CD3+, CD56+ Aggressive Variant of Large Granular Lymphocyte Leukemia

By Teresa C. Gentile, Aysegul H. Uner, Robert E. Hutchison, Jonathan Wright, Jonathan Ben-Ezra, E. Clifton Russell, and Thomas P. Loughran, Jr

Clonal expansions of CD3+ large granular lymphocytes (LGL) have been classified as T-LGL leukemia. The majority of patients with T-LGL leukemia have a chronic disease (years) manifested often by severe neutropenia, rheumatoid arthritis, and mild-to-moderate splenomegaly. The characteristic phenotype of the leukemic LGL is CD3+, CD8+, CD16+, CD57+, and CD56+. In this report we describe an aggressive variant of T-LGL leukemia in which leukemic LGL also expressed CD56, as identified by two-color flow-cytometry analysis. In contrast to the chronic nature typical of T-LGL leukemia, these patients presented with a severe systemic illness that was rapidly progressive and resistant to treatment. Atypical clinical features included rapidly increasing spleen size to massive proportions, extensive lymphadenopathy, and the presence of B symptoms (fever, night sweats, weight loss). Hematologic and pathologic features were also unusual for T-LGL leukemia. These patients had very high LGL counts at diagnosis (range 11,692 to 26,312 μL), which increased rapidly despite treatment. Histopathologic examination of splenic sections showed extensive infiltration of red pulp cords and sinuses by leukemic cells with atrophy of the white pulp. These clinicopathologic features are similar to those described for patients with natural killer cell (NK)-LGL leukemia, whose cells are also CD56+. However, unlike NK-LGL leukemia, we could not show a direct pathogenic role for Epstein-Barr virus (EBV), as Southern-blot analyses using an EBV-joined termini probe were negative in these patients. Our findings suggest that CD3+, CD56+ LGL leukemia is a distinct clinicopathologic entity separate from the usual CD3-, CD56- T-LGL leukemia. The expression on leukemic LGL of CD56, an adhesion molecule, may determine the aggressive biologic nature of this newly described disease.

© 1994 by The American Society of Hematology.

CASE REPORTS

Patient no. 1. A 14-year-old girl, originally from Jordan, presented in May 1992 with massive splenomegaly, adenopathy (most notably in the cervical and axillary areas), and fever. Workup for infection was unrevealing and she subsequently underwent excisional biopsy of a lymph node for diagnosis and elective splenectomy for severe abdominal pain. The lymph node showed mostly mature-appearing lymphocytes and a provisional diagnosis of lymphoma was made. DNA flow cytometry of the spleen showed a diploid cell population, and cell cycle analysis showed 15% S phase. Bone marrow examination at that time showed about 50% lymphocytes similar to those seen in the lymph node. Unstimulated cultures of bone marrow showed a normal karyotype. Approximately 3 weeks after splenectomy, she developed rapidly enlarging cervical lymph nodes, a peripheral white blood cell (WBC) count of 64,300 with 79% lymphocytes, and worsening anemia and thrombocytopenia. Repeat bone marrow showed 65% small, mature-appearing lymphocytes.

Immunophenotyping and TCR gene rearrangement studies were subsequently performed on her peripheral blood mononuclear cells and led to the diagnosis of LGL leukemia. Immunophenotypic abnormalities including polyclonal hypergammaglobulinemia, circulating immune complexes, and positive tests for rheumatoid factor, antinuclear antibodies, antineutrophil antibodies, and antiplatelet antibodies.

In contrast, manifestations of systemic disease are often the initial clinical finding in NK-LGL leukemia with fever without signs of infection, night sweats, and weight loss are common. Most patients with NK-LGL leukemia die of disseminated disease within a few months of presentation, despite therapy. Rheumatoid arthritis has for the most part not been evaluated.

In this report we describe three patients with clonal CD3+ LGL leukemia whose leukemic cells also co-expressed CD56, a marker usually found in CD3- LGL leukemia. Despite expressing CD3 and showing clonal TCR gene rearrangements typical of patients with T-LGL leukemia, the clinicopathologic features of these patients were similar to those of NK-LGL leukemia.

From the Departments of Medicine, Microbiology and Immunology, and Pathology, Health Science Center, State University of New York at Syracuse; Department of Veterans Affairs Medical Center, Syracuse, NY; and the Departments of Pediatrics and Pathology, Medical College of Virginia, Richmond.

Submitted March 11, 1994; accepted June 9, 1994.

Supported by Grants No. CA46930 and CA54352 awarded by the National Cancer Institute, National Institutes of Health.

Address reprint requests to Teresa C. Gentile, MD, PhD, Veterans Affairs Medical Center, 800 Irving Ave, Syracuse, NY 13210.

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.

© 1994 by The American Society of Hematology.

Blood, Vol 84, No 7 (October 1), 1994: pp 2315-2321
globulins showed normal IgG, IgM, and IgA concentrations. Serum protein electrophoresis (SPEP) was normal. Over the next 7 to 8 months she was treated with varying doses of prednisone, vincristine, methotrexate, and 6-mercaptopurine, with temporary disease control. In January 1993 she again had rapid progression of her disease as evidenced by dramatic increases in the size of axillary and cervical lymph nodes. She was treated with induction chemotherapy similar to that administered for childhood acute lymphoblastic leukemia (vincristine, prednisone, Adriamycin, L-asparaginase) followed by five cycles of consolidation therapy with intermediate-dose methotrexate and cytosome arabinoside. Her disease control was again temporary and she has recently had increasing adenopathy. She is currently undergoing conditioning for allogeneic bone marrow transplantation.

**Patient no. 2.** A 52-year-old white man presented to his physician in July 1993 complaining of a cough and postnasal drip. Examination at that time showed an enlarged spleen (palpated 11 to 12 cm below the left costal margin) and an elevated WBC count. On further questioning the patient reported a 20-pound weight loss over the prior 2 months and some mild sweating episodes. Bone marrow examination showed a hypercellular marrow (70% to 80%) with markedly increased numbers of lymphoid cells. Unstimulated cultures of bone marrow showed a normal karyotype. Immunophenotyping and T-cell gene rearrangement studies led to the diagnosis of LGL leukemia. RF and ANA tests were negative. Serum immunoglobulins were mildly decreased (IgG: 551 mg/dL, normal 591 to 1965 mg/dL; IgM: 60 mg/dL, normal 77 to 400 mg/dL; IgA: 44 mg/dL, normal 50 to 311 mg/dL). SPEP showed an increased alpha fraction at 6.0% (normal 1.9% to 4.5%). Over the next month the patient continued to lose weight and complained of progressively increasing severe left upper quadrant and left shoulder pain. Because of his worsening symptoms, therapy was instituted and he subsequently received 3 courses of cytotoxan 1,400 mg, Adriamycin 95 mg, vinorestine 2 mg, and prednisone 100 mg (CHOP) every day for 5 days, resulting in a decrease in his peripheral WBC count but no significant improvement in his splenomegaly. In November 1993 he underwent elective splenectomy. Cell-cycle analysis showed a G0 phase of 5.5%. Markedly increased numbers of LGL (in the range of 70,000 to 100,000/µL) with unusually prominent nucleoli, were noted post-splenectomy. Thrombocytopenia did not improve. High-dose cytotoxan (4.5 g/m²) was administered, and he is being evaluated for HLA-identical allogeneic bone marrow transplantation.

**Patient no. 3.** A 41-year-old Brazilian man was noted to have an enlarged spleen (palpated 15 cm below the left costal margin) during a routine physical exam in September 1992. Subsequent laboratory evaluation showed an elevated WBC count with 80% lymphocytes, the majority of which had the morphology of LGL. Immunophenotyping of peripheral blood and TCR gene rearrangement studies confirmed the diagnosis of LGL leukemia. The RF test was positive at a titer of 1:320, and ANA was negative. Quantitative immunoglobulins and SPEP were within normal limits. Over the next 3 months he developed systemic symptoms of low-grade fever and night sweats as well as increasing abdominal pain from an enlarging spleen and splenic infarctions. In January he was treated with daily oral cytotoxan which decreased his peripheral WBC count (from 32,000 to 18,000/µL) but did not much improve his splenomegaly. Subsequently he was treated with chloroethoxydatum (2-CdA), again with little improvement in the symptomatology caused by enlarging spleen. In March 1993 he underwent splenectomy, which was complicated by sepsis, and led to his eventual death.

**MATERIALS AND METHODS**

**Cell phenotyping and separation.** Peripheral blood mononuclear cells (PBMCs) were first isolated from whole blood using Ficoll-Hypaque density gradient centrifugation (Phar迈, Swedesboro, NJ). These cells were subsequently analyzed for the presence of cell-surface antigens using FACSscan (Becton-Dickinson, Mountainview, CA) and a panel of monoclonal antibodies (MoAbs) directly conjugated with either fluorescein isothiocyanate or phycoerythrin. These MoAbs included PE-leu 4 (CD3 specific; Becton-Dickinson), FITC-T3 (CD3 specific; Olympic, Lake Success, NY), FITC-leu 3 (CD4 specific; Becton-Dickinson), FITC-leu 2 (CD6 specific; Becton-Dickinson), FITC-leu 11 (CD16 specific; Becton-Dickinson), FITC-leu 7 (CD57 specific; Becton-Dickinson) and PE-leu 19 (CD56 specific; Becton-Dickinson). Data were collected and analyzed using Consort 30 software (Becton-Dickinson), gating on leukocytes using Leukogate (Becton-Dickinson). Results are reported as percentage positive based on threshold values of fluorescence determined using suitable isotype-specific control antibodies (Becton-Dickinson or Olympic).

**Histopathology and Immunopathology.** Four micron sections were cut and mounted on Probe-on Plus (Fisher Scientific, Pittsburg, PA) slides for immunohistochemical staining. One slide was stained with hematoxylin and eosin for histopathology. The antibody panel used was a modification of a panel shown to identify lymphocyte lineage with approximately 95% accuracy. Antibodies included leukocyte common antigen (LCA, CD45; DAKO, Carpentryera,CA), L26 (CD20; DAKO), 4KB5 (CD45R; DAKO), MB2 (Biostest, Swedesboro, NJ), UCHL-1 (CD45Rc; DAKO), CD3 (DAKO), MT1 (CD43; Biostest), Ber-H2 (CD30; Biostest), CD7 (Becton-Dickinson), CD1 (Becton-Dickinson), and CD22 (DAKO) were applied to frozen sections. Each antibody was applied at optimal dilutions followed by application of a biotinylated secondary antibody. Alkaline phosphatase–conjugated streptavidin was then reacted with the biotinylated secondary antibody and the complex detected using fast red TR. Counterstaining was with hematoxylin followed by a polymeric covering. Each reagent was applied in uniform volume by capillary action to paired slides on a code-on immunostainer (Fisher Scientific, Rochester, NY). Appropriate blocking agents, washes, and enzyme treatments were integrated. Reactions were scored by light microscopy.

**Clonal analyses.** High molecular weight DNA was isolated by subjecting cells to lysis and digestion in 1% sodium dodecyl sulfate and 200 µg/mL of Proteinase K (BRL Life Technologies, Gaithersburg, MD) and incubating overnight at 37°C. DNA was extracted with Tris-saturated phenol (0.5 mol/L Tris, pH 7.5) followed by chloroform:isoamyl alcohol (24:1) and then precipitated in 0.3 mol/L sodium acetate and 2 mol of 95% ethanol. DNA was next digested with the restriction enzymes BamHI (Onco, Gaithersburg, MD), BglII, and AgeI.

**Table 1. Clinical Features of Patients With CD3*, CD56+ LGL Leukemia.**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age at diagnosis (yrs)</th>
<th>Sex</th>
<th>Disease duration (mos)</th>
<th>Recurrent infections</th>
<th>Rheumatoid arthritis</th>
<th>Systemic B symptoms</th>
<th>Massive splenomegaly (g)†</th>
<th>Lymphadenopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>F</td>
<td>19</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>52</td>
<td>M</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>41</td>
<td>M</td>
<td>8*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

† Normal spleen weight <250 g.
CD3+, CD56+ LGL LEUKEMIA

Table 2. Hematologic Features of Patients With CD3+, CD56+ LGL Leukemia

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes/µL</td>
<td>14,800</td>
<td>29,900</td>
<td>19,000</td>
</tr>
<tr>
<td>LGL/µL</td>
<td>11,692</td>
<td>26,312</td>
<td>15,200</td>
</tr>
<tr>
<td>Neutrophils/µL</td>
<td>2,368</td>
<td>2,891</td>
<td>3,800</td>
</tr>
<tr>
<td>Hematocrit %</td>
<td>30.8</td>
<td>47.8</td>
<td>35.1</td>
</tr>
<tr>
<td>Platelets (1 × 10^12/µL)</td>
<td>97</td>
<td>101</td>
<td>208</td>
</tr>
</tbody>
</table>

At presentation:

Within 3 mos of presentation:

Table 2. An absolute lymphocytosis was present in all patients.

Table 3. One- and Two-Color Flow Cytometry Analysis of PBMC

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Patient No.</th>
<th>% Positive</th>
<th>Normals</th>
<th>Absolute No./µL</th>
<th>Normals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>1</td>
<td>94</td>
<td>99</td>
<td>74 + 6</td>
<td>10,990</td>
</tr>
<tr>
<td>CD4</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>47 + 4</td>
<td>935</td>
</tr>
<tr>
<td>CD8</td>
<td>77</td>
<td>93</td>
<td>85</td>
<td>25 + 5</td>
<td>9,003</td>
</tr>
<tr>
<td>CD16</td>
<td>3</td>
<td>66</td>
<td>88</td>
<td>9 + 4</td>
<td>351</td>
</tr>
<tr>
<td>CD57</td>
<td>6</td>
<td>0</td>
<td>40</td>
<td>13 + 6</td>
<td>701</td>
</tr>
<tr>
<td>CD3+CD16+</td>
<td>ND</td>
<td>66</td>
<td>89</td>
<td>1 + 1</td>
<td>ND</td>
</tr>
<tr>
<td>CD3+CD56+</td>
<td>45*</td>
<td>94</td>
<td>95</td>
<td>2 + 1</td>
<td>1,439*</td>
</tr>
<tr>
<td>CD3+CD57+</td>
<td>ND</td>
<td>0</td>
<td>41</td>
<td>10 + 4</td>
<td>ND</td>
</tr>
</tbody>
</table>

Normals values are mean ± SD of 10 normal subjects.

Abbreviation: ND, not done.

* Value obtained after treatment.

RESULTS

Table 1 summarizes the clinical features of the patients. The acute, aggressive nature of the disease is evidenced by the presentation with systemic B symptoms, massive splenomegaly, extensive lymphadenopathy, and resistance to therapy. Features also atypical for T-LGL leukemia include absence of recurrent infections or rheumatoid arthritis.

The hematologic profiles of the patients are outlined in Table 2. An absolute lymphocytosis was present in all patients at presentation, with relatively high numbers of LGL compared with values typically observed in patients with T-LGL leukemia (median 4200/µL). Of note, there were rapid increases (within 3 months) in circulating LGL to high levels in all patients. Such a finding is not observed in T-LGL leukemia but is typical for NK-LGL leukemia. None of the patients was neutropenic. All patients had moderate degrees of thrombocytopenia, either at presentation or with disease progression.

Leukemic cells in all three patients coexpressed CD3 and CD56 (Table 3). In patient no. 1, the percentage of double-labeled CD3+CD56+ cells was lower; however, this analysis was not done on presentation but after extensive treatment with multiple chemotherapeutic agents. It is reasonable to assume that this value may have been higher at presentation because decreases in circulating LGL were seen, at least transiently, as a consequence of treatment. Leukemic cells were also CD8+. Variable expression of CD16 and CD57 were noted on leukemic LGL.

Characteristic histopathologic features of CD3+, CD56+ T-LGL leukemia are shown in Fig 1. Massive splenomegaly was a prominent feature of the disease in each patient. Spleen weights ranged from 1,700 to 2,900 g, with normal being less than 250 to 300 g. Histologically, each spleen specimen showed extensive, diffuse infiltration of the splenic red pulp cords and sinuses by lymphoid cells (Fig 1c and d). These findings are similar to those seen in typical T-LGL leukemia. Variable degrees of atrophy of the white pulp were also noted in these patients, in contrast to follicular hyperplasia usually noted in T-LGL leukemia. Immunoperoxidase staining of frozen section from each spleen confirmed that infiltrating lymphoid cells had the same phenotype as circulating LGL (not shown).

DNA hybridization studies showed clonal rearrangement of TCR β gene in each patient (Fig 2). There was no hybridization with the NIH6 probe (not shown), indicating that the clonal expansion of LGL was not related to Epstein-Barr virus (EBV) infection. Using PCR analysis we were able to detect EBV sequences in PBMC from only one of these patients, at a low copy number. Subsequent testing by in situ hybridization showed only occasional positive cells consistent with a background of previous EBV infection.

From www.bloodjournal.org by guest on November 16, 2017. For personal use only.
Serologic reactivity to human T-cell leukemia virus I/II antigens was tested in all three patients. Serum from each patient was enzyme-linked immunosorbent assay negative and Western-blot indeterminant, similar to previously published observations in patients with T-LGL leukemia.

**DISCUSSION**

We describe a new disease, CD3+, CD56− LGL leukemia, which is an aggressive variant of T-LGL leukemia. The characteristic features of this disorder include clonally expanded CD3+, CD56− LGL that increase rapidly after diagnosis, systemic B symptoms (fever, night sweats, weight loss), and massive splenomegaly. Moderate thrombocytopenia and lymphadenopathy are also observed. Common features of T-LGL leukemia such as neutropenia, recurrent bacterial infections, and rheumatoid arthritis did not occur in the CD3+, CD56− variant. Indeed, the clinical presentation of this disease is unlike the usual clinical course of T-LGL leukemia, but remarkably similar to features of NK-LGL leukemia.

The cellular origin of the malignant LGL clone in our
patients is not known. CD56 is expressed on approximately 15% of normal PBMC, which have LGL morphology and mediate non-major histocompatibility complex-restricted cytotoxicity. The majority of these cells are NK cells, i.e. CD3⁻, CD56⁺. However, a small percentage of normal PBMC coexpress CD3 and CD56.²² It is likely that these LGL represent the normal counterparts of the leukemic LGL. Alternatively, it is conceivable that leukemic cells arose from normal T cells (CD3⁺, CD56⁻), with upregulation of CD56 after activation. Supporting this hypothesis, marked expansion of CD3⁺, CD56⁺ cells can be generated in vitro from normal CD3⁺, CD56⁻ T-cell precursors after activation with interleukin (IL)-1, IL-2, and interferon γ.²³

Our data suggest that expression of CD56 is an important biological marker in LGL leukemia. LGL leukemias expressing CD56, whether of NK (CD3⁻) or T-cell (CD3⁺) origin, have similar aggressive clinical features. In contrast, T-LGL leukemias not expressing CD56 have different manifestations and a chronic clinical course. A relationship between NK immunophenotype and biological behavior has been previously reported. Sheibani et al²⁴ observed that lymphoblastic lymphomas expressing NK-associated antigens showed...
Figure 2. Southern-blot analysis using 32-P-labeled TCR β probe. Lane 1: DNA from patient no. 2 digested with BamHI restriction enzyme. Lane 2: DNA from patient no. 2 digested with HindIII restriction enzyme. Lane 4: DNA from patient no. 2 digested with HindIII restriction enzyme. Similar results were obtained for patients no. 1 and 3 (not shown).

Molecular analyses of EBV-fused termini in CD56 expressing NK-LGL leukemias and AIL have shown that viral episomes have a uniform configuration. These data support a clonal process that implicates EBV directly in the pathogenesis of both these diseases. In contrast, such analyses were negative in our patients. Furthermore, after PCR amplification we detected EBV sequences in only one patient, at a low copy number. These results do not support a role for EBV infection in CD3ε, CD56ε LGL leukemia. Further studies are needed to elucidate the pathogenesis of this newly delineated disease.

REFERENCES


CD3+, CD56+ aggressive variant of large granular lymphocyte leukemia [see comments]

TC Gentile, AH Uner, RE Hutchison, J Wright, J Ben-Ezra, EC Russell and TP Jr Loughran