Granulated T Cell Lymphocytosis With Neutropenia: Malignant or Benign Chronic Lymphoproliferative Disorder?

By Robert W. McKenna, Diane C. Arthur, Kazimiera J. Gajl-Peczalska, Patrick Flynn, and Richard D. Brunning

Granulated T cell lymphocytosis with neutropenia is a chronic lymphoproliferative disorder characterized by moderate blood and bone marrow lymphocytosis, absence of lymphadenopathy and, usually, splenomegaly and polyclonal hypergammaglobulinemia. The proliferative cells have been identified as T lymphocytes or as reacting with monoclonal antibodies to suppressor/cytotoxic T lymphocytes. In the first descriptions of this disorder, it was generally considered a T cell chronic lymphocytic leukemia. However, the chronicity and lack of progression of the disease in most patients has caused controversy regarding its malignant or benign nature. Authors of several recent reports have favored a benign, reactive, or immunoregulatory disorder as the etiology.

In this study of seven patients, the clinical, morphological, ultrastructural, immunologic, and cytogenetic findings of granulated T cell lymphocytosis with neutropenia are detailed. Partial data on four of the patients have been previously described; our original studies were inconclusive regarding the biology of this disease. Additional observations reported here are suggestive of a neoplastic nature in some cases, but others have manifested a clinical course more characteristic of a reactive process.

MATERIALS AND METHODS

The clinical review, routine morphological evaluation, and electron microscopic studies were performed by methods previously detailed. Immunologic studies. Specimens were processed and studied for cell membrane markers as previously reported. The phenotyping by monoclonal antibodies was done using an indirect immunofluorescence procedure with quantitation of positive cells either by fluorescence microscopy or flow cytometry, as previously reported. Lymphocyte functional studies were performed on lymphocytes from patient 6. The suppressor or helper effect of patient T cells on immunoglobulin secretion by allogeneic B cells in co-cultures, following pokeweed mitogen stimulation, was studied using previously published techniques. Mitogenic responses were studied in four- or five-day cultures on density gradient-separated patient lymphocytes, using standard techniques.

Cytogenetic studies. Karyotype analysis was performed on peripheral blood specimens from patients 5, 6, and 7; on bone marrow specimens from patients 5 and 6; and on skin from patient 5. Metaphase cells were harvested from direct preparations, 24-hour unstimulated cultures, and 72-hour phytohemagglutinin (PHA)-stimulated cultures of heparinized whole bone marrow, using previously published methods. Peripheral blood cultures were done in the laboratory of Dr Lisa Filipovich. Mononuclear cells were separated from heparinized venous blood over an Isolymph gradient (Gallard-Schlesinger, SG 1.077) and plated at a concentration of 1.0 x 10^6/200 μL RPMI 1640 medium (GIBCO, Grand Island, NY) in 96-well round bottom microtitre plates. Cells were cultured for 72 hours at 37°C in 5% humidified CO2 with the following mitogenic agents: 1 μg/mL phytohemagglutinin (HA-17, Burroughs Wellcome, Research Triangle Park, NC), 100 ng/mL phorbol myristate acetate (PMA, Sigma, St Louis), 5 x 10^-8 molar calcium ionophore A23187 (Sigma), and 50% T cell conditioned medium as a source of interleukin-2 (IL-2) (48-hour supernatants of lymphocytes pooled from multiple donors, irradiated, and stimulated with 1 μg/mL phytohemagglutinin). Cells from approximately ten wells were pooled and harvested by the direct method described above for bone marrow. Skin fibroblasts from a 3-mm punch biopsy of patient 5 were cultured in 25-cm² sterile flasks with Dulbecco’s modified Eagle’s medium with glutamine (GIBCO) supplemented with 10% fetal calf serum and penicillin (100 U/mL)-streptomycin (100 μg/mL). After approximately three weeks, these cultures were trypsinized, subcultured, and harvested by routine techniques.

From the Department of Laboratory Medicine and Pathology, University of Minnesota Health Sciences Center, Minneapolis. Submitted Nov 26, 1984; accepted Jan 19, 1985.

Address reprint requests to Dr Robert W. McKenna, Department of Laboratory Medicine and Pathology, Box 198, Mayo Memorial Bldg, University of Minnesota, Minneapolis, MN 55455.
Table 1. Clinical Findings

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex/Age</th>
<th>Diagnosis</th>
<th>Splenomegaly</th>
<th>Hepatomegaly</th>
<th>Lymphadenopathy</th>
<th>Serum Immunoglobulins (mg/dL)*</th>
<th>Heterophile Antibody</th>
<th>Coomb’s Test</th>
<th>Rheumatoid Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M, 19</td>
<td>Cellulitis</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>IgG 2,300</td>
<td>−</td>
<td>−</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>M, 26</td>
<td>Rectal abscess</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>1,200 (total)</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>3</td>
<td>M, 58</td>
<td>Back pain</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>IgG 1,730</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>M, 75</td>
<td>Weakness, macrocytic anemia, dizziness</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>IgG 1,710</td>
<td>IgA 178</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>5</td>
<td>F, 76</td>
<td>Infected knee, anal fissures</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>IgG 1,240</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>M, 36</td>
<td>Asymptomatic</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>IgG 4,100</td>
<td>−</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>F, 55</td>
<td>Macrocytic anemia</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>IgG 1,420</td>
<td>IgA 281</td>
<td>−</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not done.

*Normal values: IgG, 372 to 1,356; IgA, 40 to 468; IgM, 48 to 250.

Metaphase chromosomes from all tissues were G-banded using the Wright's method of Sanchez et al. 33

RESULTS

The presenting clinical and laboratory findings of the seven patients are detailed in Tables 1 and 2. The patients ranged in age from 19 to 76 years; three were <40 years. Five were men. Three of the patients presented with infection-related complaints. Six had splenomegaly, three had hepatomegaly, and none had lymphadenopathy. Six patients had polyclonal hypergammaglobulinemia. All had negative heterophile antibody studies. One patient developed mild Coomb's positive hemolysis, and one had a higher rheumatoid factor without clinical arthritis.

The initial leukocyte count ranged from $5.2 \times 10^9/L$ to $35.5 \times 10^9/L$ (median, $11.7 \times 10^9/L$); four of the patients had leukocytosis. Four were slightly to moderately anemic; three had macrocytic red cell indices without evidence of B12 or folate deficiency. One patient was mildly thrombocytopenic. All patients had lymphocytosis with absolute lymphocyte counts ranging from $5 \times 10^9/L$ to $34.5 \times 10^9/L$ (median, $10.6 \times 10^9/L$). Six of the patients had severe or moderate neutropenia. The absolute neutrophil counts varied from $0.2 \times 10^9/L$ to $2.1 \times 10^9/L$; four patients had neutrophil counts of $<0.5 \times 10^9/L$.

Morphology. In all seven cases, the majority of the blood lymphocytes were medium sized to large sized, with a moderate amount of lightly basophilic cytoplasm. The cytoplasm of 83% to 94% of the lymphocytes contained variable numbers of coarse azurophilic granules (Fig 1). The nuclei were round or oval with regular margins and coarse chromatin; nucleoli were generally lacking. On ultrastructural examination, more than 75% of the lymphocytes in all seven cases contained cytoplasmic bundles of parallel tubular arrays (PTAs) (Fig 2). These PTAs appeared to correspond to the azurophilic granulation seen in light microscopy.

The bone marrow was slightly hypercellular in all patients. The lymphocyte percentage in the marrow ranged from 22% to 61% (median, 53%). The cytology of the majority of the bone marrow lymphocytes was identical to the granulated lymphocytes in the blood. In trephine biopsy sections, there was generally diffuse lymphoid infiltration with focal accentuation in some cases (Fig 3). The granulocyte precursors were moderately decreased in all cases, and erythroid precursors appeared diminished in six. The neutrophil maturation sequence appeared normal except in patient 5, whose marrow

Table 2. Initial Laboratory Data

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Blood Values</th>
<th>Bone Marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Leukocytes ($\times 10^9/L$)</td>
<td>Absolute Lymphocytes ($\times 10^9/L$) (%)</td>
</tr>
<tr>
<td>1</td>
<td>11.7</td>
<td>11.0 (94)</td>
</tr>
<tr>
<td>2</td>
<td>35.5</td>
<td>34.5 (96)</td>
</tr>
<tr>
<td>3</td>
<td>9.0</td>
<td>6.0 (67)</td>
</tr>
<tr>
<td>4</td>
<td>12.3</td>
<td>10.6 (86)</td>
</tr>
<tr>
<td>5</td>
<td>5.2</td>
<td>5.0 (96)</td>
</tr>
<tr>
<td>6</td>
<td>13.0</td>
<td>11.7 (90)</td>
</tr>
<tr>
<td>7</td>
<td>7.2</td>
<td>6.4 (89)</td>
</tr>
</tbody>
</table>
showed only occasional cells beyond the neutrophil myelocyte stage of development.

Spleen tissue from patient 2 was available for examination. The spleen weighed 850 g. The splenic microarchitecture was basically intact. Follicles appeared normal in number and size and lacked germinal centers. The red pulp was expanded and heavily infiltrated with lymphocytes that were morphologically uniform and mature in appearance. The lymphocytic infiltrate was diffuse and predominantly sinusoidal in distribution but also involved the splenic cords.

**Immunology.** Sixty percent to 88% of the blood lymphocytes from the seven patients had receptors for unsensitized sheep erythrocytes; 1% to 7% had surface immunoglobulin. In three of three cases studied (cases 2, 3, and 4), 55%, 83%, and 95% of the lymphocytes manifested receptors for the Fc portion of IgG, and in three of three additional cases (cases 5, 6, and 7) 57%, 74%, and 78% of the lymphocytes reacted with monoclonal antibody OKT8 (Table 3). In these three patients, 9% to 14% of the lymphocytes reacted with monoclonal antibody OKT4. Thus, in six of the six cases, the majority of blood T lymphocytes were either FcγR⁺ or OKT8⁺ suppressor/cytotoxic cells. The other case (case 1) was not studied for Fc receptors or with monoclonal antibodies.

Functional studies for proliferative responses and suppressor/helper activity of lymphocytes were performed in case 5 (Table 4). The patient lymphocytes showed reduced responsiveness with concanavalin A, pokeweed mitogen, and phytohemagglutinin, and manifested strong suppression of pokeweed mitogen-induced immunoglobulin production when cocultured with normal allogeneic tonsilar B lymphocytes.

**Cytogenetics.** Nonbanded chromosome studies on the blood lymphocytes from patient 1 were performed and previously reported by Brody et al.26 These investigators identified an abnormal clone with a 47,XY,+C karyotype. G-banded chromosome analysis was done on bone marrow, peripheral blood, and skin from patient 5. Seventeen metaphase cells (two from a direct preparation and 15 from PHA-stimulated cultures) from bone marrow had a normal 46,XX female karyotype, and three from PHA-stimulated cultures had 45 chromosomes with only one X (45,X).
Table 4. Blood Lymphocyte Functional Studies (Patient 6)

<table>
<thead>
<tr>
<th>Mitogenic Response</th>
<th>Suppressor/Helper Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstimulated Cells</td>
<td>Tonsil B Lymphocytes (1 x 10^6) Incubated With 1.5 x 10^6 T Lymphocytes</td>
</tr>
<tr>
<td>Concanavalin A (5 mg/mL)</td>
<td>IgM</td>
</tr>
<tr>
<td>Pokeweed Mitogen (10 mg/mL)</td>
<td>IgG</td>
</tr>
<tr>
<td>Phytohemagglutinin (20 mg/mL)</td>
<td>IgG</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Case No.</th>
<th>OKT3</th>
<th>OKT4</th>
<th>OKT6</th>
<th>OKT8</th>
<th>OKT11</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>75</td>
<td>11</td>
<td>0</td>
<td>57</td>
<td>74</td>
</tr>
<tr>
<td>6</td>
<td>84</td>
<td>14</td>
<td>2</td>
<td>78</td>
<td>85</td>
</tr>
<tr>
<td>7</td>
<td>83</td>
<td>9</td>
<td>0</td>
<td>74</td>
<td>86</td>
</tr>
</tbody>
</table>

A total of 50 G-banded metaphase cells were analyzed from direct preparations, 24-hour unstimulated, and 72-hour PHA-stimulated cultures of bone marrow from patient 6. All had a normal 46,XY male karyotype. Twenty metaphase cells were analyzed from each of the peripheral blood cultures stimulated with HA-17, calcium ionophore, and IL-2, and all had normal karyotypes. Of 12 metaphase cells obtained from phorbol myristate acetate (PMA)-stimulated cultures, seven had a normal karyotype and five had the following abnormal karyotype which is shown in Fig 4: 46,Y,-X,-14,del(2)(p13),+der(X),t(X;14)(q22;q11),+mar,t(5;12)(?;p11). Peripheral blood chromosome analysis was repeated five months after the initial evaluation, and one metaphase cell from the PMA-stimulated cultures again showed this same abnormal karyotype.

Thirty G-banded metaphases were analyzed from each of the four stimulated peripheral blood cultures from patient 7. Six of the metaphases from the HA-17-stimulated cultures and five of the metaphases from the IL-2-stimulated cultures were missing one X chromosome; however, random loss of other chromosomes was significant, occurring in 57% and 67% of the metaphases analyzed, respectively. Random structural rearrangements were found in three metaphases from the calcium ionophore-stimulated cultures, two metaphases from the HA-17-stimulated cultures, and one meta-
GRANULATED T CELL LYMPHOCYTOSIS

Fig 4. Karyotype of one metaphase cell from PMA-stimulated cultures of peripheral blood mononuclear cells from patient 6 showing abnormalities of chromosomes 2, 14, and X.

The lymphocytosis and severe neutropenia recurred when steroids were stopped. The other two patients were treated less intensively and sporadically with steroids, with a moderate decrease in the lymphocytosis in one and no appreciable response in the other. Four patients had not been treated for their lymphoproliferative disorder.

Patient 4, a 79-year-old man, died four years from diagnosis with persistence of his lymphoproliferative disorder. His death was cardiac related. He had an increasingly severe refractory anemia for several years as well as persistent neutropenia.

Five patients are known to be living from three months to 17 years from diagnosis. Four of them (cases 1, 5, 6, and 7) have a persistence of their lymphoproliferative disorder but...
have remained clinically stable, including the two patients (cases 1 and 6) with a clonal chromosome abnormality in blood lymphocytes. One patient (case 2) appears to have obtained a spontaneous regression of his lymphoproliferative process and neutropenia after 11 years and has remained well for six years. Another patient, case 3, was lost to follow-up at 11 months. When the patient was last seen, his neutrophil and lymphocyte counts were normal.

DISCUSSION

Granulated T cell lymphocytosis with neutropenia is a chronic lymphoproliferative disorder characterized by moderate blood and bone marrow lymphocytosis, absence of lymphadenopathy, and usually splenomegaly, and hypergammaglobulinemia.1-3,13 The neutropenia is often severe and accompanied by infectious complications.2,4,6,7,14 A moderate degree of macrocytic anemia and/or mild thrombocytopenia is present in some cases.2,3,12 The disease appears to have a prolonged course with little progression of symptomatology in most instances. The proliferative lymphocytes have generally typed as Tγ cells and have reacted with monoclonal antibodies to suppressor/cytotoxic lymphocytes.2,4-14 Functionally, the lymphocytes have manifested in vitro antibody-mediated cytotoxicity in most cases4,6,8-10,14,16,28; occasionally, they have expressed suppressor or natural killer activity.9,10,16,29

The two important questions regarding granulated T cell lymphocytosis with neutropenia that remain unresolved are whether the lymphocytosis is of a malignant or benign nature and what the etiology of the neutropenia is. In several reports, the process has been designated T cell chronic lymphocytic leukemia because of the chronic course, presence of organomegaly, and lack of an identifiable infectious or immune etiology.3,13,11,13 In other studies, a reactive or immunoregulatory disorder has been proposed because of the lack of progression of the disease, moderate lymphocytosis and minimal degree of marrow infiltration, and in one report, the morphological and immunohistologic heterogeneity of the blood lymphocytes.4,6,7,14 In none of these studies, however, has a specific infectious or immune cause been demonstrated. The presence of blood lymphocyte clonal chromosomal abnormalities in two patients in the present study suggests a neoplastic etiology in these cases.

Cytogenetic studies have been reported in a small number of cases of granulated T cell lymphocytosis.4,6,9,15,26,30 In most of these cases, the chromosomes have been normal. In the few cases in which karyotypic abnormalities have been found, no consistent pattern has been identified. Loughran et al found in two of three patients; an extra number 8 chromosome in one and an extra 14 in another.9 Catovsky and associates reported one case with cytogenetic abnormalities involving chromosomes 15, 18, and 19.9 There was no consistent chromosome abnormality in the two patients in the present study. Patient 6 in this study has a structural rearrangement of chromosome 14 with a break in band 14q11. This break point has been reported in other patients with T cell disease.31

There are additional reports of cytogenetic abnormalities in T cell chronic lymphoproliferative processes; however, the majority of these disorders appear to be clinically and morphologically distinct from granulated T cell lymphocytosis. Finan and associates found chromosome abnormalities in seven of seven cases of chronic T cell leukemia.32 No consistent change was found, but alterations in chromosomes 2 and 14 were the most common. It is unclear how many of these seven may have been examples of granulated T cell lymphoproliferative disorders because detailed clinical and morphological descriptions were lacking. Ueshima et al also found rearrangements of chromosome 14 in four of four patients with chronic T cell leukemia/lymphoma; however, none of these patients appeared to have had granulated T cell lymphocytosis with neutropenia.31 Although no consistent karyotypic pattern has been identified, there appears to be a relatively high frequency of rearrangements involving chromosomes 2 and/or 14 in the various chronic T cell lymphoproliferative disorders, including granulated T cell lymphocytosis with neutropenia.

The reports of normal chromosomes in the blood lymphocytes in some cases of granulated T cell lymphocytosis may indicate an absence of a clonal abnormality in those cases or that the mitoses evaluated were not from the proliferative lymphocytes. The lymphocytes in this disorder have been demonstrated to have a decreased response to mitogen stimulation in this study and others.3,14,28 Difficulty may be encountered in finding adequate mitoses for study, particularly when only PHA-stimulated lymphocytes are evaluated. Finan et al noted that the clone of chromosomally abnormal lymphocytes in some patients with chronic T cell leukemia gave variable responses and yield of mitoses with different mitogens.32 Multiple mitogens were used in the cytogenetic analyses in three of the cases in the present study, and the abnormal clone of patient 6 was identified only in cultures stimulated with phorbol myristate acetate.

The etiology of the neutropenia in this disorder has not been elucidated. Neutrophil precursors in the bone marrow have been shown to be decreased in most instances; in some cases, the marrow has shown granulocytic hyperplasia.30 In six of the seven cases in the present study, normal granulocyte maturation was observed in the marrow. In one of our patients and in several in other studies, an apparent bone marrow maturation arrest at the myelocyte stage of development was noted.3,4,6,7,14 Neutrophil antibodies have been demonstrated in the serum of some patients9,30; in one study, the neutropenia was attributed to a neutrophil-reactive IgG antibody.9 In other cases, antineutrophil antibodies have not been detected.9,7

It has been suggested that the proliferative lymphocytes may exert a suppressor effect on granulopoiesis; however, definitive evidence of this is lacking. Functionally, the lymphocytes have manifested in vitro antibody-mediated cytotoxicity in most instances; in only a small percentage of cases have they expressed suppressor activity. In one study for in vitro suppressor effect on granulocyte colony formation, the lymphocytes failed to inhibit granulopoiesis.7 The lymphocytes in a few other cases have been shown to suppress immunoglobulin production by B lymphocytes, similar to case 5 in the present study, and/or to suppress erythropoiesis in vitro.3,5,12 Several patients with a granulated T cell lympho-
Granulated T cell lymphocytosis with neutropenia is a lymphoproliferative disorder characterized by a prolonged and relatively stable clinical course. Because of the lack of progression of the disease in most patients, the benign or malignant nature of the entity has been the subject of controversy. The observation of lymphocyte chromosome abnormalities in two of the patients in the present study is supportive evidence for a neoplastic nature in at least some of the cases of chronic granulated T cell lymphocytosis with neutropenia. However, two other patients in this study appear to have undergone a spontaneous regression of their disorder. In one of these patients, regression occurred after several years of persistent lymphocytosis and neutropenia; a spleenectomy specimen from this patient taken early in his course showed heavy lymphoid infiltrate in a histopathologic pattern generally found in neoplastic processes. There were no distinguishing features noted in clinical presentations or the morphological and immunologic characteristics of the lymphocytes between the patients with lymphocyte chromosome abnormalities and persistent disease and the patients in whom the disease underwent a spontaneous regression. It is probable that cases of granulated T cell lymphocytosis with neutropenia, although morphologically and immunologically similar, are biologically heterogeneous. Regardless of the reactive or neoplastic nature of this disorder, it is generally associated with relatively mild clinical manifestations and a stable, long course. Chemotherapy does not appear to be indicated. Neutropenia is the most important and persistent problem and is often associated with recurrent infections. A favorable response in one of our patients suggests that a trial of pulse steroid therapy is warranted in patients with severe neutropenia and a serious and unresponsive infection.

**ACKNOWLEDGMENT**

The authors gratefully acknowledge the helpful suggestions of Dr Peter Nowell regarding mitogenic stimulation of lymphocytes from these patients and the expert technical assistance of Ms Joy Machnicki in performing the cytogenetic studies.

**REFERENCES**

20. Gajl-Peczalska KJ, Bloomfield CD, Coccia PF, Sosin H,
Granulated T cell lymphocytosis with neutropenia: malignant or benign chronic lymphoproliferative disorder?

RW McKenna, DC Arthur, KJ Gajl-Peczalska, P Flynn and RD Brunning