Clinical, Morphologic, Immunophenotypic, and Molecular Cytogenetic Assessment of CD4-/CD8- γδ T-Cell Large Granular Lymphocytic Leukemia

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Key Words: γδ T-cell large granular lymphocytic leukemia; Morphologic features; Clinical features; Immunophenotype; Bone marrow; Spleen

Abstract

γδ T-cell large granular lymphocytic (T-LGL) leukemia of the CD4-/CD8− subtype is rare, and data are limited in the literature. This study evaluated the clinical, morphologic, immunophenotypic, and molecular cytogenetic features of 7 cases of CD4-/CD8− γδ T-LGL leukemia. Although this variant shares several clinical and morphologic features with the more common T-LGL leukemias, the incidences of autoimmune hemolytic anemia and pure red cell aplasia are higher. Another striking feature observed in our study was the lack of increased large granular lymphocytes in the peripheral blood in the majority of cases despite prominent bone marrow or splenic involvement by CD4-/CD8− γδ T-LGL leukemia, and discuss the morphologic features that are helpful in distinguishing CD4-/CD8− γδ T-LGL leukemia from HSTCL. List findings in peripheral blood, bone marrow, and spleen in patients with CD4-/CD8− γδ T-LGL leukemia and describe clinical manifestations that may be seen.

T-cell large granular lymphocytic (T-LGL) leukemia represents 2% to 3% of mature lymphocytic leukemia and is defined by the current World Health Organization (WHO) classification as a persistent (>6 months) clonal expansion of peripheral blood T-LGL cells, usually between 2 and 20 × 10^9/L, without a clearly identified cause.1 The underlying pathologic mechanisms of the disease are not well understood. However, its frequent association with autoimmune disorders suggests that it may arise from clonal expansion of cytotoxic T cells in a setting of sustained immune stimulation.

T-LGL leukemia cells retain many phenotypic and functional properties of normal cytotoxic effector cells. The majority of cases are derived from T-cell receptor (TCR)αβ+ and CD8+ T cells, and the neoplastic T cells are often CD16+ and CD57+.1,7 TCRγδ+ T-LGL leukemia is rare, and similar to the αβ subtype, the majority of cases are also CD8+.8,9 It has been reported that γδ T-LGL leukemia, in general, shows clinical features similar to those of its αβ counterpart.8,9 A rare subgroup of T-LGL leukemia arising from CD4-/CD8− γδ T cells has been recognized. To our knowledge, there have been only approximately 30 such cases reported in the literature, either as case reports or as a small subset of cases included with a larger group of CD8+ γδ T-LGL leukemia.8,17 Therefore, the data on the clinical and laboratory features of CD4-/CD8− γδ T-LGL leukemia are limited.

The present study evaluated 7 cases of CD4-/CD8− γδ T-LGL leukemia identified in our institution between 1992 and 2010. Although the number of cases is small, our study represents the largest and most extensively studied
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series of CD4–/CD8– γδ T-LGL leukemia from a single institution. The evaluation includes morphologic features of peripheral blood, bone marrow, and spleen; flow cytometric immunophenotyping; immunohistochemical studies; molecular and cytogenetic findings; and clinical features, including manifestations, treatment, and outcome. We also compared these features with those reported in αβ and γδ T-LGL leukemia (mostly CD8+).3,8,9

Materials and Methods

Case Identification

This study was approved by the Northwestern Memorial Hospital Institutional Review Board, Chicago, IL. Cases diagnosed on peripheral blood and bone marrow biopsy samples as CD4–/CD8– γδ T-LGL leukemia or γδ T-cell neoplasm with a CD4–/CD8– phenotype between January 1992 and July 2010 were identified from the database at the Department of Pathology, Northwestern Memorial Hospital. Slides of all diagnostic specimens, including peripheral blood smear, bone marrow aspirate smear, core biopsy sample, splenectomy specimen, immunohistochemical staining, flow cytometric immunophenotyping, and the associated molecular, cytogenetic, and fluorescence in situ hybridization (FISH) results, were retrieved and reviewed.

For this study, a cutoff for increased large granular lymphocytes (LGLs) in the peripheral blood was arbitrarily defined as a total LGL count of 0.4 × 10^9/L or more based on the upper limit of the total lymphocyte count (4,000/μL [4.0 × 10^9/L]) and the proportion of LGLs (<10%) usually present within the total lymphocyte population in a healthy subject. Severe neutropenia was defined as a neutrophil count less than 500/μL (0.5 × 10^9/L) and severe anemia as a hemoglobin level of less than 8 g/dL (80 g/L).

Flow Cytometric Immunophenotyping

Fresh cell suspensions were prepared from peripheral blood, bone marrow aspirate, or spleen and analyzed as previously described.18 The T and NK cell–associated antigens analyzed included CD2, CD3, CD5, CD7, CD4, CD8, CD16, CD56, CD57, TCRαβ and TCRγδ, cytoplasmic cytotoxic granule–associated proteins (TIA-1 and granzyme B), and CD158 (a, b, and e), a member of killer cell immunoglobulin-like receptors (KIR).

Immunohistochemical Staining

Immunohistochemical staining for CD3, CD2, CD5, CD57, and TIA-1 on the bone marrow core and spleen sections was performed on the BenchMark XT (Ventana Medical System, Tucson, AZ) and for CD7, CD4, CD8, CD56, and βF1 on the DAKO Autostainer (DAKO, Carpinteria, CA). The following primary antibodies were from Novocastra/Leica, Bannockburn, IL: CD3 (clone PS1), CD2 (clone AB75), CD5 (clone 4C7), CD7 (clone LP15), CD4 (clone 1F6), and CD56 (clone 1B6). Additional primary antibodies were CD8 (clone C8/144B, DAKO North America, Carpinteria, CA), CD57 (clone HNK-1, BD Biosciences, San Jose, CA), TIA-1 (clone TIA, Immunotech-A, Beckman Coulter, Marseille, France), βF1 (clone 8A3, Thermo Scientific, Rockford, IL), and TCR C gamma M1 (clone γ3.20, Thermo Scientific).

Molecular and Genetic Studies

For TCR gene rearrangement analysis, DNA was extracted from fresh cells from the peripheral blood or bone marrow aspirate or formalin-fixed spleen tissues using the QIAsymphony automated extractor and the QIAsymphony DNA Mini Kit (Qiagen, Valencia, CA) according to the manufacturer’s protocol. Polymerase chain reaction was performed in an automated thermocycler PTC-100 (Bio-Rad, Hercules, CA) according to the InVivoScribe multiplex TCRG gene clonality assay (InVivoScribe, San Diego, CA). Two multiplex master mixes were used to target the variable and joining regions surrounding the hypervariable region of the TCR genes. The amplified products were run on the ABI 3130xl genetic analyzer (Applied Biosystems, Carlsbad, CA) for analysis.

Cytogenetic studies and FISH analysis for isochromosome 7q (i7q) were performed at Mayo Medical Laboratories.

Results

Case Identification

A total of 16 cases of CD4–/CD8– γδ T-LGL leukemia or CD4–/CD8– γδ T-cell neoplasm, not otherwise subclassified, diagnosed on peripheral blood and bone marrow biopsy samples were identified. Of the 16 cases, 7 were confirmed to be CD4–/CD8– γδ T-LGL leukemia according to the current WHO classification based on a combination of clinical, morphologic, immunophenotypic, and molecular data. The remaining 9 cases represented various other types of peripheral γδ T-cell lymphoma/leukemia involving peripheral blood and bone marrow (data not shown).

The peripheral blood smear, bone marrow aspirate smear, core biopsy and immunophenotyping data were available for all 7 cases of CD4–/CD8– γδ T-LGL leukemia. Splenectomy samples were available in 3 cases. Molecular analysis for TCR gene rearrangement, cytogenetic studies, and FISH analysis for i7q were performed in 7, 5, and 5 cases, respectively [Table II].

[Table II]
Patient Characteristics and Clinical and Hematologic Findings

The clinical and laboratory findings for the 7 cases (5 men and 2 women) with CD4–/CD8– γδ T-LGL leukemia are summarized in Table 1. Of the 7 patients, 6 were 50 years or older. In 4 of 7 cases, the patients had systemic symptoms at initial examination, including B symptoms in 1 (case 6), fatigue in 2 (cases 2 and 4), and fever in 1 (case 7). The main physical finding was splenomegaly, which was identified in 6 of 7 cases. None of the patients had lymphadenopathy. Associated autoimmune manifestations were identified in all 7 cases, including 3 with autoimmune hemolytic anemia (AIHA; cases 2-4), 2 with a long-standing history of rheumatoid arthritis (cases 1 and 6), 1 with positive antinuclear antibody and Coombs tests but no clinical evidence of hemolysis (case 5), and 1 with immune thrombocytopenic purpura (case 7). Two patients (cases 2 and 4) with AIHA and pure red cell aplasia (PRCA) became transfusion-dependent. Splenectomy was done for 3 patients, 2 for AIHA (cases 3 and 4) and 1 for progressive splenomegaly and cytopenia (case 6) before T-LGL leukemia was recognized.

The hematologic findings included neutropenia and/or anemia in all 7 cases, 5 with severe neutropenia (cases 1, 3, and 5) or severe anemia (cases 2 and 4). Thrombocytopenia was present in 3 patients (cases 3, 6, and 7) and was mild. One patient (case 7) had mild thrombocytopenia at initial examination, but marked thrombocytopenia developed secondary to immune thrombocytopenic purpura during the initial clinical workup.

Morphologic Findings in Peripheral Blood, Bone Marrow, and Spleen

Review of the CBC and peripheral blood smears showed that only 1 patient (case 1) had absolute lymphocytosis (lymphocyte count, 5,800/μL [5.8 × 10^9/L]) composed predominantly of LGLs (3.7 × 10^9/L) with prominent cytoplasmic azurophilic granules. The remaining 6 patients had no absolute increase in total lymphocyte count or the number of LGL. In fact, 3 patients had absolute lymphopenia and 3 additional patients had a lymphocyte count near the lower limit of the normal reference range (1,000/μL [1.0 × 10^9/L]). Unlike the LGLs in the peripheral blood in cases 1 and 7, the occasional LGLs identified in the remaining 5 cases were relatively small and the cytoplasmic granules were less prominent.

All 7 cases showed bone marrow involvement by the neoplastic T cells. The patterns of the infiltration were

<table>
<thead>
<tr>
<th>Case No.</th>
<th>1</th>
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<td>(splenectomy)</td>
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<td>30</td>
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<td>MTX; FCR</td>
<td>PSE</td>
<td>MTX; PSE</td>
<td>MTX; PSE</td>
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<tr>
<td>Follow-up (mo)</td>
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<td>77</td>
<td>22</td>
<td>120</td>
<td>32</td>
<td>25</td>
<td>6</td>
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</table>

AIHA, autoimmune hemolytic anemia; ANA, antinuclear antibody; BM, bone marrow; CYPH, cyclophosphamide; DCF, pentostatin; FCR, fludarabine; FISH, fluorescence in situ hybridization; ITP, immune thrombocytopenic purpura; LGLs, large granular lymphocytes; MTX, methotrexate; NA, not available; PB, peripheral blood; PRCA, pure red cell aplasia; PSE, prednisone; RA, rheumatoid arthritis; TCR, T-cell receptor; T-LGL, T-cell large granular lymphocytic.

* Mild thrombocytopenia was present at diagnosis, but marked thrombocytopenia developed secondary to ITP during the initial diagnostic workup.
mainly interstitial and, to a lesser extent, intrasinusoidal. The neoplastic infiltration was difficult to recognize on routine H&E-stained sections in 6 of 7 cases but was well highlighted by immunohistochemical staining for CD3 or TIA-1. Intrasinusoidal involvement was present in all 7 cases and was characterized by a linear array of CD3+ or TIA-1+ lymphocytes within the sinuses. Octanoid aggregates were also present in 2 cases, but they were composed of nonneoplastic CD4+ T cells with occasional CD20+ B cells. The extent of bone marrow involvement was 20% to 40% in 4 cases and slightly less than 20% in the remaining 3 cases. In addition, 2 cases (cases 2 and 4) with severe anemia were found to have PRCA in the bone marrow biopsy specimens.

Splenectomy specimens from 3 patients (cases 3, 4, and 6) showed significant expansion of the red pulp by the neoplastic T cells; the white pulp was unremarkable. The infiltration was predominantly localized in the splenic cords, but focal intrasinusoidal infiltration was also present in all 3 cases. The lymphocytes were small with dense chromatin, and no significant cytologic atypia was identified. These lymphocytes were strongly positive for CD3 and TIA-1 by immunohistochemical staining.

**Immunophenotype**

The immunophenotype of the neoplastic T cells was determined by flow cytometric and immunohistochemical studies in 5 cases (cases 1, 2, 4, 5, and 7) and by immunohistochemical studies in 2 (cases 3 and 6). The results are summarized in Table 2.

All 7 cases were positive for the pan–T-cell antigens, CD3, CD2, and CD7, but negative for CD5 (cases 3-7) or only partially dim positive for CD5 (cases 1 and 2). All 7 cases were negative for CD4 and CD8. TCRαβ and TCRγδ were analyzed by flow cytometry in 5 cases, and all were positive for γδ but negative for αβ. The remaining 2 cases (cases 3 and 6) were analyzed by immunohistochemical studies and were positive for TCRγ and negative for βF1, a marker for TCRβ.

T and NK cell–associated antigens, CD57, CD56, and CD16, were also examined. CD57 was positive in 2 of 6 cases, CD56 in 2 of 7 cases, and CD16 in 2 of 4 cases. The cytotoxic granule–associated proteins, TIA-1 and granzyme B, were positive in 7 of 7 and 4 of 6 cases, respectively. The expression of CD158, a member of the KIR family, was assessed by flow cytometry in 3 cases (cases 1, 2, and 7). One case (case 2) showed exclusive expression of a single KIR antigen (CD158b) in the CD4−/CD8− γδ T cells, which is immunophenotypic evidence for a clonal T-cell proliferation. Another case (case 7) demonstrated aberrant coexpression of 2 KIR antigens (CD138a and CD138e) with uniform intensities in the same cell population. The remaining case (case 1) showed absent expression of all 3 KIR antigens, which overlaps with normal T cells. The results of flow cytometric immunophenotyping of a representative case (case 4) are shown in Image 4I.
Molecular, Cytogenetic, and FISH Data

Polymerase chain reaction for TCR gene rearrangement was performed on all 7 cases and all showed a clonal TCR gene rearrangement (Table 1). Owing to the overlapping immunophenotype between CD4–/CD8– γδ T-LGL leukemia and hepatosplenic T-cell lymphoma (HSTCL), cytogenetic studies and FISH analysis for isochromosome 7q (i7q), a common genetic abnormality in HSTCL, was performed. None of the 6 cases analyzed had evidence of i7q based on cytogenetic and/or FISH results (Table 1).

Therapy and Outcome

The clinical follow-up of patients ranged from 6 months to 10 years. All 7 patients required treatment before, at the time of, or within 12 months of diagnosis (Table 1). Three patients underwent splenectomy (cases 3, 4, and 6) for severe AIHA or progressive splenomegaly and cytopenia before the diagnosis of T-LGL leukemia.

Five patients were initially treated with methotrexate as a single agent (cases 1, 3, and 7) or in combination with prednisone (cases 5 and 6; Table 1). One patient...
Image 3I (Case 4) Splenic involvement by CD4−/CD8− γδ T-cell large granular lymphocytic (T-LGL) leukemia (minimal therapy and 10-year follow-up). A, The red pulp was expanded by the neoplastic cells (H&E, ×200). B, The neoplastic cells in the red pulp were positive for CD3; the lymphocytes in the white pulp (left lower corner) were negative (×200). C, The white pulp was composed of CD20+ B cells (×200). D, Focal intrasinusoidal infiltration of the neoplastic cells (arrows) (×600). E, The neoplastic cells in the sinusoid were positive for CD3 (arrows) (×600). F, The neoplastic cells in the sinusoid were positive for TIA-1 (arrows) (×600).
(case 1) received methotrexate therapy owing to progressive neutropenia and splenomegaly after a 10-month observation, and 1 patient (case 7) began treatment at the time of diagnosis. The severe neutropenia in case 1 resolved after 3 weeks of low-dose methotrexate therapy, but neutropenia and thrombocytopenia continued in case 7 after 4 weeks of treatment. Another 2 of the 5 patients (cases 3 and 5) had a partial response to the initial treatment, and their therapy was changed to cyclophosphamide and prednisone (case 5) or fludarabine (case 3). The remaining patient (case 6) was treated with methotrexate and prednisone for cytopenia, splenomegaly, and B symptoms before T-LGL leukemia was diagnosed. His symptoms improved; however, a splenectomy was performed 8 months later for progressive splenomegaly and cytopenia before the diagnosis of T-LGL leukemia was made.

Of the 2 patients not initially treated with methotrexate, 1 (case 2) was treated with pentostatin and cyclophosphamide owing to relatively advanced disease clinically and pathologically (severe AIHA, PRCA, and 40% bone marrow

**Table 2**

Immunophenotype in Seven Cases of CD4−/CD8− γδ T-LGL Leukemia*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>1</th>
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<tbody>
<tr>
<td>CD3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>CD8</td>
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<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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<td>–</td>
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<td>Granzyme B</td>
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</table>

KIR, killer cell immunoglobulin-like receptors; NA, not available; T-LGL, T-cell large granular lymphocytic; +, positive; –, negative.

* The immunophenotype was determined by immunohistochemical analysis in 2 cases (cases 3 and 6) and by flow cytometry and immunohistochemical analysis in the remaining 5 cases.

**Image 4** (Case 4) Flow cytometric immunophenotyping of CD4−/CD8− γδ T-cell large granular lymphocytic (T-LGL) leukemia. A distinct CD4−/CD8− T cell population (red) was detected and was CD3+, CD2+, CD7+, and CD5−. These cells were positive for T-cell receptor (TCR)γδ, but negative for TCRαβ. Blue indicates normal αβ T cells.
involvement). The PRCA resolved, and the patient has been in clinical remission. The other patient (case 4) had severe AIHA that was treated with prednisone and splenectomy. His severe anemia improved, and he achieved transfusion-independence. The long-term follow-up of this patient showed no significant disease progression. He died of cardiovascular disease 10 years after the diagnosis of T-LGL leukemia. The remaining 6 patients are all alive with no evidence of significant disease progression.

**Comparison of Clinical and Laboratory Features of CD4–/CD8– γδ T-LGL Leukemia With Features Reported in γδ T-LGL Leukemia and αβ T-LGL Leukemia**

The clinical and laboratory features in the 7 cases CD4–/CD8– γδ T-LGL leukemia were compared with the features of γδ T-LGL leukemia reported in the 2 largest series of γδ T-LGL leukemia (mostly CD8+) in the literature and the features of αβ T-LGL leukemia reported by Loughran. The data are summarized in Table 3.

The cases of CD4–/CD8– γδ T-LGL leukemia in our study showed several clinical features similar to cases of αβ T-LGL leukemia and γδ T-LGL leukemia that are primarily CD8+. It preferentially affected older people and usually manifested with neutropenia (5/7 [71%]), anemia (4/7 [57%]), and splenomegaly (6/7 [86%]) but not lymphadenopathy (0/7 [0%]). It was often associated with autoimmune disorders (7/7 [100%]) including rheumatoid arthritis (2/7 [29%]), AIHA (3/7 [43%]), and PRCA (2/7 [29%]). However, the incidences of AIHA (43%) and PRCA (29%) were higher compared with γδ T-LGL leukemia (mostly CD8+; 5% and 6%, respectively), and the incidence of PRCA was also higher than in αβ T-LGL leukemia (7%) as reported by Dhodapkar et al.5

A significant difference in the numbers of LGLs in the peripheral blood was also noted. As shown in Table 3, in the vast majority of cases of αβ (89/89 [100%]) and γδ T-LGL leukemia (42/44 [95%]), the LGLs were at least 0.4 × 10⁹/L, the cutoff defined in this study for increased LGLs in the peripheral blood. In 25 (57%) of 44 γδ T-LGL leukemia cases (mostly CD8+), the LGLs were at least 2.0 × 10⁹/L, the count indicated by the current WHO as frequently associated with a clonal proliferation. However, only 1 of the 7 cases of the CD4–/CD8– subtype of γδ T-LGL leukemia in our study had increased LGLs (≥0.4 × 10⁹/L) in the peripheral blood.

**Discussion**

T-LGL leukemias most frequently arise from αβ T cells (95%), with only a small subgroup (~5%) from γδ T cells. It is interesting that unlike the normal γδ T cells that...
are predominantly CD4−/CD8−, the γδ T-LGL leukemias reported so far are mostly CD8+.8,9 A rare subgroup of γδ T-LGL leukemia with a CD4−/CD8− phenotype has been recognized, but the data are limited, with only approximately 30 cases reported in the literature as case reports or as a small subset included with a larger group of CD8+ γδ T-LGL leukemias.8,17

The present study evaluated 7 cases of CD4−/CD8− γδ T-LGL leukemia identified in our institution between 1992 and 2010. Although the number of cases is small, our study represents the largest and most extensively studied series of CD4−/CD8− γδ T-LGL leukemia from a single institution. Our data showed that similar to other types of T-LGL leukemia reported in the literature, CD4−/CD8− γδ T-LGL leukemia preferentially affects older people, is frequently associated with autoimmune disorders, and often manifests with neutropenia, anemia, and splenomegaly. However, the incidences of AIHA (43%) and PRCA (29%) in CD4−/CD8− T-LGL leukemia observed in our study are much higher than reported in γδ T-LGL leukemia (primarily CD8+; 5% and 6%, respectively),8,9 and the incidence of PRCA (29%) was also higher than in αβ T-LGL leukemia as reported by Dhodapkar et al (7%).3 In fact, in 2 of the 3 cases in our series, splenectomy was done for transfusion-dependent AIHA before T-LGL leukemia was recognized. However, the higher incidences of AIHA and PRCA observed in our study need to be confirmed by accumulation of data from a larger number of cases of CD4−/CD8− γδ T-LGL leukemia.

Of importance, one of the most striking features of CD4−/CD8− γδ T-LGL leukemia observed in our study was the lack of lymphocytosis (total lymphocyte count, >4,000/μL [4.0 × 10⁹/L]) or increased LGLs (LGL count, ≥0.4 × 10⁹/L) in the peripheral blood in the majority of cases (6/7) despite prominent bone marrow or splenic involvement. In the bone marrow, CD4−/CD8− γδ T-LGL leukemia shows predominantly interstitial and, to a lesser extent, intrasinusoidal infiltration similar to that of the common αβ T-LGL leukemia as described by Morice et al.19 The neoplastic infiltrate could be difficult to detect on routine H&E-stained sections; the presence of a distinct CD4−/CD8− T-cell population detected by flow cytometry often serves as an initial alert and prompts further evaluation. Immunostaining for CD3 and/or TIA-1 is helpful in highlighting the abnormal lymphoid infiltrate, particularly the linear pattern of intrasinusoidal infiltration.

The frequent absence of increased LGLs in the peripheral blood and the relative subtlety of the neoplastic infiltrate in H&E-stained bone marrow sections owing to the interstitial and intrasinusoidal infiltration may cause difficulty in early recognition of this subgroup of T-LGL leukemia. In our series, in only 1 of 7 cases was the disease initially suspected based on the examination of the peripheral blood smear that showed LGL lymphocytosis. The remaining 6 cases had absolute lymphopenia or a low normal lymphocyte count with no increase in LGLs. In 4 of 6 cases, the initial workups, including bone marrow biopsies at the referring institutions, were all reported as unremarkable. The accurate diagnosis of CD4−/CD8− γδ T-LGL leukemia was delayed from 1 to 4 years in 4 of 7 cases.

Splenectomy involvement by T-LGL leukemia has been described and characterized by expansion of the red pulp and sinusoids by neoplastic T cells with sparing of the white pulp.20,21 The splenectomy specimens available in 3 of our cases of CD4−/CD8− γδ T-LGL leukemia demonstrated marked expansion of the red pulp by neoplastic T cells. Although the cordal infiltration is the main pattern of involvement, intrasinusoidal infiltration, a common finding in HSTCL, was also present focally. Because CD4−/CD8− γδ T-LGL leukemia and HSTCL share a similar immunophenotype, ie, CD3+, CD4−, CD8−, CD5−, and TCRγδ+, and certain overlapping infiltrative patterns in the spleen, distinction between the 2 diseases could be difficult in some cases. In fact, 2 of the 7 cases in our series were initially misdiagnosed as HSTCL on the splenectomy specimens. However, CD4−/CD8− γδ T-LGL leukemia may be readily differentiated from HSTCL once this rare variant of T-LGL leukemia is considered in the differential diagnosis.

Morphologically, HSTCL typically shows medium-sized lymphocytes with a less mature chromatin pattern resembling blasts,1,22 whereas the neoplastic T cells of CD4−/CD8− γδ T-LGL leukemia exhibit mature lymphocyte morphologic features. The preponderant pattern of splenic and bone marrow involvement of HSTCL is intrasinusoidal, while CD4−/CD8− γδ T-LGL leukemia, as demonstrated in our study, shows predominant cordal infiltration in the spleen and interstitial spread in the bone marrow with significantly less prominent intrasinusoidal involvement. The intrasinusoidal infiltration of CD4−/CD8− γδ T-LGL leukemia in the bone marrow is manifested as a linear array rather than expansion of the sinusoids by the neoplastic T cells. Molecular and/or cytogenetic studies may be helpful in difficult cases. In our study, 6 of 6 cases were negative for i7q, a common genetic abnormality in HSTCL.

It has been reported that the majority of T-LGL leukemias, including the γδ subtype, are CD3+/CD8+/CD57+.1,8,9 The expression of CD57 has been suggested as a specific feature helpful in the differentiation of T-LGL leukemia from other aggressive γδ T-cell malignancies, including HSTCL.17 However, our study showed that the expression of CD16, CD56, and CD57 in CD4−/CD8− γδ T-LGL leukemia was variable, and in the case of CD57, 4 of 6 cases were negative.

T-LGL leukemia is generally a chronic disease with a 10-year survival of more than 80%, and approximately 30% to 50% of patients do not require therapy.5,8,9 Spontaneous regression of the disease has been reported.10,23 Patients...
often respond to treatment with methotrexate, cyclosporin, or cyclophosphamide, as a single agent or in combination with prednisone. Splenectomy has also been found to be an effective therapeutic option in certain cases. Our follow-up data indicate that similar to the common type of T-LGL leukemia, CD4–/CD8– γδ T-LGL leukemias behave in an indolent manner. However, treatment was required in all 7 cases in our study before, at the time of, or within 12 months after diagnosis owing to severe hemolysis, progressive splenomegaly, or severe cytopenias. Of the 7 cases, 5 responded to immunomodulatory agents, but in 2 cases, cytotoxic chemotherapy was required owing to disease progression or relatively advanced disease at diagnosis. At last follow-up, 6 of 7 patients were alive with no evidence of significant disease progression, and 1 died of unrelated disease 10 years after the diagnosis of T-LGL leukemia.

The clinical course and outcome of our patients indicate that CD4–/CD8– γδ T-LGL leukemia has overall indolent behavior, but it seems to be relatively more aggressive than the common type T-LGL leukemia such that treatment is often required. This finding and the higher incidences of AIHA and PRCA observed in CD4–/CD8– γδ T-LGL leukemia in our study may be related to the higher cytotoxic activity observed in the CD5– subset of normal γδ T cells than in the CD5+ subset. It has been reported that T-LGL leukemia with CD56 expression has a more aggressive clinical course and may represent a distinct entity in the spectrum of LGL disorders. However, this is controversial. In our study, CD56 expression was detected in 2 of 7 cases; neither of the patients had a more aggressive clinical course than the other patients.

It is also worth mentioning that similar to other T LGLs, an expansion of CD4+/CD8– γδ T cells in the peripheral blood can be seen in various reactive conditions, a significant proportion of which are CD5– (data not shown). Therefore, the lack of CD5 expression, an immunophenotypic feature often associated with a neoplastic proliferation in αβ T cells, does not necessarily indicate immunophenotypic aberrancy in γδ T cells because a subset of normal γδ T cells does not express CD5.

Our study demonstrated that CD4–/CD8– γδ T-LGL leukemia exhibits several clinical and morphologic features similar to the more common types of T-LGL leukemia; however, the incidences of AIHA and PRCA in CD4–/CD8– γδ T-LGL leukemia are higher. Another striking feature observed in our study is the lack of lymphocytosis or increased LGLs in the peripheral blood in the majority of cases despite prominent bone marrow or splenic involvement, making early recognition of the disease difficult. CD4–/CD8– γδ T-LGL leukemia displays an immunophenotype and pattern of splenic involvement overlapping with HSTCL, which may lead to misdiagnosis. Clinically, CD4–/CD8– γδ T-LGL leukemia has an overall indolent course but seems to be relatively more aggressive than common T-LGL leukemia such that treatment is often required. Awareness of the clinical, morphologic, and laboratory features of this rare subgroup of γδ T-LGL leukemia is important for early recognition and accurate diagnosis of the disease.

References


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