True T-Cell Chronic Lymphocytic Leukemia: A Morphologic and Immunophenotypic Study of 25 Cases

By J.D. Hoyer, C.W. Ross, C.-Y. Li, T.E. Witzig, R.D. Gascoyne, G.W. Dewald, and C.A. Hanson

We studied 25 T-cell chronic lymphocytic leukemia (T-CLL) cases collected over a 15-year period. Immunophenotypic analysis was performed in each case; 12 cases were evaluated by cytogentetics, and gene rearrangement studies were performed in 14 cases. The median age was 57 years with a male predominance (M:F, 15:10). The median presenting lymphocyte count was 36.3 × 10^9/L (range, 3.3 to 438 × 10^9/L). Fourteen patients (56%) had shotty adenopathy and ten (40%) had mild-to-moderate splenomegaly at presentation; four (16%) had erythematous skin lesions. The lymphocytes were predominantly small; some cases had a minor component of medium-sized cells (<10%). The nuclear:cytoplasmic ratios were uniformly high with round to oval nuclei; however, a wide spectrum of nuclear outlines could be found, ranging from minimally to markedly convoluted. Nucleoli were either absent or small and inconspicuous. These lymphocytes did not have the morphology of prolymphocytes and did not contain cytoplasmic granules. Bone marrow infiltration was generally in an interstitial pattern; the degree of involvement ranged from 15% to 90%. Immunophenotyping showed that the lymphocytes were mature T-cells with a predominant CD4^+ immunophenotype. Three cases displayed a CD8^+ immunophenotype. The patients were treated with a variety of chemotherapeutic regimens with only a minimal response observed in two of 20 patients. We conclude that T-CLL is an uncommon chronic lymphoproliferative disorder (CLPD) that can be morphologically similar to B-CLL, is distinct from T-prolymphocytic leukemia, and has an aggressive clinical course that is refractory to therapy. It may also be difficult to distinguish T-CLL from other T-CLPD, especially the leukemic phase of peripheral T-cell lymphomas and some cases of Sézary syndrome.

© 1995 by The American Society of Hematology.

MATERIALS AND METHODS

Case Selection

The laboratory files of the Mayo Clinic, the University of Michigan, and the British Columbia Cancer Agency were reviewed to identify cases with an absolute lymphocytosis and a T-cell immunophenotype. From 1978 to 1994, a total of 25 cases were identified that fulfilled these criteria; 14 were from the Mayo Clinic, 6 from the University of Michigan, and 5 from the British Columbia Cancer Agency. Six of the cases have been previously reported. Patient medical records were available in each case and were reviewed to obtain complete blood count and other laboratory findings, clinical data, and treatment and survival data.

Peripheral Blood, Bone Marrow (BM), and Tissue Samples

Peripheral blood and BM aspirate smears were stained by either Wright's or Wright-Giemsa stains using standard techniques. BM trephine biopsies were fixed in B5 fixative, processed by standard decalcification techniques, and stained using hematoxylin-eosin. Lymph node biopsies from two cases were fixed in 10% formalin and/or B5 fixative; skin biopsies from two cases were fixed in 10% formalin. Both tissues were stained by standard hematoxylin-eosin techniques.

Immunophenotyping

Immunophenotyping was performed on peripheral blood using flow cytometric analysis (16 cases), immunocytochemical stains of air-dried smears (seven cases), or by immunoperoxidase studies on paraffin sections (two cases). Flow cytometric analysis was performed on either a FACS IV or FACSscan Analyzer (Becton Dickinson, Mountain View, CA), using standard techniques. The immunocytochemical technique on air-dried slides used either an alkaline phosphatase-antialkaline phosphatase method with fast red violet as the chromogen or a labeled streptavidin-biotin method using aminoethylcarbazole as the chromogen. The following antibodies were used with either flow cytomtery or immunocytochemical techniques: CD2, CD3, CD4, CD5, CD7, CD8, CD16, CD19, CD20, CD56, CD57, and HLA-DR.

Two cases were identified before the availability of immunophenotyping. A T-cell phenotype of the lymphocytes was presumed by detecting T-rosette formation and focal, punctate α-naphthyl acetate esterase (ANAE) staining. Retrospectively, the BM biopsy blocks in these two cases were retrieved and paraffin section immunostaining using a labeled streptavidin-biotin method with aminoethylcarbazole as the chromogen was performed. The following antibodies were used for these two paraffin block studies: CD20 (L26), CD3, CD4, CD5, CD7, CD8, CD16, CD19, CD20, CD56, CD57, and HLA-DR.

From the Departments of Laboratory Medicine and Pathology and Internal Medicine, Mayo Clinic, Rochester, MN; the Department of Pathology, University of Michigan, Ann Arbor, MI; and the Department of Pathology, British Columbia Cancer Agency, Vancouver, BC, Canada.

Submitted October 13, 1994; accepted March 21, 1995.

Address correspondence and reprint requests to J. D. Hoyer, MD, 1020 Hilton, Mayo Clinic, 200 First St SW, Rochester, MN 55905.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1995 by The American Society of Hematology. 0006-4971/95/8603-0001$3.00/0

Blood, Vol 86, No 3 (August 1), 1995: pp 1163-1169
CD45RO (UCHL-1, OPD4), CD43 (Leu-22), CD3 (polyclonal), and CD57 (Leu-7).

Cytogenetics
Chromosomal analysis was performed on either peripheral blood (nine cases), BM aspirates (two cases), or both (one case). Specimens collected before 1984 were processed using standard direct and short-term unstimulated culture methods. Specimens collected after 1984 were cultured and procured using a standard direct technique and in vitro unstimulated culture method. Metaphases were stained by either Giemsa stain with trypsin pretreatment (GTG-banding) or fluorescent staining with quinacrine mustard (QFQ-banding). Photomicrographs of representative metaphases were taken in all cases for documentation purposes. The cytogenetic results were recorded in accordance with the standard rules for the International System for Human Cytogenetic Nomenclature (ISCN).

Molecular Genetics
T-cell receptor (TCR) and Ig gene rearrangement studies were performed on either peripheral blood (13 cases) or both BM aspirate and peripheral blood (one case) using standard methods. The TCR β chain region probes used were either a Jβ1 (0.7-kb XbaI fragment)/Jβ3 (4.4-kb EcoRI fragment) probe mixture (four cases) or a Cβ1 (0.7-kb EcoRI fragment) (five cases) or a Cβ1 (3.5-kb HindIII fragment) (five cases). The TCRγ chain region probe used was a 0.7-kb EcoRI/Hind III fragment (four cases). Ig gene rearrangements were evaluated with the following DNA probes: Jκ (1.8-kb SacI fragment); Cκ (1.06-kb BamHI fragment); Cμ (1.3-kb EcoRI fragment); and Jκ (2.5-kb Sau3A fragment) (14 cases). The restriction enzymes used for the Southern blot analysis included: (1) EcoRI for Jβ1/Jβ2, TCRγ, and Cκ; (2) BamHI for Cμ and Jκ; (3) BglII, BamHI, and HindIII for Jκ; and (4) EcoRI, BamHI, and HindIII for Cβ1.

RESULTS
Patient Profiles/Laboratory Findings
The median age range of the patients was 57 years (range, 26 to 80 years) with a male predominance (M:F, 15:10). The median white blood cell count at presentation was 48.1 × 10^9/L (range, 10.9 to 488.0 × 10^9/L), with a median absolute lymphocyte count of 36.3 × 10^9/L (range, 3.9 to 438.0 × 10^9/L). Twelve patients had a marked lymphocytosis exceeding 100.0 × 10^9/L at some point in the course of their disease, with the highest lymphocyte count recorded being 762.0 × 10^9/L. Six patients presented with both anemia and thrombocytopenia, three patients with anemia alone, and two patients with thrombocytopenia alone. In those patients with anemia and/or thrombocytopenia, the hemoglobin values ranged from 7.1 to 12.6 g/dL and platelet counts from 42 to 135 × 10^9/L. None of the patients had an absolute neutropenia. Ten patients showed elevated serum liver function tests at some time in the course of their disease, usually manifested as a mild elevation of both alkaline phosphatase and aspartate aminotransferase with or without mild elevation of lactate dehydrogenase. One patient had an elevation of lactate dehydrogenase to 750 U/L. None of the patients had significant hypercalceemia or hyperproteinemia. One patient had a small bicalon gammopathy by immunoelctrophoresis, which was not clinically significant. Ten patients had serologic testing for HTLV-1; all were serologically negative.

Physical Findings
Fourteen patients had mild lymphadenopathy at presentation, characteristically with small shotty nodes (<1 to 2 cm) in multiple lymph node chains; two cases underwent lymph node biopsies. Ten patients presented with mild-to-moderate splenomegaly and two others with mild-to-moderate hepatosplenomegaly. Two additional patients developed hepatosplenomegaly later in their clinical course. Three patients presented with a maculopapular rash and one patient with erythematous plaques over the trunk.

Morphology
Peripheral blood. The peripheral blood smears in all cases showed a predominant small lymphocytic proliferation (Fig 1). In eight cases, there was a minor component of medium-sized lymphocytes, comprising less than 10% of the lymphoid cells. The small lymphocytes were characterized by a high nuclear:cytoplasmic ratio, with scant pale-blue cytoplasm. Cytoplasmic granularity was absent in all cases. In four specimens, the lymphocytes displayed cytoplasmic blebs. The malignant lymphocytes exhibited a wide variety of nuclear shapes and contours. In 16 of the cases the lymphocytes were round to oval and exhibited only mild nuclear irregularity, consisting of a slight indentation to one side of the nucleus. Two cases in this group had a minor component of lymphocytes with a more pronounced indentation almost resembling a cleaved cell. The remaining nine exhibited moderate nuclear irregularity with the nuclear membranes being either folded or undulated. In these cases, a minority of lymphocytes were markedly convoluted. In all cases the nuclear chromatin was condensed, with nucleoli either absent or small and inconspicuous. The medium-sized lymphocytes displayed a lower nuclear:cytoplasmic ratio with pale-blue cytoplasm, had irregular nuclear outlines, and had identifiable, but not prominent, nucleoli.

BM. BM aspiration and biopsy samples were available from 17 of the 25 patients. The BM aspirate smears showed an increase in small lymphocytes similar in appearance to that seen on the peripheral blood smears. No abnormalities were seen in the myeloid, erythroid, or megakaryocytic cell lines. Four of the 17 BM biopsy samples were normocellular with the rest being hypercellular. The degree of lymphocytic involvement ranged from 15% to 90%, with two cases showing a nodular pattern, 12 an interstitial pattern, and three a diffuse pattern of infiltration (Fig 2). The lymphocytic infiltrate had a homogeneous appearance in all but one case; in most cases, slight-to-moderate nuclear irregularity of the small lymphocytes could be appreciated. In one case, a small (10%) component of larger cells was present. Nucleoli were absent and mitoses were not seen. Only one case had prominent vascularity within the lymphoid infiltrate. None of the cases had associated necrosis, granulomas, or plasma cell/ eosinophilic infiltrates.

Other specimens. Lymph node biopsy samples were available for review in two cases. Both cases showed effacement of the normal lymph node architecture by a monomorphic population of small lymphocytes. Small, sometimes sclerotic vessels were prominent in both cases. One case was
characterized by small lymphocytes with minimal nuclear irregularity. There were occasional small clusters of larger cells present. In the other case, prominent nuclear irregularity in the small lymphoid cells was noted. In two of the cases with skin involvement, skin biopsy samples were available for review (neither of these had lymph node biopsies performed); both showed a perivascular lymphocytic infiltrate without epidermotropism. The patient with the erythematous plaques did not undergo a biopsy; these plaques regressed after initial treatment.

Immunophenotyping

All cases showed a mature T-cell immunophenotype (Table 1). No aberrant expression or loss of pan-T-cell antigens was identified. In 20 cases, the lymphocytes were CD4⁺; however, three cases displayed a CD8⁺ phenotype. These three cases were morphologically indistinguishable from the CD4⁺ cases, did not have a LGL morphology, and were CD16⁺, CD56⁺, and CD57⁺.

The two cases identified before the availability of immunohistochemical studies showed positive E-rosettes and focal ANAE staining, in a pattern shown to be common in T-lymphocytes. Retrospective immunohistochemical studies performed on the BM biopsy sample paraffin sections showed a lymphocytic infiltrate composed of T-lymphocytes that were positive for CD3 and weak CD45RO (both UCHL-1 and OPD4). These lymphocytes were CD20⁻, CD43⁻, and CD57⁻.

Treatment/Survival

Complete follow-up information was available on 20 of the 25 patients; partial information was available for the
Table 1. Summary of Immunophenotypic Results

<table>
<thead>
<tr>
<th>Antigen</th>
<th>No. Positive/No. Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD2</td>
<td>16/16</td>
</tr>
<tr>
<td>CD3</td>
<td>21/21</td>
</tr>
<tr>
<td>CD4</td>
<td>20/23</td>
</tr>
<tr>
<td>CD5</td>
<td>18/18</td>
</tr>
<tr>
<td>CD7</td>
<td>14/14</td>
</tr>
<tr>
<td>CD8</td>
<td>3/23</td>
</tr>
<tr>
<td>CD16</td>
<td>0/11</td>
</tr>
<tr>
<td>CD19</td>
<td>0/13</td>
</tr>
<tr>
<td>CD20</td>
<td>0/16</td>
</tr>
<tr>
<td>CD56</td>
<td>0/10</td>
</tr>
<tr>
<td>CD57</td>
<td>0/11</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>0/14</td>
</tr>
</tbody>
</table>

other five. The patients were treated with a variety of chemotherapeutic regimens, the most common being chlorambucil (or cyclophosphamide) plus prednisone. Other regimens included CHOP, CVP, m-BACOD, CVB, PROMACE, COPA, CAP-BOP, VP-16, fludarabine, and pentostatin. One patient was treated with intrathecal methotrexate, prednisone, and whole brain irradiation (3,000 rads) because of suspected meningeval involvement (bilateral seventh nerve palsies). Two patients received allogeneic BM transplantation.

Seventeen of the 20 patients with available follow-up information have died as a result of their disease (Fig 3). The median survival of those who died was 13 months (range, less than 1 month to 6 years). In this group, only two patients showed minimal responses to chemotherapy; all others were refractory. Currently three patients are still alive (9 to 14 months after diagnosis). One of these patients received a BM transplantation and is alive 9 months after diagnosis.

Cytogenetics

Cytogenetic studies were performed on 12 cases (Table 2). Recurring structural abnormalities involving bands 14q11 and 14q32, because of either translocation or inversion, were identified in six cases. Two cases also had abnormalities involving band 7p15. Three cases had abnormalities involving the long arm of chromosome 8; two of these were iso-

chromosome of the long arm. In all cases involving bands 14q11 and 7p15, additional complex chromosomal abnormalities were present.

Molecular Genetics

Gene rearrangement studies were performed in 14 cases. In all instances there was rearrangement of the TCRβ chain without rearrangement of the Ig heavy or light chain. Rearrangement of the TCRγ chain was also detected in two cases. Interestingly, there were two cases that had cytogenetic abnormalities involving 14q32 that also had molecular genetic studies performed. These two cases did not show rearrangement of the Ig heavy chain gene.

DISCUSSION

The results from this multi-institutional study show that T-CLL is an uncommon entity with features distinct from other T-CLPD. Based on the total number of cases seen at our institutions, T-CLL accounts for less than 1% of all CLPDs. T-CLL is characterized by a marked lymphocytosis of primarily small, mature T-lymphocytes having some degree of nuclear irregularity, and inconspicuous nucleoli that do not meet the diagnostic criteria for prolymphocytes. Lymphadenopathy and splenomegaly are uncommonly found and, when present, are always mild-to-moderate without striking organomegaly. The immunophenotype is that of a mature T-cell that is usually CD4+, but may occasionally be CD8+. The patients from our study did poorly; a number of different chemotherapeutic agents were administered with only a minimal response being obtained in a minority of patients.

A number of CLPDs enter into the differential diagnosis of T-CLL, including: T-PLL, leukemic phase of peripheral T-cell lymphoma (PTCL), mycosis fungoides/Sézary syn-

Table 2. Summary of Cytogenetic and Molecular Genetic Studies

<table>
<thead>
<tr>
<th>Cytogenetics (n = 12)</th>
<th>Molecular Genetics (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome abnormality*</td>
<td>T-cell receptor β chain</td>
</tr>
<tr>
<td>inv(14q11.2;q32)</td>
<td>14/14</td>
</tr>
<tr>
<td>t(14;14)(q28;q11)</td>
<td>0/14</td>
</tr>
<tr>
<td>t(X;14)(q28;q11)</td>
<td>2/4</td>
</tr>
<tr>
<td>Normal</td>
<td>3</td>
</tr>
</tbody>
</table>

* Some cases had more than one of these abnormalities, ie, i(19p) and inv(14)(q11.2;q32). Many cases also displayed additional random abnormalities not listed here.

Fig 3. Survival curve of T-CLL patients is shown.
TRUE T-CLL

A recurring chromosomal abnormality in patients with these T-cell malignancies involves 14q11, the site of the T-cell receptor \( \alpha \) and \( \delta \) chain genes, and 14q32. Other cases had abnormalities involving 7p15, the site of the TCR\( \gamma \) chain gene. Although some authors have claimed that inv(14) and t(14;14) abnormalities are specific for T-PLL,\(^{23,33}\) it appears more likely that these findings are common to T-PLL proliferations in general, including T-PLL,\(^{14-17}\) PTCL,\(^{34-41}\) T-cell acute lymphoblastic leukemia,\(^{42-44}\) T-cell lymphoblastic lymphoma,\(^{40}\) ATL,\(^{44}\) and Sézary syndrome.\(^{45}\) Although 14q32 is the location of the Ig heavy chain gene, it may not be intimately involved in inv(14)(q11;q32) or t(14;14)(q11;q32).\(^{21,123}\) This concept is supported by two of our cases in which simultaneous cytogenetic and molecular genetic studies were performed. In both cases, the 14q32 locus was involved and yet no Ig heavy chain gene rearrangements were detected by molecular studies, implying that the breakpoint on band 14q32 is outside the Ig heavy chain gene locus detected by commonly used probes.

Studies in the literature have reported an association between ataxia telangiectasia and T-CLPDs\(^{35,36,46-48}\); our series included one such patient. Early chromosome studies on this patient before the T-CLL diagnosis showed a t(14;14)(q11;q32), but with no additional chromosomal abnormalities and a normal white blood cell count and differential. She continued to have a protracted clinical course; 8 years later, a repeat karyotype showed numerous complex abnormalities in addition to the t(14;14). She subsequently entered a more aggressive stage of her disease, was diagnosed with a T-CLL, and died within 2 years. This case...
supports the theory previously presented that 14q11 abnormalities may represent a precursor event in ataxia telangiectasia, and that additional cytogenetic abnormalities signal the development of a more aggressive phase in this disease.  

This patient also had an older sister with ataxia telangiectasia, who died of an atypical T-cell lymphoproliferation that was not sufficient for the diagnosis of T-CLL.  

Cytogenetics on this older sister showed a t(14;14)(q11;q34) abnormality as well as other karyotypic abnormalities.

The basic classification scheme for B-CLPDs is well established.  

The separation of B-CLL, B-CLL with prolymphocytoid features, and B-PLL is based on identifying an increasing percentage of prolymphocytes: less than 15%, 15% to 55%, and greater than 55%, respectively. Other components of the B-cell chronic lymphocytic proliferation scheme are based on unique morphologic as well as immunophenotypic characteristics. In contrast, there has been a different approach regarding the classification of BM-based T-cell proliferations.

T-prolymphocytic leukemia has remained a distinct T-cell malignancy with characteristic clinical and laboratory features. In recent years, evidence has accumulated that T-prolymphocytic leukemia may be a distinct entity as well.

Cytogenetic studies also suggest that there may be karyotypic subgroups within the T-cell malignancies worth further study. Future classification schemes of the T-CLPDs may need to incorporate a variety of morphologic, immunologic, cytogenetic, and molecular features in subclassifying this heterogeneous group of malignancies.

REFERENCES


9. Matutes E, Catovsky D: CLL should be used only for the disease with B-cell phenotype. Leukemia 7:917, 1993


27. Tajima K: Malignant lymphomas in Japan: Epidemiological
True T-cell chronic lymphocytic leukemia: a morphologic and immunophenotypic study of 25 cases [see comments]

JD Hoyer, CW Ross, CY Li, TE Witzig, RD Gascoyne, GW Dewald and CA Hanson