depends in part on the integrity of a functional TCR complex. Activation of the TCR leads to turnover of cellular phosphatidylinositol as measured by calcium flux between the free cytoplasmic and extracellular compartments with the sequestered intracellular compartment. Calcium flux, as measured by changes in Indo-1 fluorescence, was shown in DOC leukemic cells, as well as in the TCRγδ T-cell line, PEER (data not shown), and the TCRαβ T-cell line, Jurkat, when incubated with 5 µg/mL soluble anti-CD3 MoAb (Table 1). Low levels of calcium flux were found by incubating DOC cells with δTCS-1 and the weakly TCRγδ cross-reactive MoAb, WT31, an anti-TCRαβ MoAb. Cooperative interactions between DOC T-cell surface antigens were also documented because no changes in cytosolic calcium were found with MoAb Leu-1, an anti-CD5 MoAb. However, an enhancement of the anti-δTCS-1 response was found when cells were preincubated with anti-CD5 (Table 1). Preincubation with anti-CD43 did not give similar enhancement (data not shown).

Thus, the cell surface antigen, CD5, can cooperate to
Fig 4. Southern blot analysis of the c-myc locus-chromosome 8. Genomic DNA of the patient (ALL) and of HeLa (GL) was cut with varying restriction enzymes and hybridized to four c-myc locus probes. (A) was digested with EcoRI/HindIII and probed with an exon 2 probe. The exon 2 containing the fragment of about 9 kb includes the c-myc structural gene from 1 kb 5' to c-myc to the end of exon 3. (An EcoRI digest alone that includes an additional 10 kb 5' to c-myc also was in the germline configuration [data not shown]). (B) was cut with EcoRI/Kpn I and probed with p-CA1.7S. The probe is located near the 5' end of an approximately 15-kb fragment. It abuts at the EcoRI site of the fragment detected in (A). (C) was cut with BamHI and probed with pPA1.3SB that detects an approximately 7-kb fragment whose 5' end overlaps the KPNI site in (B). (An Xba I digest also is in germline configuration when examined with the probe and detects a 13-kb fragment that overlaps the 3' end of the fragment shown in (C) and extends to 32 kb downstream of c-myc [data not shown]). (D) was digested with EcoRI and BamHI, respectively, probed with pPAO.2S, and spans a genomic distance from approximately 20 kb to 43 kb 3' of c-myc (see map and probes described in Sun et al33). (A) and (B) and (C) and (D) represent two independent Southern blots that were used for this study. Additional bands noted in (A) and (B) are nonspecific and do not map within the c-myc locus.

Table 1. Intracellular Calcium Flux in Response to Agonist MoAb

<table>
<thead>
<tr>
<th>MoAb</th>
<th>Peak % Cells With Increased [Ca^{2+}]</th>
<th>Jurkat</th>
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<tbody>
<tr>
<td>OKT3</td>
<td>26.4</td>
<td>89.4</td>
</tr>
<tr>
<td>Leu1 x 10 min, OKT3</td>
<td>27.8</td>
<td>84.1</td>
</tr>
<tr>
<td>WT31</td>
<td>11.8</td>
<td>86.1</td>
</tr>
<tr>
<td>Leu1 x 10 min, WT31</td>
<td>25.2</td>
<td>74.7</td>
</tr>
<tr>
<td>δTCR-1</td>
<td>8.2</td>
<td>13.5</td>
</tr>
<tr>
<td>Leu1 x 10 min, δTCR-1</td>
<td>39.4</td>
<td>9.5</td>
</tr>
<tr>
<td>Leu1 x 10 min</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Calcium flux was determined as described in Materials and Methods. *The resting state of the tumor cells was identified before the addition of any MoAbs. Gated populations were then assessed for alterations of intracellular calcium concentration after the addition of agonist MoAb.

†At the time of analysis, DOC cells had been maintained in culture for several weeks in the presence of lymphokine-enriched media. The enhancement of TCR expression was documented in these conditions.

‡Marked alterations of intracellular calcium concentrations were noted with the Jurkat cell line. It is of note that this particular cell line has been selected for its responsiveness after TCR activation.66

Table 2. Redirected Lysis of the Resistant, Fc Receptor-Rich Tumor Target, P815, via TCR Activation of the Leukemic Cells

<table>
<thead>
<tr>
<th>Target Panel</th>
<th>Effector:Target Cell Ratio</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>100:1</td>
</tr>
<tr>
<td>P815</td>
<td>8*</td>
</tr>
<tr>
<td>+ anti-HLA class I (W6/32)</td>
<td>0</td>
</tr>
<tr>
<td>+ anti-CD43 (L-10)</td>
<td>11</td>
</tr>
<tr>
<td>+ anti-CD3 (OKT3)</td>
<td>100</td>
</tr>
<tr>
<td>+ anti-V_{γ} (δTCS-1)</td>
<td>95</td>
</tr>
<tr>
<td>+ anti-C_{γ} (δTCR-1)</td>
<td>93</td>
</tr>
<tr>
<td>+ anti-TCRαβ (WT31)</td>
<td>21</td>
</tr>
</tbody>
</table>

Tumor cells had been carried in 10% IL-2–enriched complete media at the time of this assay for approximately 4 weeks. Percent specific lysis is recorded within this table as described in Materials and Methods.

*Spontaneous release was 20%.
†Cultured MoAb supernatant (W6/32, L-10, OKT3) was added undiluted for a final 25% concentration. Purified MoAb δTCR-1, δTCS-1, and WT31 was added for a final concentration of 1 μg/mL.

These cells were next assayed for the functional ability to perform redirected lysis of murine P815 cells, via Fc receptor-mediated, MoAb-dependent triggering of the TCR.35 No lysis was found when leukemic cells were incubated with the ^{51}Cr-labeled P815 target cells. However, both anti-CD3 and anti-δ chain MoAbs stimulated the lysis of the P815 target cell by DOC cells, while the MoAb, WT31, appeared to generate minimal cross-reactive lysis above background (Table 2).77 Thus, the DOC leukemic cells not only have a functional TCR capable of transmembrane signalling, but antibodies against the TCR are capable of activating the cell to generate target cell destruction.

Normal human CD4 CD8 TCRγδ T cells have been shown to readily generate non–MHC-restricted lysis in the presence of lymphokines,46 but not when isolated directly from peripheral blood.48 To analyze the functional capacity of these leukemic blasts, fresh tumor cells were assayed directly on a panel of chromium^{51} labeled target cells in a ^{51}Cr release cytolysis assay. Lysis of K562, Jurkat, Hs294T, and H69 cell lines was found, but not of JY or Daudi cells, two B-cell lymphoblastoid target cells (data not shown). Because the possibility remained that contaminating circulating mononuclear cells were the source of the demonstrated non–MHC-restricted lysis (although at presentation, the peripheral WBC was 95% blasts and 5% neutrophils), the leukemic blasts were maintained in continuous culture with the addition of T-cell growth factors. Repeat flow cytometric analysis confirmed the presence of 100% TCRγδ leukemic cells in these cultures with notable augmentation of TCR surface expression. Cytolysis on the same tumor cell panel was measured after 28 days and found to be enhanced at least threefold on HS294T and over 10-fold enhanced on K-562 as determined by the comparison of equivalent effector:target ratios (data not shown). No new tumor cell lines were lysed that were not lysed previously (data not shown). Antibody blocking data enhance the reactivity of the TCR in the leukemic blasts upon anti-TCR stimulation, as has been shown in normal TCRαβ T cells.43
showed that MoAb against CD18 (β chain of human LFA-1) inhibited tumor cell lysis, whereas MoAbs to the CD2, CD3, CD4, CD8, and CD58 (LFA-3) antigens did not block lysis (data not shown), suggesting that these latter antigens were not used. Thus, the maintenance of the leukemic blasts in the presence of IL-2–enriched media markedly enhanced their lytic capacity.

These functional activities of the TCRyδ DOC leukemic cells, ie, (1) the presence of a functional TCR capable of generating Fc-redirected lysis, and (2) the non–MHC-restricted tumoricidal activity in the presence of lymphokine that did not rely on T cell structures known to be involved in cytolytic activity by TCRαβ T cells, are characteristic of normal CD4-CD8- T-cell function. A comparison of lytic capacity, therefore, was made between freshly isolated DOC TCRyδ leukemic blasts obtained at relapse and two well-defined human T-cell lines with known NK-like activity, a CD4 CD8- TCRyδ line isolated from human skin, and a CD4 CD8- TCRyδ line isolated from human thymus. Nearly identical tumoricidal activity was noted (Table 3). However, the DOC leukemic cells did lyse a small cell carcinoma tumor cell, H-69, while the other two T-cell lines did not. Seven different melanoma lines (including HS294T demonstrated here), with known susceptibility to NK-like and lymphokine-activated cytolysis, were lysed by the DOC tumor cells (data not shown). Thus, the leukemic T-cell blasts were capable of non–MHC-restricted lysis nearly identical to that by known CD4-CD8- T-cell lines with NK-like activity that originally were derived from normal human tissue.

DISCUSSION

This study addresses the underlying biology of a highly malignant T-cell leukemia. The patient’s disorder had several distinct clinical characteristics. First, the tumor burden was massive and the leukemia showed an extremely high proliferative index. His relapse developed from a remission bone marrow (<5% blasts noted in the bone marrow aspirate) to a circulating WBC of $8 \times 10^5$/mm$^3$ in 96 hours. Secondly, the morphologic, histochemistry, and surface antigen expression studies indicated that this T-cell leukemia demonstrated “lineage-infidelity” as manifest by its myeloblast-like appearance and the expression of rare granules, the histochemical expression of low levels of myeloperoxidase and specific esterase, and the coexpression of myeloid markers on some T cells. An alternative explanation suggests that promiscuity in a transformed hematopoietic precursor allows for the multiple lineage phenotype, although this appears less likely in this case where a clear demonstration of T-cell function had been shown (see below).

Recently, an increasing number of these undifferentiated leukemias have been assigned to the T-cell lineage by cell surface antigen analysis or by TCR gene rearrangements. Characteristically, these leukemias can be biphenotypic and have been capable of being differentiated in vitro along with either the myeloid or lymphoid pathways depending on the culture conditions. Griesinger et al$^{15}$ reported a T-cell line that expressed myeloid differentiation in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF). Similarly, O’Connor et al$^{16}$ have described a TCRyδ T-cell leukemia from which cell lines have been derived that are IL-3 dependent, and express bifunctional differentiation depending whether they are exposed to GM-CSF or IL-2.

The t(8;14)(q24;q11) translocation observed in this patient’s leukemic blasts is reminiscent of the molecular derangement found in Burkitt’s lymphoma. This chromosomal rearrangement has been previously identified in T-cell leukemias, and certainly the clinical course of this patient is similar to the rapid progression of advanced B-cell Burkitt’s lymphoma. The rearrangement described in this report is novel, however, and represents the first demonstration of a rearrangement involving the TCRδ and the c-myc locus. It is not surprising to identify this rearrangement because in B-cell Burkitt’s lymphomas, c-myc- associated translocations have been found with both the light (κ and λ) as well as the heavy Ig genes. A large number of nonrandom rearrangements have been identified in T-cell leukemias, but to date only the TCR-α locus fragments have been associated with c-myc rearrangements. These TCRα rearrangements have been identified in cell lines as well as fresh tissue, but as of yet, no documented TCRδ, TCRβ, or TCRγ rearrangements have been previously reported to involve the c-myc gene locus.

The TCRδ locus is an active site for recombination and is felt to represent part of the TCR of a distinct T-cell lineage that has ontologic and physiologic functions distinct from TCRαβ T cells. Multiple T-cell leukemias have been found with δ rearrangements, some occurring as random chromosomal breaks, while other δ translocations have occurred via heptamer:nonamer recombinant events that have generated an aberrant trans-chromosomal recombination event.$^{11,12}$ It has been suggested that these leukemias may be more often associated with immature phenotypes and mixed lineage markers because of the

Table 3. Comparative Lysis of Tumor Targets of the TCRyδ Leukemia With Known CD4+CD8- TCRαβ and CD4+CD8- TCRyδ Lines

<table>
<thead>
<tr>
<th>Target Panel</th>
<th>PT ALL</th>
<th>T-cell Effector Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effector:Target Cell Ratio</td>
<td>TCRγδ</td>
<td>TCRαβ</td>
</tr>
<tr>
<td>K-562</td>
<td>33:1</td>
<td>17:1</td>
</tr>
<tr>
<td>Hs294T</td>
<td>23</td>
<td>12</td>
</tr>
<tr>
<td>JY</td>
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<td>Jurkat</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P815</td>
<td>42</td>
<td>38</td>
</tr>
<tr>
<td>E95A-BL</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>H-69</td>
<td>27</td>
<td>22</td>
</tr>
</tbody>
</table>

The patient’s leukemic blasts were assayed in comparison to known T-cell lines with NK-like activity in a 4-hour $^{51}$Cr cytolytic release assay. Percent specific release is as noted. Spontaneous release was less than 25% in all targets.
developmentally earlier expression of the TCRγδ complex.

This particular rearrangement is unusual in that the Jγ translocation to chromosome 8 appears to lie at a distance far from the structural c-myc gene. Southern blot analysis shown here (Fig 4) identifies no genomic rearrangements on chromosome 8 from approximately 10 kb 5′ to c-myc to approximately 43 kb downstream of c-myc. The TCRδ enhancer is located 3′ to Jγ, by approximately 7 kb, and lies in the intron between Jγ and Cγ, and was transferred with Jγ at the time of recombination. It is known that this Jγ rearrangement moves to chromosome 8 because the cloned Jγ fragment binds chromosome 8 sequences (Kasai, Maziarz, Strominger, manuscript in preparation). It is also suggested that c-myc remains on chromosome 8 because the macro-molecular bidirectional rearrangement occurred telomeric from the Jγ locus at 14q11. Thus, this T-cell leukemia appeared to be similar to the recently described variant Burkitt lymphoma lines in which translocations far downstream (140 kb) from c-myc have been identified in a region designated bvr (Burkitt variant rearrangement locus) or into another human proto-oncogene locus, pvr-1, located even further downstream (260 kb 3′ to c-myc). Whether breakpoints into this region deregulate c-myc or another, as of yet, undefined proto-oncogene remains to be elucidated. Presently, efforts are underway to further delineate the molecular events occurring in this TCRγδ leukemia. Molecular cloning of the novel 1.4 kb Jγ fragment has been accomplished (Kasai, Maziarz, Strominger, manuscript in preparation) and preliminary analysis of the breakpoint via pulsed-field gel electrophoresis suggests that the actual breakpoint is near the pvr-1 locus, 260 kb 3′ of c-myc (Kasai, Maziarz, Strominger, manuscript in preparation). Determination of the structural integrity of the c-myc gene on chromosome 8 remains to be assessed. If this translocation does turn out to be responsible for cellular transformation via activation of c-myc, it remains unclear how deregulation of a cellular proto-oncogene occurs at such great chromosomal distances.

It is noteworthy that, despite the multiple characteristics of an undifferentiated state, this T-cell leukemia maintained multiple functions associated with the mature TCRγδ cell. The TCR was functional (Table 1) and could direct T-cell-mediated target cell lysis (Table 2). In addition, these cells were capable of lysing target cells spontaneously, and when maintained in vitro in the presence of interleukins, they also demonstrated a target cell spectrum nearly identical to known T-cell lines with NK-like activity (Table 3). This latter point is significant because, in all likelihood, this NK-like activity is not TCR-mediated, but is a result of activation of other surface structures, as noted previously. This finding is further suggested by the identical lytic capacity of CD4⁺CD8⁺ T cells that express different T-cell receptors (Table 3).

Several reports have shown that large granular lymphocyte (LGL) leukemias can generate non-MHC-restricted tumor cell lysis. The LGL is the circulating lymphocyte that normally provides the most significant degree of circumventing NK cell activity (although the TCRγδ T cells have also been implicated). These disorders are characterized by a slowly progressive accumulation of lymphocytes in the bone marrow as well as in the spleen and liver. This clinical course is inconsistent with the biology of the TCRγδ T-cell leukemia presented here. Other groups have reported that T-cell acute lymphocytic leukemias can lyse target cells when activated with IL-2, but have never found evidence of spontaneous lytic activity by these blasts. Finally, a TCRγδ T-cell line has been established in vitro that maintains IL-2-dependent cytotoxicity. However, in no situation has a leukemia been described that has demonstrated such a highly malignant phenotype while retaining discernible functional activity.

The underlying mechanism by which this TCRγδ leukemia with its novel t(8;14)(q24;q11) translocation maintained the functional capacity to generate NK-like activity, as well as TCR activation, is not clear. It is possible that autocrine production of IL-2 occurred and induced the NK-like activity. Autocrine production of GM-CSF has been described for several acute myelogenous leukemias and was felt to contribute to the malignant behavior of the cell. It is also possible that paracrine stimulation of the TCRγδ leukemia by lymphokine activation from a residual host immune system played an active role in the generation of NK-like activity. However, no serum remains to assay for circulating lymphokines. It is clear that the expression of non-MHC-restricted lysis by TCRγδ lymphoid cells is only normally found in the activated state; freshly isolated human TCRγδ T cells do not kill tumor cells. However, in this leukemia, NK-like activity was found in fresh blasts. Evaluation of further patients may allow the determination of whether spontaneous activity was truly autocrine, paracrine, or a manifestation of an aberrant functional behavior attributed to the leukemic cell. In addition, such an evaluation may help clarify if any of the clinical aspects of the active leukemic disease could be associated with this unusual biologic phenotype.

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