Natural Killer-Like T-Cell Lymphomas: Aggressive Lymphomas of T-Large Granular Lymphocytes

By William R. Macon, Michael E. Williams, John P. Greer, Richard D. Hammer, Alan D. Glick, Robert D. Collins, and John B. Cousar

Natural killer (NK)-like T cells are major histocompatibility complex-unrestricted cytotoxic T cells that are surface CD3-positive, express NK-cell antigens, and rearrange their T-cell receptor. Most neoplasms arising from this T-cell subpopulation have been a chronic lymphoproliferative disease referred to as T-large granular lymphocyte (LGL) leukemia. Only 10 NK-like T-cell lymphomas have been described in detail previously; this study presents the clinicopathologic features of six others and distinguishes these lymphomas from T-LGL leukemia. All patients presented with B-symptoms and often had marked hepatosplenomegaly without significant peripheral lymphadenopathy. Four of the six patients were immunosuppressed. All had CD3, CD8, CD56-positive tumors, presumably of hepatosplenocytosis (n = 3), intestinal (n = 1), pulmonary (n = 1), or nodal (n = 1) origin. Three patients had lymphomatous bone marrow infiltrates, and four had peripheral blood involvement by neoplastic large lymphocytes, some of which had a blastic appearance or resembled vireocytes. Azurophilic granules, ultrastructurally corresponding to cytoplasmic dense core and/or double density granules, were seen in all cases. T-cell clonality was shown in five tumors by Southern blot analysis, and three had abnormal karyotypes. Two untreated patients died 20 days after presentation, and three patients who received combination chemotherapy died within 5 months of presentation. One patient remains in complete remission 22 months after treatment. These findings suggest NK-like T-cell lymphomas are aggressive, are clinicopathologically distinct from T-LGL leukemia, and should be in the differential diagnosis of extranodal T-cell lymphoproliferations, including those in immunosuppressed patients. Furthermore, the LGL morphology, phenotype, and tissue distribution of some NK-like T-cell lymphomas suggest they may arise from thymic-independent T cells of the hepatic sinusoids and intestinal mucosa.

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MATERIALS AND METHODS

Patient population. Six patients with NK-like T-cell lymphomas, diagnosed at Vanderbilt University Medical Center (VUMC) since 1989, were included in this study. Four patients received their therapy at VUMC. Two others (patients 1 and 5), whose pathology materials were reviewed as Vanderbilt hematopathology consultation cases, were hospitalized elsewhere; their clinical data were retrieved through chart review and discussion with the referring pathologists.

Cytologic, histologic, and ultrastructural studies. Wright’s stained films or touch imprints were evaluated from peripheral blood and aspirated bone marrow (patients 2 through 6), pleural fluid (patient 5) and resected tumors (patients 1 and 6). Neoplastic lymphocytes in patient peripheral blood films were compared with those from four patients with clonal T-LGL leukemia and from four patients known to have infectious mononucleosis. Differential counts of 200 hematopoietic cells were performed on marrow aspirate films for patients 2, 3, and 4. The morphological features on 4 μm thick, hematoxylin and eosin-, periodic acid-Schiff (PAS), and methyl green pyronin-stained sections of formalin- or B5-fixed, paraffin-
extracted for it.

Table 1. Clinical Features of Patients With NK-Like T-Cell Lymphomas

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/Sex</th>
<th>Presentation</th>
<th>Hematologic Findings</th>
<th>Known Sites of Tumor</th>
<th>Stage</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64/M</td>
<td>Fever, abdominal pain, and rectal bleeding</td>
<td>WBC = 17.9 x 10^9/µL (75% PMN, 10% lymph), Hct = 29.2%, Pt = 333 x 10^9/µL</td>
<td>Duodenum and mesentery</td>
<td>IV</td>
<td>None</td>
<td>DOD 20 d after presentation</td>
</tr>
<tr>
<td>2</td>
<td>32/M</td>
<td>Fever, night sweats, and marked hepatosplenomegaly</td>
<td>WBC = 2 x 10^9/µL (on presentation), Hct = 32%, Pt = 8 x 10^9/µL; WBC = 20.6 x 10^9/µL (postsplenectomy) (44% PMN, 26% lymph)</td>
<td>Spleen, marrow, and blood</td>
<td>IV</td>
<td>Mega IVe</td>
<td>DNED 2 mo after presentation</td>
</tr>
<tr>
<td>3</td>
<td>9/F</td>
<td>Fever, lymphadenopathy, and marked hepatosplenomegaly; pneumonia</td>
<td>WBC = 18.2 x 10^9/µL (8% γMN, 86% lymph), Hct = 30%, Pt = 285 x 10^9/µL</td>
<td>Marrow and blood</td>
<td>IV</td>
<td>MSK-NY-II</td>
<td>CR × 22 mo</td>
</tr>
<tr>
<td>4†</td>
<td>16/M</td>
<td>Fever, night sweats, and marked hepatosplenomegaly</td>
<td>WBC = 6.0 x 10^9/µL (5 weeks preadmission) (67% PMN, 17% lymph); WBC = 26 x 10^9/µL (on admission) (42% PMN, 44% lymph), Hct = 10%, Pt = 82 x 10^9/µL</td>
<td>Marrow and blood</td>
<td>IV</td>
<td>MSK-NY-II</td>
<td>DNED 3 mo after presentation</td>
</tr>
<tr>
<td>5†</td>
<td>62/M</td>
<td>Fever, shortness of breath, pulmonary infiltrate, pleural effusions, and hepatosplenomegaly</td>
<td>WBC = 3 x 10^9/µL (75% PMN, 11% lymph), Hct = 40.3%, Pt = 66 x 10^9/µL</td>
<td>Lung, pulmonary lymph node, pericardium, pleural fluid, and blood mediastinal lymph nodes</td>
<td>IV</td>
<td>None</td>
<td>DOD 20 d after presentation</td>
</tr>
<tr>
<td>6†</td>
<td>64/M</td>
<td>Fever, night sweats, shortness of breath, mediastinal lymphadenopathy, and moderate splenomegaly</td>
<td>WBC = 2.6 x 10^9/µL (86% PMN, 9% lymph), Hct = 31.6%, Pt = 172 x 10^9/µL</td>
<td>Mediastinal lymph nodes</td>
<td>III</td>
<td>CHOP 22</td>
<td>DNED 5 mo after presentation</td>
</tr>
</tbody>
</table>

Abbreviations: WBC, white blood cell count; PMN, neutrophils; lymph, lymphocytes; Hct, hematocrit; Pt, platelet count; DOD, died of disease; DNED, died with no evidence of disease; CR, complete remission; PR, partial response.

* Immunosuppressed with azathioprine and prednisone for ulcerative colitis; Coombs'-positive hemolytic anemia on presentation.
† Immunosuppressed with cyclosporin A, azathioprine and prednisone 4 years after renal (patient 4) or 7 and 15 months after cardiac (patients 5 and 6) transplantation.

Antibodies directed against T-cell antigens, with vendor and technique indicated, included CD1 (OKT6, Ortho Diagnostics, Raritan, NJ; FACS), CD2 (Leu-5, BD; FACS and IAP), CD3 (Leu-4, BD; FACS and IAP or polyclonal CD3, DAKO, Carpenteria, CA; Pip), CD4 (Leu-3, BD; BD; FACS and IAP), CD5 (Leu-1, BD; BD; FACS and IAP or CD5, Novocastra Laboratories, Newcastle upon Tyne, United Kingdom [obtained from Vector Laboratories, Burlingame, CA]; Pip), CD7 (Leu-9, BD; BD; FACS and IAP), CD8 (Leu-2, BD; FACS and IAP), CD43 (Leu-22, BD or DF-T1, DAKO; Pip), CD45RO (UCHL1, DAKO; Pip), and T-cell receptor beta chain (βF1, T Cell Diagnostics, Cambridge, MA; Pip). Antibodies directed against NK-cell antigens included CD11b (Leu-15, BD; FACS and IAP), CD11c (Leu-M5, BD; FACS and IAP), CD16 (Leu-11, BD; FACS and IAP), CD56 (Leu-19, BD; FACS and IAP), and CD57 (Leu-7, BD; FACS, IAP and Pip). Other antibodies used included those directed at CD45 (HL-1, BD; FACS or LCA, DAKO; Pip), CD10 (CALLA, BD; FACS), CD15 (Leu-M1, BD; Pip), CD30 (Ki-1, DAKO; FACS or Ber-H2, DAKO; Pip), terminal deoxynucleotidyl transferase (TdT, Supertechs, Bethesda, MD; IF), S-100 (DAKO; Pip), and Epstein-Barr virus (EBV) latent membrane protein (LMP) (CS1-4, DAKO; Pip).

Tumors were considered slightly positive when 20% to 39% of the neoplastic cells marked with an individual antibody, moderately embedded tissue were also reviewed for each patient. Electron microscopy was performed by standard methods to assess ultrastructural features of tumor cells in patients 1 through 5.

Immunologic studies. Several techniques were used in concert for immunophenotypic analysis of tumor cells from each patient. Cell surface antigens were detected by flow cytometry (FACSScan; Becton-Dickinson (BD), Mountainview, CA) of mononuclear cells isolated from omental tumor (patient 1), bone marrow (patients 2 and 4), peripheral blood (patient 3), pleural fluid (patient 5), and lymph node (patients 5 and 6). Immunoalkaline phosphatase (IAP) studies using a labeled streptavidin-biotin system (patients 3, 4 and 5), and indirect immunofluorescence (IF) (patients 1 through 4) were performed on cytocentrifuge preparations of cells suspended from the same tissue as those analyzed by flow cytometry (FACS). Paraffin immunoperoxidase (PDP) studies were performed on 4 µm sections of omental tumor (patient 1), spleen (patient 2), bone marrow (patients 3 and 4), and lymph node (patients 5 and 6) using either a manual three-stage indirect immunoperoxidase technique for monoclonal antibodies and an indirect peroxidase-antiperoxidase procedure for polyclonal antibodies, or an avidin-biotin complex method with appropriate antigen retrieval using an automated immunostainer (Ventana 320 Automated Immunohistochemistry System, Ventana Medical Systems, Tuscon, AZ).
Peripheral blood films from patients with NK-like T-cell lymphomas, T-LGL leukemia, and infectious mononucleosis. (A) Circulating tumor cells from patient 2 were mostly large lymphocytes with round to oval nuclei having dispersed chromatin and prominent nucleoli. Occasional neoplastic cells had a blastic appearance (inset). Azurophilic cytoplasmic granules were seen easily. (B) Lymphocytes from a patient with clonal T-LGL leukemia were smaller, and the nuclei had dense chromatin with less discernible nucleoli. Azurophilic granules were present. (C) Circulating tumor cells from patient 3 were often large lymphocytes with a high nuclear:cytoplasmic ratio and marked nuclear irregularity. Azurophilic granules were scant in these cells. (D) Erythrocytes from a patient with infectious mononucleosis had a much smaller nuclear:cytoplasmic ratio and regular nuclear profiles.

Cytogenetic and molecular genetic studies. Karyotypic analysis was performed on harvested cell suspensions from omental tumor (patient 1) or bone marrow (patients 2, 3, and 4). Metaphases from 20 cells in each sample were evaluated by Giemsa staining with positive when 40% to 59% of the neoplastic cells marked, and strongly positive when 60% or more of the neoplastic cells marked.

Fig 1.
Fig 3. Bone marrow aspirate film and biopsy section from patient with an NK-like T-cell lymphoma. (A) Tumor cells were easily found in aspirate films from patient 2, including some exhibiting erythrophagocytosis. (B) Neoplastic cells were much more difficult to find in tissue sections from this patient because of the accompanying erythroid hyperplasia. Tumor cells that phagocytosed nucleated red blood cells helped identify the interstitial lymphomatous infiltrate (arrows) (PAS stain). DNA for molecular genetic analysis was isolated from omental tumor (patient 1), bone marrow (patients 2 and 4), peripheral blood (patient 3), pleural fluid (patient 5), and lymph node (patient 6) using standard phenol-chloroform extraction. Purified DNA was digested with the restriction endonucleases BamHI, EcoRI, and HindIII and electrophoresed on agarose gels. The DNA was transferred to nylon membranes, and Southern blot analysis was performed as previously reported. Blots were serially hybridized with probes for the T-cell receptor (TCR) beta-chain joining gene (Jβ) (Oncor, Gaithersburg, MD) in six patients, the TCR beta-chain constant gene (Cβ) (Oncor) in one patient, the TCR gamma-chain joining gene (Jγ) (Oncor) in three patients, and the TCR delta-chain constant gene (Cδ) (Dr Tak Mak, University of Toronto, Toronto, Ontario, Canada) in two patients. Probes for the immunoglobulin (Ig) heavy-chain joining gene (JH) (Oncor) and Ig kappa light-chain joining gene (JK) (Oncor) were used in four and one patient, respectively. A c-myc exon 1 probe (Dr Takis Papas, National Cancer Institute, Bethesda, MD) was also used for patient 2. Rearrangements indicating clonality were identified by the presence of nongermline bands, as compared with placental control DNA or with previously identified positive and negative control DNA.

RESULTS

Clinical features. Table 1 summarizes the clinical features of each patient, including the principal hematologic findings and histologically documented sites of tumor. All patients presented with advanced stage disease (Stage III or IV) by the Ann Arbor staging system and B-symptoms (fever, night sweats, weight loss). Most patients also had hepatosplenomegaly without significant peripheral lymphadenopathy and had an aggressive clinical course. Five patients were anemic, including patient 2 who presented with a Coombs'-positive hemolytic anemia. Three patients had an absolute lymphocytosis (range, 5,356 to 15,652/μL) with rapidly rising counts in patients 2 and 4. Patient 3 had an absolute neutropenia that was associated with pneumonia. Four patients were chronically immunosuppressed for longstanding ulcerative colitis (patient 2) or after solid organ transplantation (patients 4, 5, and 6).

Morphologic features. Peripheral blood involvement was extensive in patients 2, 3, and 4 and slight in patient 5. Circulating tumor cells were mostly large lymphocytes with abundant pale cytoplasm; fine to coarse azurophilic granules

Fig 4. Tissue sections from patient with small bowel/omental NK-like T-cell lymphoma (PAS stains). (A) The lamina propria of the duodenum from patient 1 is filled by lymphoma cells. A lymphoepithelial lesion is created by tumor cells that infiltrate a residual mucosal gland (arrow). (B) Medium-size tumor cells with dispersed chromatin efface the omental fat. Numerous mitoses are also evident.
were seen in some neoplastic cells in all cases from films of peripheral blood, marrow, or pleural fluid, or from touch imprints of tumor (Fig 1A). The neoplastic cells in patients 2 and 4 had oval nuclei with dispersed chromatin and prominent nucleoli (Fig 1A); large cell size and a blastic appearance in some lymphocytes distinguished these neoplastic cells from those in patients with T-LGL leukemia (Fig 1B).

Some tumor cells in patients 3 and 5 resembled "atypical" lymphocytes in patients with infectious mononucleosis; however, other neoplastic cells had increased nuclear to cytoplasmic ratios and markedly irregular nuclear profiles, which distinguished them from virocytes (Fig 1C and D). "Cloverleaf" peripheral blood tumor cells in patient 3 (Fig 2A) and markedly enlarged, dysplastic pleural fluid tumor cells in patient 5 (Fig 2B) demonstrated the extreme variability of nuclear shape and cell size.

Marrow involvement was more easily observed on films than in tissue sections. Differential counts on films from patients 2, 3, and 4 showed tumor cells comprised 59%, 28%, and 58% of marrow cells, respectively (Fig 3A). On the other hand, sections showed only subtle interstitial lymphoid infiltrates without focal lesions in hypercellular marrows from each of these patients (Fig 3B). Furthermore, erythrophagocytosis by tumor cells was more easily identified in marrow films from patient 2, but was difficult to detect in splenic or narrow sections (Fig 3).

The duodenal surface was ulcerated in patient 1. Neoplastic lymphocytes filled the deeper lamina propria and focally infiltrated residual mucosal glands producing "lymphoepithelial" lesions (Fig 4A). Lymphoma cells in patient 1 and those that effaced the splenic architecture in patient 2 were medium-size in sections (Fig 4B). Dysplastic large lymphocytes effaced pulmonary hilar or mediastinal nodes in patients 5 and 6. They also infiltrated lung parenchyma, pleura, and pericardium in patient 5. Additionally, autopsy on patient 5 showed congestive hepatosplenomegaly (liver 2,240 g and spleen 630 g) with erythrophagocytosis by histiocytes; no lymphoma was identified in the liver or spleen.

Electron microscopy demonstrated cytoplasmic dense core and/or double density granules (Fig 5) in some tumor cells from each patient studied. No parallel tubular arrays were found in obviously neoplastic cells.

Immunologic features. Table 2 summarizes the T-cell and NK-cell antigen expression of the tumor cells in each patient. The common phenotype was CD2+, CD3+, CM-, CDS', CD56', and CD57-, and there was variable expression of other T-cell and NK-cell antigens. The tumor cells were phenotypically mature as there was no expression of CD1, CD10 or TdT. Tumors cells were CD45+, and none expressed CD15 or S-100. CD30 staining of tumor cells was seen only in patient 6. LMP staining was seen in most tumor cells from patients 1, 5, and 6, but was negative in patient 2.

Genetic features. Karyotypic and genotypic data are shown in Table 3. Three of four patients studied had an abnormal karyotype. A t(2;17) was observed in two analyzed cells from patient 3. Isochromosome 7q was present in 17 and 20 analyzed cells from patients 2 and 4, respectively. Patient 2 also had a t(8;14) (q24;q24). T-cell clonality was demonstrated in five of six patients. No c-myc rearrangement was identified for patient 2, suggesting the possibility of a breakpoint 5' to the c-myc locus or of point mutations within c-myc not detected with the exon 1 probe.
DISCUSSION

We have described six T-cell lymphomas whose LGL morphology, expression of CD3 and NK-cell antigens, and ability to rearrange their T-cell receptor indicate an origin from NK-like T cells. Ten other T-cell lymphomas with the features of NK-like T-cell lymphomas have been reported in detail (Table 4).14-21 Data from our series and the other case reports suggest these lymphomas are a separate clinicopathologic entity that can be distinguished from T-LGL leukemia by multiparametric analysis. In this combined series, NK-like T-cell lymphomas were aggressive as 93% presented with advanced stage disease and median survival was less than 6 months. The median age was 48 years (range, 9 to 66 years); five patients (31%) were in the pediatric population. There was a 13:2 male:female ratio. Seventy-nine percent of patients presented with B-symptoms, and marked hepatosplenomegaly was common (60%). These lymphomas were usually extranodal (81%) and tended to be distributed in the liver, spleen, and marrow (50%) or in the gastrointestinal tract (25%). Nodal or cutaneous involvement without hepatosplenic or intestinal disease was unusual. A leukemic phase was common (63%) and developed after splenectomy in five of 10 patients. Peripheral white blood cell counts in patients with a leukemic phase had a median of 18,000/μL (range, 3,000 to 203,000/μL). Thrombocytopenia was present in seven of 12 (58%) patients. A CD4- CD8+ phenotype was most common (n = 10) as compared with CD4+ CD8- (n = 2), CD4+ CD8+ (n = 2) and CD4- CD8- (n = 2). CD56 was expressed on tumor cells in 11 of 12 (92%) lymphomas studied for this antigen, whereas CD57 positivity was observed in only four of 15 (27%) cases. Aberrant chromosomal analyses were found in five of six lymphomas studied, and four of these had an i(7q).

Clinical implications dictate distinguishing leukemic involvement by NK-like T-cell lymphomas from the more indolent T-LGL leukemia. In comparison with T-LGL leukemia, patients with NK-like T-cell lymphomas are more likely to be within the pediatric population, to present with B-symptoms and marked hepatosplenomegaly, and to have thrombocytopenia and a rapid increase in the number of leukemic cells.11-12 NK-like T-cell lymphomas have less frequent neutropenia and infections. Leukemic cells of NK-like T-cell lymphomas often exhibit marked nuclear pleomorphism or have a blastic appearance, the latter being rare in chronic LGL leukemias.34 There is a greater tendency for NK-like T-cell lymphomas to express CD56 rather than CD57, whereas the opposite has been reported for T-LGL leukemia.11 Parallel tubular arrays, frequently seen in ultrastructural studies of T-LGL leukemia,22 are apparently uncommon among NK-like T-cell lymphomas (seen in two of 11 cases studied). Cytogenetic abnormalities are common in NK-like T-cell lymphomas, but not in T-LGL leukemia.12 These differences between the two groups of neoplasms indicate that the recently reported cases of CD56+ aggressive variant of T-LGL leukemia22 may be NK-like T-cell lymphomas with a leukemic phase.

Several features seen in our series of NK-like T-cell lymphomas may have biologic and clinical significance; in
particular, CD56 (NCAM) expression, chromosomal abnormalities, immunosuppression, and EBV infection are noteworthy. NCAM, a neural cell adhesion molecule involved in homotypic interactions, has been implicated in the extranodal dissemination of some peripheral T-cell lymphomas, and its expression has been associated with an aggressive clinical course. However, some lymphomas regarded as NCAM-positive PTCLs may be true NK-cell lymphomas. Therefore, the term NK-like T-cell lymphoma might be best reserved for those lymphomas that express surface CD3 (or framework determinants of the TCR) and NK-cell antigens, particularly CD56, and have cytoplasmic granules by light or electron microscopy. NK-like T-cell lymphomas that lack surface CD3 or TCR framework determinants should exhibit TCR gene rearrangements to distinguish them from true NK-cell lymphomas.

Chromosomal abnormalities in NK-like T-cell lymphomas may be of pathogenetic and prognostic significance. For instance, the erythrophagocytic T-cell lymphoma in patient 2 had a t(8;14)(q24;q24), which may have juxtaposed the cmyc gene on 8q24. Moreover, a transcriptionally active (housekeeping) cytoskeletal protein gene such as that for α-actinin located on 14q24.39 Deregulation of c-myc, seen in some T-cell neoplasms, as well as possible alterations in the quantity and cellular localization of cytoskeletal components may have contributed to malignant transformation. Furthermore, altered cytoskeletal proteins may have permitted the phagocytosis of IgG-coated erythrocytes by the tumor cells in this patient, a function not normally ascribed to T cells.

Chromosomal abnormalities in NK-like T-cell lymphomas may be of pathogenetic and prognostic significance. For instance, the erythrophagocytic T-cell lymphoma in patient 2 had a t(8;14)(q24;q24), which may have juxtaposed the c-myc gene on 8q24.39 Deregulation of c-myc, seen in some T-cell neoplasms, as well as possible alterations in the quantity and cellular localization of cytoskeletal components may have contributed to malignant transformation. Furthermore, altered cytoskeletal proteins may have permitted the phagocytosis of IgG-coated erythrocytes by the tumor cells in this patient, a function not normally ascribed to T cells.

Of further biologic interest is the observation that hepatosplenic and intestinal NK-like T-cell lymphomas not only have cytological (LGL morphology) and immunological (CD56 + or CD57 +) features in common with thymic-independent T cells, but also have similar tissue distributions. NK-like T-cell lymphomas also may be of αβ or γδ types, as are extrathymically derived T-cells. Furthermore, thymic-independent T cells may be CD4−CD8−, CD4+CD8+, CD4+CD8−, or CD4−CD8−. As noted above, all four of these phenotypes have been observed among NK-like T-cell lymphomas. Therefore, it is possible that these lymphomas are derived from T-cell subpopulations with extrathymic pathways of differentiation.

Finally, NK-like T-cell lymphomas should be considered for all lymphomas of presumed hepatosplenic and intestinal origin if patients present with B-symptoms and advanced stage disease, including a leukemic phase. We recommend, in agreement with Warzynski et al., multiparametric analysis, as described below, to facilitate recognition of these aggressive lymphomas. Cytoplasmic granules may be demonstrated in tumor cells using Romanovsky's-type stains on peripheral blood, marrow, or other body fluid films or on touch imprints of tumors. Electron microscopy may be used to detect granules not seen by light microscopy. Immunophenotypic analysis should include a broad panel of T-cell and NK-cell antigens (see Table 2). Cytogenetic analysis may pinpoint patients with especially poor prognosis, as well as identify specific chromosomal abnormalities. T-cell receptor gene rearrangement studies are recommended to prove clonality in morphologically difficult lesions and to distinguish some NK-like T-cell lymphomas from true NK-cell lymphomas.

NOTE ADDED IN PROOF

Dr Mohamed Elkhalifa, Director of Hematopathology, University of South Alabama, Mobile, Alabama, has kindly provided us data and patient materials on another NK-like T-cell lymphoma since this manuscript was prepared. The patient was a 62-year-old female who presented with B-symptoms, hepatosplenomegaly, abdominal pain, and slight lymphadenopathy. Peripheral blood (WBC = 55 × 10^9/L) with 80% lymphocytes, hematocrit = 31.6%, and platelets

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Karyotype</th>
<th>T-cell Receptor*</th>
<th>Immunoglobulin*</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>46,XY</td>
<td>R</td>
<td>GL</td>
</tr>
<tr>
<td>2</td>
<td>46,XY,t(7)(q10);8;14(q24;q24);add(13)(p13)</td>
<td>R</td>
<td>GL</td>
</tr>
<tr>
<td>3</td>
<td>46,XX,t(2;17)(q34;q21.3),add(14)(q32)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>46,XY,t(7)(q11.1),add(12)(p11.1)</td>
<td>ND</td>
<td>GL</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>6</td>
<td>ND</td>
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Abbreviations: R, rearranged; GL, germline; Del, deleted; ND, not done.

* See Materials and Methods for probe definition.
### Table 4. Clinical Features of Previously Reported Patients With NK-Like T-Cell Lymphomas

<table>
<thead>
<tr>
<th>Age/Sex</th>
<th>Presentation</th>
<th>Hematologic Findings</th>
<th>Known Sites of Tumor</th>
<th>Stage</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>50/F</td>
<td>Fever, asthenia, and splenomegaly</td>
<td>WBC = 18.7 × 10^3/μL (80% lymph), red blood cells and platelets &quot;in normal range&quot;; developed leukemic phase postsplenectomy</td>
<td>Spleen, liver, marrow, and blood</td>
<td>IV</td>
<td>&quot;Aggressive chemotherapy&quot;</td>
<td>DOD 23 mo after presentation</td>
<td>14</td>
</tr>
<tr>
<td>52/M</td>
<td>Gastric mass and splenomegaly</td>
<td>Peripheral lymphocytes = 10.8 × 10^3/μL; developed leukemic phase postsplenectomy</td>
<td>Spleen, stomach, marrow, and blood</td>
<td>IV</td>
<td>Prednisone and undefined chemotherapy</td>
<td>CR × 31 mo</td>
<td>14</td>
</tr>
<tr>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Lymph node</td>
<td>NE</td>
<td>NA</td>
<td>NA</td>
<td>15</td>
</tr>
<tr>
<td>48/M</td>
<td>Intestinal obstruction; anorexia, weight loss, asthenia, and night sweats</td>
<td>WBC = 9.5 × 10^3/μL (&quot;Intact differential&quot;), Hgb = 13.5 g/dL, Pt = 350 × 10^3/μL</td>
<td>Jejunum, lungs, and CSF</td>
<td>IV</td>
<td>CHOP&quot;</td>
<td>DOD 4 mo after presentation</td>
<td>17</td>
</tr>
<tr>
<td>66/M</td>
<td>Abdominal pain, anorexia, and weight loss</td>
<td>WBC = 11.0 × 10^3/μL (80% lymph)</td>
<td>Spleen, liver, lymph nodes, and blood</td>
<td>IV</td>
<td>None</td>
<td>NA</td>
<td>18</td>
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<tr>
<td>50/M</td>
<td>Generalized maculopapular rash</td>
<td>NA</td>
<td>Skin</td>
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<td>Chlorambucil then whole body irradiation</td>
<td>DOD 1 yr after presentation</td>
<td>19</td>
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<tr>
<td>12/M</td>
<td>Fever, petechiae, and hepatosplenomegaly</td>
<td>WBC = 7.3 × 10^3/μL (on presentation), Hgb = 13.8 g/dL, Pt = 7 × 10^3/μL; WBC = 203 × 10^3/μL (80% lymph) (postsplenectomy)</td>
<td>Spleen, lymph nodes, marrow, and blood</td>
<td>IV</td>
<td>Prednisone</td>
<td>DOD 2.5 mo after presentation</td>
<td>19</td>
</tr>
<tr>
<td>18/M</td>
<td>Hepatosplenomegaly</td>
<td>WBC = 17.7 × 10^3/μL (postsplenectomy), Hgb = 8.5 g/dL, Pt = 10 × 10^3/μL</td>
<td>Liver, kidneys, lymph nodes, marrow, and blood</td>
<td>IV</td>
<td>Cyclophosphamide, prednisone, and vincristine; doxorubicin and etoposide</td>
<td>DOD 1.5 mo after presentation</td>
<td>20</td>
</tr>
<tr>
<td>20/M</td>
<td>Fever, anorexia, weight loss, massive hepatosplenomegaly, and mild lymphadenopathy</td>
<td>WBC = 2 × 10^3/μL, Hgb = 7 g/dL, Pt = 70 × 10^3/μL</td>
<td>Spleen, liver, lymph nodes, marrow, and blood</td>
<td>IV</td>
<td>Multiple combination chemotherapy</td>
<td>DOD 9 mo after presentation</td>
<td>21</td>
</tr>
</tbody>
</table>

Abbreviations: WBC, white blood cell count; lymph, lymphocytes; Hgb, hemoglobin content; Pt, platelet count; NA, not available; NE, not evaluable; CSF, cerebral spinal fluid; DOD, died of disease; CR, complete remission.

= 20 × 10^3/μL), bone marrow and a submental lymph node were involved by lymphoma that was CD2+, CD3+, CD4+, CD5-, CD7+, CD8+, CD11b+, CD11c+, CD16+, CD56-, CD57-, CD45RO+, βF1+, and LMP+. The T-cell receptor beta chain gene was rearranged. There was an abnormal karyotype, including an i(7q). The patient died two months after presentation despite combination chemotherapy (CHOP).

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### REFERENCES

1. Ortaldo JR, Winkler-Pickett RT, Yagita H, Young HA: Comparative studies of CD3+ and CD3+ CD56+ cells: Examination of
phocytes arise from both
form expressed on large granular lymphocytes. J Exp Med 175:203,

3. Lanier LL, Le AM, Civin CI, Loken MR, Phillips JH: The
relationship of CD16(Leu-11) and Leu-19 (NKH-1) antigen expres-
sion on human peripheral blood NK cells and cytotytic T lympho-

4. Schmidt RE, Murray C, Daley JF, Schlossman SF, Ritz J: A
subset of natural killer cells in peripheral blood displays a mature

5. Cerf-Bensussan N, Schneebberger E, Bhan AK: Immunohisto-
logic and immunoelectron microscopic characterization of the mucu-
sal lymphocytes of human small intestine by the use of monoclonal


8. Abo T: Extrathymic pathways of T-cell differentiation: A prim-

ton LK, Reinherz EL: T cell receptor complexes containing FcRRIy
homodimers in lieu of CD3ε and CD3γδ components: A novel iso-
form expressed on large granular lymphocytes. J Exp Med 175:203,
1992

10. Koyasu S: CD3+CD16+NK1.1+B220+ large granular lympho-

11. Loughran TP: Clonal diseases of large granular lymphocytes.
Blood 82:1, 1993

12. Dhodapkar MV, Li C-Y, Lust JA, Tefferi A, Phyllyk RL:
Clinical spectrum of clonal proliferations of T-large T cell lym-
phoma: A T-cell clonality of undetermined significance? Blood
84:1620, 1994

13. Nichols GE, Normanns DE, Williams ME: Lymphoprolifera-
tive disorder of granular lymphocytes: Nine cases including one
with features of CD55 (NKH1)-positive aggressive natural killer cell
lymphoma. Mod Pathol 7:819, 1994

G, Quinti I, Ranucci A, Raimondi B, Basso G, Strong DM, Fontana
L, Aiuti F: Characterization of two patients with lymphomas of

15. Watanabe S, Sato Y, Shimoyama M, Minato K, Shimosato
Y: Immunoblastic lymphadenopathy, anogammoblastic lympho-
ephropathy, and IBL-like T-cell lymphoma: A spectrum of T-cell neo-
plassia. Cancer 58:2224, 1986

AR, Proillet BC, Flandrin G, Hautefeuille P: A primary immunoblas-
tic T malignant lymphoma of the small bowel, with azurophilic
intracytoplasmic granules: A histologic, immunologic, and electron

17. Longacre TA, Listrom MB, Spigel JH, Willman CL, Dressler
L, Clark D: Aggressive jejunal lymphoma of large granular lympho-
cytes: Immunohistochemical, ultrastructural, molecular, and DNA

18. Berceanu S, Roman S, Butoianu F, Leahu S, Gabriescu E,
Ollescu A, Moldoveanu E, Moraru I: A particular case of large

19. Wong KF, Chan JKC, Ng CS, Lee KC, Tsang WYW, Cheung
MMC: CD55 (NKH1)- positive hematolympoid malignancies: An
aggressive neoplasn featuring frequent cutaneous/mucosal involve-
ment, cytoplasmic azurophilic granules, and angiocentricity. Hum
Pathol 23:798, 1992

Muuse W, Hombal S, Teichberg S, Broome J: A study of lymphoma
of large granular lymphocytes with modern modalities: Report of

RN, Rezuke WN, Johnson ML: An acute form of Ty lymphoprolif-
erative disease presenting with massive splenomegaly―Importance
of immunophenotyping for diagnosis. Clin Immunol Immunopathol
67:100, 1993

22. Gentile TC, Uner AH, Hutchinson RE, Wright J, Ben-Ezra J,
Russell EC. Loughran TP: CD3-, CD56+ aggressive variant of large

T cells: Facts and puzzles, in Dixon FJ (ed): Advances in Immunol-

RA, Hansen PW: Rapid enumeration of T lymphocytes by a flow-

25. Hammer RD, Collins RD, Ebrahimi S, Casey TY: Rapid im-
munocytochemical analysis of acute leukemias. Am J Clin Pathol
97:876, 1992

26. Bollum FJ: Terminal deoxynucleotidyl transferase as a hema-
topoietic cell marker. Blood 54:1203, 1979

27. Kurtin PJ, Pinkus GS: Leukocyte common antigen—a diag-
nostic discriminant between hematopoietic and nonhematopoietic
neoplasms in paraffin sections using monoclonal antibodies: Correla-
tion with immunologic studies and ultrastructural localization. Hum
Pathol 16:353, 1985

immunoglobulin in paraffin sections of multiple myeloma and mac-
roglobinemia using an immunoperoxidase technique. Am J Pathol
87:47, 1977

complex (ABC) in immunoperoxidase techniques: A comparison
between ABC and unlabeled antibody (PAP) procedures. J Histo-
chem Cytochem 29:577, 1981

30. Lee JT, Innes DJ, Williams ME: Sequential bcl-2 and c-myc
carcinogene rearrangements associated with the clinical transformation

aspiration of non-Hodgkin’s lymphoma: Southern blot analysis
for antigen receptor, bcl-2, and c-myc gene rearrangements. Am J
Clin Pathol 93:754, 1990

32. Macon WR, Williams ME, Greer JP, Cousar JB: Paracortical
nodular T-cell lymphoma: Identification of an unusual variant of

33. Carbone PP, Kaplan HS, Musshoff K, Smithers DW, Tubiana
M: Report of the committee on Hodgin’k disease staging. Cancer
Res 31:1860, 1971

34. Ohno Y, Amakawa R, Fukuhara S, Huang C-R, Kamesaki
H, Amano H, Imanaka T, Takahashi Y, Arita Y, Uchiyama T, Kitah
Miwa H: Acute transformation of chronic large granular lymphocyte
leukemia associated with additional chromosome abnormality. Can-
cer 64:63, 1989

35. Prasthofer EF, Barton JC, Zarcone D, Grossi CE: Ultrastruc-
tural morphology of granular lymphocytes (GL) from patients with
immunophenotypically homogeneous expansions of GL populations
Natural killer-like T-cell lymphomas: aggressive lymphomas of T-large granular lymphocytes [see comments]

WR Macon, ME Williams, JP Greer, RD Hammer, AD Glick, RD Collins and JB Cousar