Clonal Diseases of Large Granular Lymphocytes

By Thomas P. Loughran, Jr

LARGE GRANULAR lymphocytes (LGL) are a morphologically recognizable lymphoid subset comprising 10% to 15% of peripheral blood mononuclear cells. Initially, LGL were synonymous with natural killer (NK) cells. However, it is now clear that LGL can be divided into two major lineages: CD3- and CD3+. CD3- LGL are NK cells that mediate non-major histocompatibility complex (MHC)-restricted cytotoxicity and do not express the CD3/T-cell receptor (TCR) complex or rearrange TCR genes. CD3+ LGL are T cells that do express the CD3/TCR complex and rearrange TCR genes. These cells mediate non-MHC-restricted cytotoxicity in vitro and are thought to represent in vivo-activated cytotoxic T lymphocytes.

A syndrome of increased numbers of circulating LGL associated with chronic neutropenia was recognized as a distinct clinical entity in 1977. A major question was whether the LGL proliferation represented a reactive or a neoplastic disorder. The first evidence that this disease resulted from a clonal process was the finding of clonal cytogenetic abnormalities in unstimulated cultures of peripheral blood and/or splenic mononuclear cells from two of our patients. We proposed the term LGL leukemia for this disorder based on the observation of clonality and the demonstration of tissue invasion by LGL of marrow, spleen, and liver.

It is now recognized that LGL proliferations may be clonally derived from either of their normal counterparts, ie, CD3- or CD3+ LGL. Confusion exists in the literature about the natural history of these disorders because previous studies have used a variety of names to describe such patients and have included patients without documented clonal disease. The purpose of this review is to focus on the clinical features of patients with clonal diseases of CD3+ or CD3- LGL to provide a basis for a uniform classification for these disorders.

NOMENCLATURE

Terms used to describe patients with LGL proliferative disorders have included: chronic T-cell lymphocytosis with neutropenia, T8 chronic lymphocytic leukemia (CLL), T-cell CLL, granulated T-cell lymphocytosis with neutropenia, T-suppressor cell CLL, neutropenia with T lymphocytosis, and NK and suppressor T-cell CLL. The most common names, besides LGL leukemia, have been Tγ lymphocytosis or Tγ lymphoproliferative disorder, T-CLL, and lymphoproliferative disorder of granulocytes. Indeed, this disorder was last reviewed in Blood in 1984 under the term Tγ lymphocytosis. Tγ was first used to describe normal peripheral blood mononuclear cells that were E-rosette (CD2) and EA-rosette (CD16) positive. This term is somewhat of a misnomer because normal cells with this phenotype usually lack CD3 and are not T cells but NK cells. Furthermore, some normal Tγ cells are not LGL and occasional Tγ lymphoproliferative disorders are reported that do not involve LGL.

In 1975 Brouet et al identified patients with T-CLL; in some of these patients the abnormal lymphocytes were LGL. The French-American-British (FAB) Cooperative Group's proposal for classification of chronic T-lymphoid leukemias designated four subgroups: (1) T-cell CLL or LGL leukemia, (2) T-prolymphocytic leukemia, (3) adult T-cell leukemia/lymphoma, and (4) Sezary's syndrome. In 1990, this FAB classification was reviewed by the Morphologic, Immunologic, Cytogenetic (MIC) Cooperative Study Group and it was agreed that the term LGL leukemia should replace T-CLL as the preferable terminology.

Given these considerations and in light of the evidence that clonal LGL proliferations may be either CD3+ (T-cell origin) or CD3- (NK cell origin), it is proposed that the following terminology be adopted: (1) T-LGL leukemia, characterized by clonal CD3+ LGL proliferation and (2) NK-LGL leukemia, characterized by clonal CD3- LGL proliferation. The clinical implications of this classification are reviewed below.
Table 1. Clinical Features of Patients With T-LGL Leukemia

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<tr>
<th>Feature</th>
<th>Observed</th>
<th>%</th>
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<tr>
<td>Median age, range</td>
<td>57, 15-88</td>
<td></td>
</tr>
<tr>
<td>Male/female</td>
<td>57/71</td>
<td>45/55</td>
</tr>
<tr>
<td>Recurrent infections</td>
<td>50/129</td>
<td>39</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>35/129</td>
<td>28</td>
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METHODS

A Medline computerized search of the published literature was conducted using as key words the terminology described above. The cited literature of the primary references identified in this search was examined to identify additional pertinent publications. One hundred twenty-nine patients (including 33 from our series) with clonal CD3+ LGL, as determined by cytogenetic or TCR gene rearrangement studies, were classified as having T-LGL leukemia.11,18,26,35-79 Eleven patients with clonal CD3+ LGL, as determined by cytogenetic studies, were classified as having NK-LGL leukemia.3,77,80-86 The clinical features of these two groups of patients are summarized below.

T-LGL LEUKEMIA (CLONAL CD3+)

Clinical features. Clinical features are summarized in Table 1. The median age is 55 years, with a range of 4 to 88. Although the disease is infrequently reported in the pediatric literature, it is apparent that clonal onset may occur in childhood.45,74 Male-to-female distribution is about equal. Recurrent bacterial infections, occurring as a consequence of neutropenia, are a hallmark of the disease and the major reason why patients come to medical attention. These infections typically involve skin, sinuses, and perirectal areas, but may also be more deeply invasive, resulting in pneumonia or sepsis. Opportunistic infections with fungal, viral, or parasitic pathogens are rare.

Of particular interest, rheumatoid arthritis occurs in patients with T-LGL leukemia.11,13,34,47,49,52,65,72,75,78,79 These patients may resemble patients with Felty's syndrome, with the clinical triad of rheumatoid arthritis, neutropenia, and splenomegaly. Previous studies had suggested that longstanding severe erosive arthritis and extraarticular manifestations of rheumatoid arthritis were rarely seen in T-LGL leukemia, distinguishing these patients from patients with typical Felty's syndrome.65,78 However, it is now apparent that such features may also be seen in rheumatoid patients with T-LGL leukemia.97 The prevalence of T-LGL leukemia in Felty's syndrome is not well characterized. We identified a clonal CD3+ LGL population in 4 of 12 patients with Felty's syndrome;65 Gonzales-Chambers et al95 found clonal disease in 8 of 23 such patients. Although clonal studies were not performed, 6 of 18 patients with Felty's had increased numbers of LGL; a more recent study has suggested that the true prevalence of this syndrome is greatly underestimated.89

The cause of T-LGL leukemia (see below) and the events initiating the development of rheumatoid arthritis are not known; it is hypothesized that there may be a common etiology underlying both diseases. Because T-LGL leukemia is a newly recognized disorder, it is difficult to determine the temporal sequence of onset of T-LGL leukemia to onset of rheumatoid arthritis. Several patients seem to have a simultaneous onset of rheumatoid arthritis and T-LGL leukemia.62,78 In one patient we have noted that clonal LGL proliferation (diagnosed after an abnormal complete blood cell count was found on routine annual examination) preceded the development of rheumatoid arthritis by several years. Subsequent studies performed after the diagnosis of rheumatoid arthritis demonstrated a normal lymphocyte phenotype and disappearance of the abnormal clone by Southern blot hybridization analyses.90 These results raise the possibility that rheumatoid arthritis may be preceded by a clonal lymphoproliferation that is no longer apparent at diagnosis. In this regard, it is of interest that increased numbers of LGL with a CD3+, CD16+ phenotype (typical of T-LGL leukemia, see below) are observed in synovial fluid but not peripheral blood obtained from patients with rheumatoid arthritis.91

Hematologic features. Examination of the peripheral blood smear is critical in establishing the diagnosis of LGL leukemia. The characteristic finding is the presence of increased numbers of LGL, usually identified by a size greater than normal lymphocytes, abundant pale cytoplasm, and prominent azurophilic granules (Fig 1A). However, these features may vary, even among cells from the same patient. For example, granulation can range from fine to coarse, and some cells may have otherwise characteristic features but lack granules (sometimes called large agranular lymphocytes).92 Occasionally, clonally expanded lymphocytes with a characteristic CD3+, CD57+ phenotype may not have LGL morphology on peripheral smear.35,49 It is also important to realize that most patients have only a modest absolute lymphocytosis (median, 7.800/μL, range 1,000 to 49,000/μL); because of this the diagnosis may be overlooked unless the blood smear is examined carefully. Nevertheless, the absolute numbers of circulating LGL are usually markedly increased (median 4,200/μL; normal values, 223 ± 99/μL),90 however, clonal disease has been documented in patients with absolute LGL counts as low as 700/μL (Table 2).

Hematologic findings are shown in Table 3. Most patients have neutropenia, often severe. The incidence of neutropenia may be related to the referral pattern; patients without neutropenia may be asymptomatic and not come to medical attention. Patients with adult-onset cyclic neutropenia also have clonal LGL disease, in contrast to the findings in patients with childhood-onset cyclic neutropenia.59,63 The mechanism of neutropenia is not completely understood. Most patients show "maturation arrest" of the myeloid series in the marrow as well as lymphocytic infiltration (see below); however, the degree of lymphocytic infiltration cannot account for neutropenia. There is some evidence for autoimmune neutropenia: antineutrophil antibodies are common11,49,62,72 and decreased neutrophil survival using kinetic studies has been demonstrated.74 The specificity of the antineutrophil antibodies is not determined; it is conceivable that these antibodies may reflect
Fig 1. Characteristic histopathologic features of T-LGL leukemia. (A) Peripheral blood smear showing typical LGL, note differences in granulation, with some LGL exhibiting fine granulation and other LGL having course granules (Wright-Giemsa stain, original magnification × 990). (B) Sinusoidal infiltration of liver by LGL (Hematoxylin-eosin stain, original magnification × 400). (C) Bone marrow biopsy showing focal lymphocytic infiltration (Hematoxylin-eosin stain, original magnification × 100).
Fig 1. (Cont’d) (D) Marrow aspiration showing ‘maturation arrest’ of myeloid series (Hematoxylin-eosin stain, original magnification X 400). (E) Infiltration of splenic red pulp cords and sinuses by LGL and follicular hyperplasia of splenic white pulp (Hematoxylin-eosin stain, original magnification X 100). (F) CD8-positive LGL infiltrate of red pulp of spleen (immunoperoxidase stain with methyl green counterstain, original magnification X 100). (Figs B through F courtesy of Dr Marshall Kadin, Department of Pathology, Beth Israel Hospital. Fig A courtesy of Dr Robert Hutchison, Department of Pathology, Health Science Center, State University of New York, Syracuse.)
immune complex deposition on neutrophils rather than neutrophil-specific antibodies. Because normal LGL are known to have multiple regulatory roles in normal hematopoiesis, it is tempting to speculate that leukemic LGL may be suppressing granulopoiesis in vivo. However, coculture studies have generally failed to show such suppression in vitro.9-11,45,51,69,74,79

The prevalence of abnormal LGL proliferations in patients presenting with chronic neutropenia has been addressed in several small series96-98 although it should be noted that determination of clonality was not performed in any of these studies. Chan et al96 evaluated 16 consecutive patients who had marrow examinations for unexplained neutropenia; 8 of these had lymphocyte surface marker studies. Of these 8, 5 had increased circulating LGL. T-cell phenotyping was performed in 15 consecutive patients with neutropenia and antineutrophil antibodies; 2 had the CD3+, CD8+ phenotype characteristic of T-LGL leukemia (see below).97 Finally, Picker et al performed immunoperoxidase staining of marrow sections of 15 consecutive patients with neutropenia and antineutrophil antibodies; 2 had the CD3+, CD8+ phenotype characteristic of T-LGL leukemia (see below).97 Of note, these four patients did not have an abnormal percentage of circulating LGL. Taken together, these studies would suggest that T-LGL leukemia is not an uncommon cause of chronic “idiopathic” neutropenia.

Anemia and thrombocytopenia occur less frequently than neutropenia in T-LGL leukemia. Some of these patients develop pure red blood cell (RBC) aplasia; in contrast to the findings in patients with neutropenia, a direct inhibitory effect of leukemic LGL on erythropoiesis in vitro can be demonstrated.56,70,99,100 Hemolytic anemia, both Coombs’ positive and Coombs’ negative, has been observed in a few patients with T-LGL leukemia.11,38,40 Thrombocytopenia, when present, is usually of moderate degree, but can present as idiopathic thrombocytopenia purpura, with adequate numbers of marrow megakaryocytes and antiplatelet antibodies.

### Table 2. LGL Counts in Patients With T-LGL Leukemia

<table>
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<tr>
<th>LGL Count (cells/μL)</th>
<th>Observed</th>
<th>%</th>
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<tbody>
<tr>
<td>600 - 1,000</td>
<td>7/89</td>
<td>8</td>
</tr>
<tr>
<td>1,001 - 4,000</td>
<td>36/89</td>
<td>40</td>
</tr>
<tr>
<td>4,001 - 10,000</td>
<td>35/89</td>
<td>37</td>
</tr>
<tr>
<td>10,001 - 20,000</td>
<td>13/89</td>
<td>14</td>
</tr>
<tr>
<td>&gt; 20,001/μL</td>
<td>1/89</td>
<td>1</td>
</tr>
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Note: Normal LGL counts in our laboratory are 223 ± 99 (mean ± SD, n = 10).9,96

### Table 3. Hematologic Features of Patients With T-LGL Leukemia

<table>
<thead>
<tr>
<th>Hematologic Feature</th>
<th>Observed</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>Neutropenia (&lt;2,000/μL)</td>
<td>109/129</td>
<td>84</td>
</tr>
<tr>
<td>Severe neutropenia (&lt;500/μL)</td>
<td>62/121</td>
<td>48</td>
</tr>
<tr>
<td>Anemia (Hct &lt;33%)</td>
<td>48/97</td>
<td>49</td>
</tr>
<tr>
<td>Thrombocytopenia (&lt;150,000/μL)</td>
<td>13/89</td>
<td>19</td>
</tr>
<tr>
<td>Lymphocytosis (&gt;4,000/μL)</td>
<td>93/126</td>
<td>74</td>
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</tbody>
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Abbreviation: Hct, hematocrit.

### Table 4. Organ Involvement in T-LGL Leukemia

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<tr>
<th>Organ Involvement</th>
<th>Observed</th>
<th>%</th>
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<tbody>
<tr>
<td>Splenomegaly</td>
<td>64/127</td>
<td>50</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>29/127</td>
<td>23</td>
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<tr>
<td>Lymphadenopathy</td>
<td>1/127</td>
<td>1</td>
</tr>
<tr>
<td>Skin involvement</td>
<td>0/127</td>
<td>0</td>
</tr>
<tr>
<td>Bone marrow infarction</td>
<td>81/82</td>
<td>88</td>
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### Table 5. Serologic Abnormalities in Patients With T-LGL Leukemia

<table>
<thead>
<tr>
<th>Serologic Abnormality</th>
<th>Observed</th>
<th>%</th>
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<tbody>
<tr>
<td>Positive RF</td>
<td>34/80</td>
<td>57</td>
</tr>
<tr>
<td>Positive ANA</td>
<td>19/50</td>
<td>38</td>
</tr>
<tr>
<td>Polyclonal hypergammaglobulinemia</td>
<td>30/66</td>
<td>45</td>
</tr>
<tr>
<td>Hypogammaglobulinemia</td>
<td>4/66</td>
<td>6</td>
</tr>
<tr>
<td>Circulating immune complexes</td>
<td>22/39</td>
<td>56</td>
</tr>
<tr>
<td>Antineutrophil antibody</td>
<td>17/41</td>
<td>41</td>
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Abbreviations: RF, rheumatoid factor; ANA, antinuclear antibody.

### Histopathologic features

Distinctive pathologic findings of T-LGL leukemia involve the marrow, spleen, and liver (Fig 1). Skin infiltration and lymphadenopathy are distinctly unusual (Table 4). Most cases show a diffuse lymphocytic infiltration of marrow; however, lymphoid aggregates have been noted in some patients (Fig 1D).11,101 Merlio et al102 have suggested that these lymphoid aggregates may represent reactive B cell nodules, with the leukemic LGL having an extranodal, interstitial infiltration as determined by paraffin immunohistochemistry. In some patients the marrow may be difficult to aspirate; reticular stains have shown some degree of fibrosis.20 Characteristic findings in the spleen include lymphocytic red pulp cord infiltration and often reactive germinal follicles (Fig 1E). These findings need to be distinguished from those of hairy cell leukemia.101 Touch imprints of splenic tissue show that the infiltrating lymphocytes are LGL; immunoperoxidase staining shows that splenic LGL have the same phenotype as circulating LGL (Fig 1F).11 In one study a proportion of leukemic splenic LGL were actively in cell cycle21; cytogenetic and TCR gene rearrangement studies have demonstrated the clonal nature of the splenic infiltrate.11,42,46,65 Plasmacytosis of splenic red pulp and marrow is frequently observed.101 Liver involvement typically shows a lymphocytic infiltration of hepatic sinusoids (Fig 1B); the portal areas may also be involved in more extensive disease. A few unusual cases have shown lymphomatous involvement of the small bowel with CD3+ LGL of either CD45R or CD848J03 phenotype; one patient initially presented with an intracutaneous lymphoma.48 These lymphomas may arise from LGL that reside in normal intestinal mucosa.104,105 Few autopsies have been performed in these patients; we have seen lymphocytic infiltration in tissue other than marrow, spleen, and liver, such as kidneys, thyroid, and adrenal glands.101

### Immune abnormalities

Serologic abnormalities are frequent and include polyclonal hypergammaglobulinemia, circulating immune complexes, and positive tests for rheumatoid factor.
matoid factor or antinuclear antibody (Table 5). As discussed above, antineutrophil antibodies as well as antiplatelet antibodies are also common. Less frequently, patients may present with hypogammaglobulinemia, an occasional patient will have a monoclonal gammopathy. Clonal B-cell disorders, including hairy cell leukemia, have been described rarely in association with T-LGL leukemia. More work is needed to define the mechanism of humoral immune abnormalities occurring in T-LGL leukemia.

Patients with T-LGL leukemia also have defects in cellular immunity. Absolute numbers of CD3+ LGL (normal NK cells) are markedly diminished. NK activity is also diminished or absent. However, leukemic LGL can be stimulated in vitro by anti-CD3 monoclonal antibody (MoAb) interleukin-2 or (IL-2) to acquire non–MHC-restricted cytotoxicity. Antibody-dependent cellular cytotoxicity activity is usually preserved, because leukemic LGL possess IgG Fc receptors (CD16). T-cell defects include diminished proliferative responses to mitogens such as phytohemagglutinin and impairment in formation of high-affinity IL-2 receptors, despite constitutive expression of functional p75 intermediate-affinity IL-2 receptors.

**Phenotype of leukemic LGL.** CD3+ leukemic LGL express LGL antigens, such as CD16 and CD57, to varying extents (Fig 2A). Another LGL antigen, CD56, is uncommonly expressed on CD3+ leukemic cells. It is likely that CD3+ leukemic LGL from all patients express CD16; however, discrepancies have been noted in the staining pattern of leukemic cells using a panel of anti-CD16 MoAbs. For example, CD3+ leukemic LGL are often Leu 11 negative but VD2 or 8.28 positive. This variable expression of CD16 as defined by MoAb staining is most likely caused by the structural differences of CD16 antigen on T cells compared with NK cells. Two-color analyses are helpful in identifying leukemic LGL as CD3+, CD57+ or CD3+, CD16+. Cells coexpressing these antigens are a rare component of normal blood.

Subclassification of CD3+ leukemic LGL is possible based on expression of CD4, CD8, and TCR antigens; however, it is not known whether such a classification has any clinical relevance. Greater than 95% of cases have a CD3+, TCRαβ+, CD4+, CD8- phenotype. Leukemic LGL may also be CD3+, TCRγδ-, being CD4+, CD8- or CD4+, CD8+. These TCRγδ+ cases seem to have similar clinical presentations to TCRαβ+ cases, including neutropenia, rheumatoid arthritis, and pure RBC aplasia, although only a few patients with this phenotype have been described. Rare cases of CD3+, TCRβ+, CD4+, CD8- have also been published; it is of interest that these patients have normal or only moderately decreased neutrophil counts. Occasionally, CD3+ leukemic LGL are both CD4+ and CD8+.

**Etiology.** The cause of the clonal LGL proliferation is not known. CD3+, CD57+ cells, the rare normal counterparts of leukemic LGL, are thought to represent in vivo activated CTL of unknown antigen specificity. CD3+ leukemic LGL will proliferate in vitro after activation with anti-CD3 MoAb (mimicking antigen activation) or lymphokines such as IL-2 or IL-4. Optimum stimulation is provided by anti-CD3 MoAb plus lymphokines. It is conceivable that antigen activation plus lymphokine secretion could lead to leukemic LGL proliferation in vivo. Clonal evolution would depend on additional genetic or environmental factors. The development of additional cytogenetic abnormalities observed in one patient with progression from chronic to an acute phase would support such a model.

Retroviral infection may represent a pathway of antigen activation. In 1987 we found that 6 of 12 patients with T-LGL leukemia had serologic reactivity to human T-cell leukemia virus I/II (HTLV-I/II) antigens. Reactivity was directed against HTLV-I/II gag proteins, p19 and/or p24. Seroreactivity to HTLV-I/II gag proteins was subsequently reported in 7 of 27 European patients with LGL disease. No seropositivity was observed in Japanese patients with this disease. We recently showed that seroreactivity in one patient was caused by HTLV-II and not HTLV-I infection. We also cloned and sequenced HTLV-II from marrow mononuclear cell DNA of this patient using polymerase chain reaction (PCR) amplification. This patient had not received prior blood transfusions and was not an intravenous drug user. In another patient with T-LGL leukemia, HTLV-I was isolated; however, that patient had received multiple blood transfusions before the detection of HTLV-
In view of familial transmission of retroviral infection, it is of interest that clonal LGL proliferation has been documented in a father and his two children. We have also observed T-LGL leukemia in a mother and her son; serum from the son was indeterminate for HTLV-1/2 infection, reacting with gag protein p19. Taken together, these findings suggest an infectious cause of T-LGL leukemia. Disease manifestations seen in association with clonal LGL proliferation such as pure RBC aplasia, chronic or cyclic neutropenia, rheumatoid arthritis, or immunodeficiency might then depend on host-virus interactions or viral genotype, as occurs for animal retroviral infections.

Prognosis and therapy. It is difficult to assess the natural history of T-LGL leukemia because few patients have been followed up by any one group of investigators for sufficient time. One study indicated a 20% mortality after 4 years of prospective follow-up; however, that study included patients without documented clonal disease as well as patients with CD3+ LGL proliferative disorders. There has been only one report of a spontaneous "remission" in a patient with clonal disease; that patient had clinical remission of neutropenia and anemia but still had evidence for a persistent clone by Southern blot analysis. Subsequently, neutropenia and anemia recurred in that patient (E. Winton, personal communication, September 1992). We have observed only one case in which there was a disappearance of clonal disease without treatment; however, that patient developed rheumatoid arthritis (see above).

In contrast to the impression that T-LGL leukemia is an indolent disease not requiring treatment, most patients in our series (24 of 33) have eventually needed therapy. The primary indication for treatment has been recurrent infections caused by severe neutropenia. Optimum treatment for severe neutropenia remains undefined. Splenectomy usually has only limited benefit in correcting neutropenia and results in increased numbers of circulating leukemic cells. Improvement in neutrophil counts with prednisone therapy has been observed in a few patients, but most reported cases show no improvement. Experience with hematopoietic growth factors is limited; granulocyte-macrophage colony-stimulating factor (GM-CSF) does not seem to be effective, whereas a few patients have responded to G-CSF. We are conducting a clinical trial of use of low-dose methotrexate. Preliminary results seem encouraging in correcting severe neutropenia.

Pure RBC aplasia associated with T-LGL leukemia can be treated effectively with single-agent chemotherapy such as methylprednisolone, chlorambucil, or cyclophosphamide. Correction of neutrophil cycling in patients with adult-onset cyclic neutropenia associated with T-LGL leukemia has been achieved with alternate day steroid therapy; however, clinical remission did not result in the complete disappearance of the abnormal clone.

Nine of our 25 patients followed up prospectively for at least 2 years have died, usually of sepsis. Thus, it would seem that the majority of patients have a chronic disease with significant morbidity and mortality resulting from the consequences of neutropenia. However, some patients have a more aggressive and rapid clinical course, often marked by systemic B symptoms. Foroni et al have noted that the aggressive course may correlate with the extent of clonal disease as evaluated by Southern blot analysis, i.e., the lack of germline band indicates no residual normal cells, was associated with aggressive disease. Treatment of these aggressive cases with combination chemotherapy has not been successful.

Differential diagnosis. The diagnosis of T-LGL leukemia should be suspected in patients with chronic neutropenia, pure RBC aplasia, adult-onset cyclic neutropenia, or rheumatoid arthritis. Careful examination of the peripheral blood smear is mandatory, because some of these patients may not have an absolute lymphocytosis. In most patients with T-LGL leukemia, an increase in percentage and absolute numbers of LGL will be evident. However, clonally derived lymphocytes in some patients may not have LGL morphology but have the characteristic CD3+, CD8+, CD16+, CD57+ phenotype. Therefore, lymphocyte phenotyping is recommended in all patients with clinical features suggesting T-LGL leukemia, particularly in patients with neutropenia and rheumatoid arthritis. The diagnosis is established in patients with characteristic lymphocyte phenotypes by demonstrating clonal cytogenetic abnormalities or clonal TCR gene rearrangement. Other conditions causing atypical lymphocytosis, particularly viral infections, should be considered. Epstein-Barr virus (EBV) infection causes an atypical lymphocytosis; however, the atypical lymphocytes are not CD57+. Cytomegalovirus infection can cause a mild increase in CD3+, CD57+ LGL; however, patients with active cytomegalovirus disease do not have evidence for TCR gene rearrangement. Patients with human immunodeficiency virus infection may also have increases in CD16+ LGL; however, these LGL are polyclonally expanded (G. Semenzato, personal communication, September 1992).

NK-LGL LEUKEMIA (CLONAL CD3+)

Patients with NK-LGL leukemia also have clonally expanded LGL, but of CD3+ phenotype. The clinical presentation is quite different than CD3- LGL leukemia, as reviewed below.

Clinical and hematologic features. The median age of 39 years is younger than that observed in T-LGL leukemia (Table 6). There is an equal male/female distribution. Manifestations of systemic disease are often the initial clinical presentations; high fever without signs of infection and other B symptoms are common. Rheumatoid arthritis has not been observed in any patient; however, NK-LGL leukemia has an acute clinical course. Mild neutropenia is common; however, in contrast to

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<th>Table 6. Clinical Features of Patients With NK-LGL Leukemia</th>
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<td><strong>Observed</strong></td>
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<td>-------------------</td>
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<tr>
<td>Median age, range</td>
</tr>
<tr>
<td>Male/female</td>
</tr>
<tr>
<td>Fever</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
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T-LGL leukemia, severe neutropenia is unusual. Anemia and thrombocytopenia are more common and pronounced in NK-LGL leukemia; thrombocytopenia may be severe, in the range of 10,000 to 20,000/μL. It has been reported that one patient had hemolytic anemia. Most patients have had an absolute lymphocytosis at presentation (Table 7). There are often rapid increases in LGL counts (>50,000/μL) occurring over a few weeks in this disease.

Other clinical features in NK-LGL leukemia are also different than T-LGL leukemia (Table 8). Massive hepatosplenomegaly is common in NK-LGL leukemia. These patients often have involvement of the gastrointestinal system, with jaundice and ascites, analysis of peritoneal fluid showed LGL in one case. Jejunal perforation has also been reported. Coagulopathy is also frequent. Lymph node involvement is observed more often in NK-LGL leukemia than T-LGL leukemia. Central nervous system disease, with spinal fluid examination showing LGL, has been documented in one case. Like T-LGL leukemia, the marrow shows LGL infiltration in NK-LGL leukemia. Immune function has not been evaluated in many of these patients, although two patients have had polyclonal hypergammaglobulinemia and a monoclonal protein was observed in another patient. These clinical features of NK-LGL leukemia are similar to those described for the F344 rat model of CD3- LGL leukemia.

**Phenotype of NK-LGL leukemia.** LGL from these patients are indistinguishable by light microscopy from LGL of patients with T-LGL leukemia (Fig 2B). One group of investigators has indicated that ultrastructural features may differ: CD3 leukemic LGL often have parallel tubular arrays, whereas cells from patients with CD3 LGL proliferations do not. The usual phenotype is CD3, CD4, CD8, CD16, CD56, and CD57, in contrast to T-LGL leukemia in which cells are CD3, CD8, CD57, and usually CD56 (see above).

**Etiology.** A remarkable finding is that most of the NK-LGL leukemia patients with clonal cytogenetic abnormalities described are from Japan. Kawa-Ha and colleagues have implicated EBV infection in the pathogenesis of NK-LGL leukemia in these Japanese patients, because they found clonal integration of EBV in leukemic cells. This finding has recently been confirmed in an NK-LGL leukemia patient reported from New Zealand. We were unable to find EBV genome using Southern blot analyses in DNA from European or American patients with chronic CD3- LGL lymphocytosis (a subset of whom were informative for X-linked gene analysis and were shown not to have a clonal disease, see below) or in DNA from our T-LGL leukemia patients, although all patients were seropositive for EBV.

**Prognosis and therapy.** In contrast to the usual chronic course of T-LGL leukemia, most patients with NK-LGL leukemia have an acute presentation of a systemic illness (see above). Nine of the 11 reported patients died, usually within 1 to 2 months after diagnosis, despite aggressive treatment with multiagent chemotherapy. The main cause of death is disseminated disease with associated coagulopathy and multiorgan failure. Autopsy examinations have shown LGL infiltration of spleen, marrow, and liver as well as lymph nodes, small bowel, and kidneys. There have been two apparent complete responses to combination chemotherapy. One patient was treated with ProMACE-Cytarabine and died of *Candida tropicalis* infection during neutropenia; autopsy showed no infiltration of spleen, marrow, or liver by LGL. The other patient presented with nephrotic syndrome, and was treated with cyclophosphamide, vincristine, and prednisone. Complete remission was of 10-months duration at the time of the report.

Although NK-LGL leukemia is usually diagnosed after an acute presentation, there is some evidence that there may be an antecedent chronic phase. A few patients have had a more chronic clinical course for several years before acceleration of disease. Furthermore, additional cytogenetic abnormalities have been noted in one patient at the time of transformation from chronic phase.

**LGL LYMPHOCYTOSIS (CD3- OR CD3-; NO EVIDENCE OF CLONALITY)**

Some patients have persistently increased numbers of either CD3- or CD3 LGL, which cannot be proven to be clonally expanded. In large series most patients with the typical clinical syndrome associated with increased numbers of CD3- LGL have a clonal disorder (for example, 15 of 15 by Rambaldi et al and Pelicci et al and 33 of 34 in our series). Nevertheless, clonality cannot be proven in some typical cases of CD3- LGL patients using TCR rearrangement studies. Failure to demonstrate clonality using such methodology may be caused by technical artifact or failure to use appropriate TCR gene probes. For example, in some patients the CD3- LGL are not TCRαβ+ but rather TCRγδ+ (see above). In such cases, TCRβ probes will not show clonality but TCRγ probe will. Lack of clonality using TCR gene probes may also reflect the ontogeny of the transformed cells. Brito-Babapulle et al found a clonal cytogenetic abnormality in a patient with CD3- LGL; a germline pattern for TCRγ gene and polyclonal rearrangement of the TCRγ gene was shown. They specu-

**Table 7. Hematologic Features of Patients With NK-LGL Leukemia**

<table>
<thead>
<tr>
<th></th>
<th>Observed</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutropenia (&lt;2,000/μL)</td>
<td>7/11</td>
<td>64</td>
</tr>
<tr>
<td>Severe neutropenia (&lt;500/μL)</td>
<td>2/11</td>
<td>18</td>
</tr>
<tr>
<td>Anemia (Hct &lt;30%)</td>
<td>9/9</td>
<td>100</td>
</tr>
<tr>
<td>Thrombocytopenia (&lt;150,000/μL)</td>
<td>6/8</td>
<td>75</td>
</tr>
<tr>
<td>Lymphocytosis (&gt;4,000/μL)</td>
<td>9/10</td>
<td>90</td>
</tr>
</tbody>
</table>

**Table 8. Organ Involvement in Patients With NK-LGL Leukemia**

<table>
<thead>
<tr>
<th></th>
<th>Observed</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splenomegaly</td>
<td>10/11</td>
<td>91</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>7/11</td>
<td>64</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>3/11</td>
<td>27</td>
</tr>
<tr>
<td>Marrow infiltration</td>
<td>9/9</td>
<td>100</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>1/11</td>
<td>9</td>
</tr>
</tbody>
</table>
lulated that a TCRγδ precursor was the target of the malignant change. It

Inability to demonstrate clonality may also mean that the disease has a polyclonal phase antecedent to clonal evolution. Because normal CD3+ LGL are thought to represent CTL of unknown antigen specificity, it is hypothesized that CD3+ LGL in these patients are CTL that first undergo polyclonal expansion in response to antigen stimulation (see above). In support of this theory, Agostini et al demonstrated a polyclonal expansion of CD3+, CD8+ LGL directed against hepatitis B virus-infected CD4+ lymphocytes in one patient. Subsequent clonal transformation might then depend on additional genetic or environmental factors. However, it is important to emphasize that polyclonal-to-clonal progression has not been documented in this disease.

It is more difficult to prove clonality in patients with proliferative disorders of CD3- LGL, because of the lack of a clonal marker. TCR gene rearrangement studies cannot be used to assess clonality, because normal CD3- LGL and LGL from patients with CD3- lymphoproliferation do not rearrange TCRα, β, γ, or δ genes. Clonal cytogenetic abnormalities occurring in patients with an acute presentation involving CD3- LGL have supported the diagnosis of NK-LGL leukemia (see above). Patients with similar clinical features have also been reported; however, cytogenetic studies were not performed. It would seem reasonable to designate such patients as having NK-LGL leukemia without needing to rely on cytogenetic studies, particularly because no unique cytogenetic abnormalities have yet been identified in this disease. In contrast, most patients with increased numbers of CD3- LGL have a different clinical syndrome, marked by a chronic course. These patients have mild-to-moderate neutropenia, but would seem to have a much lower incidence of rheumatoid arthritis and autoimmune phenomena than T-LGL leukemia patients. It was not known whether such patients with the chronic CD3- LGL proliferation have a clonal disorder. Recently, we studied seven women with chronic CD3- LGL from Europe and the United States who were heterozygous for certain X-linked loci. Using such X-linked gene analyses, we could not demonstrate clonality in any of these patients. These data suggest a reactive rather than neoplastic origin of the LGL lymphocytosis. However, it is conceivable that such a polyclonal proliferation may represent a preneoplastic state. As noted above, some of the patients with NK-LGL leukemia may have a chronic phase before acute transformation. Serial studies of clonality using this methodology are needed to address whether clonal progression can be demonstrated.

OTHER MALIGNANCIES WITH LGL PHENOTYPE

There are other malignant diseases in which tumor cells express some LGL antigens. The ontogenetic relationship of these diseases to T- or NK-LGL leukemia is not known. Acute lymphocytic leukemia (ALL). There have been several case reports of ALL cells with a CD3-, CD16+, CD56+ phenotype, CD3+, CD8+, CD16+ phenotype, and CD3-, CD16-, CD57+ phenotype. Blast morphology was FAB L2; the blasts did not have LGL morphology. Kaplan et al described 14 cases of "T"-ALL; 4 were CD57+. All 4 had L2 morphology; 1 was CD3+, and in 1 case, tumor cells contained granules. Non-Hodgkin’s lymphoma. There seems to be a distinct group of non-Hodgkin’s lymphomas in which the tumor cells are CD16+ or CD56+. These tumors usually are diffuse large cell lymphomas. Ng et al reviewed 149 cases of non-Hodgkin’s lymphoma occurring in Chinese patients; 17 were CD16+ and/or CD56+. Seventy-six percent of the CD16+/CD56+ tumors involved the nasopharynx; conversely, 52% of nasopharyngeal lymphomas were CD16+ and/or CD56+. The predilection for nasopharynx of CD56+ lymphomas was confirmed in a series of 11 patients from the United States; other unusual sites for these tumors included central nervous system and muscle.

Several cases of CD4+, CD16+, CD57+ lymphoblastic lymphomas have also been described; in one series, 6 of 38 patients with lymphoblastic lymphoma had this phenotype. Finally, three of seven CD8+ immunoblastic lymphomas were also CD57+; imprints from one of these cases showed LGL.

SUMMARY

Three distinct clinical syndromes occur in patients with increased numbers of circulating LGL. Patients with T-LGL leukemia have clonal proliferations of CD3+ LGL typically associated with chronic neutropenia and autoimmune features. NK-LGL leukemia is characterized by clonal CD3- LGL proliferation with an acute clinical presentation marked by massive hepatosplenomegaly and systemic illness. However, most patients with increased numbers of CD3- LGL do not have clinical features of NK-LGL leukemia and have a chronic clinical course. X-linked gene analyses have supported a polyclonal LGL lymphocytosis in this syndrome. Further studies are needed to determine whether clonal progression can occur in these patients.

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Clonal diseases of large granular lymphocytes [see comments]
TP Jr Loughran

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