T-Cell Imbalance in Neutropenia of Uncertain Etiology

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The authors studied 16 consecutive patients with chronic neutropenia of uncertain etiology in whom bone marrow biopsies were performed. Using monoclonal antibodies, they measured the levels of the two major subclasses of T lymphocytes, cells with the T-suppressor/cytotoxic cell phenotype (Ts), and cells with the T helper/inducer cell phenotype (Th) in the blood of eight of these patients. Five patients had Ts lymphocytosis or increased proportions of Ts cells and Th lymphocytopenia.

All five patients had greatly increased proportions of large lymphocytes with prominent cytoplasmic azurophilic granules. Since cells with similar morphology from normal individuals have been shown to express cytotoxic activities, the authors measured the cytotoxic function of cells from three of the patients with Ts lymphocytosis. All three possessed antibody-dependent cell-mediated cytotoxicity (ADCC) but not natural killer (NK) cell activity.

Many patients had evidence of autoimmune disorders, conditions in which Ts cells usually are decreased. None had hypogammaglobulinemia, a disorder that has been associated with increased Ts activity.

The high incidence of an imbalance in T lymphocyte subpopulations in patients with neutropenia suggests that disturbed immunoregulation involving T cells may play an important role in the pathogenesis of this disorder. (Key words: Chronic neutropenia; Large granular lymphocytes; T-cell imbalance; T-suppressor/cytotoxic cell lymphocytosis; Antibody-dependent cell mediated cytotoxicity (ADCC); NK cells) Am J Clin Pathol 1984; 81: 54-61

NEUTROPENIA SOMETIMES MAY RESULT from an underlying immunologic disturbance. Some patients have associated autoimmune disorders such as rheumatoid arthritis or systemic lupus erythematosus, and others appear to have isolated neutropenia. Antibodies to neutrophils have been implicated in the pathogenesis of the neutropenia in some of both types of patients.1,2,19,34,39

Both humoral and cellular immune mechanisms may play a role in impaired marrow production of hematopoietic cells. Some patients, for example, have serum antibodies that reduce the number of autologous bone marrow colony and burst-forming units.1,14,16,25 Occasionally, plasmapheresis has resulted in clinical improvement.1,25 In other patients, abnormalities of T-lymphocyte function may cause neutropenia. T cells are known to regulate a variety of B- and T-cell functions, and recent studies suggest that they also may regulate hematopoiesis.1,2,8,21,24,30 Using monoclonal antibodies, it is possible to identify two major subclasses of T lymphocytes in peripheral blood: those with the T-helper/inducer cell phenotype (Th) and those with the T-suppressor/cytotoxic cell phenotype (Ts).32 Bagby recently reported that removal of bone marrow Ts cells resulted in an increase in colony forming units in marrow cultures from two patients with chronic neutropenia.5 T-cell lymphocytosis has been observed in a number of patients with neutropenia2,2,6,8,21,24,30 and in three reports, the T lymphocytes were predominantly Ts cells.2,21,30

To elucidate further the potential role of T-cell imbalance in chronic neutropenia of uncertain etiology, we determined the levels of the two major subclasses of T lymphocytes in the peripheral blood of eight such patients using monoclonal antibodies. We found that five of these patients had Ts lymphocytosis or a decreased Th/Ts ratio and Th lymphocytopenia. A sixth patient had a marginally abnormal Th/Ts. During the period these patients were seen at our institution, only eight additional patients were evaluated by bone marrow examination, but not by T cell studies, for unexplained neutropenia. This high incidence (at least 5 of 16 patients) of T-cell imbalance in neutropenic patients suggests that disturbed immunoregulation involving T cells plays a role in the pathogenesis of this disorder.

Materials and Methods

Case Selection

We identified 21 patients who had bone marrow biopsies to investigate chronic neutropenia by reviewing the diagnostic files of the Emory University Hospital clinical hematology laboratory for 1979–1981 (1850 cases). All patients had neutropenia for at least six months. Five patients were excluded because a probable cause for their neutropenia was identified (three drug or chemically-induced, one splenomegaly secondary to portal hyperten-
sion, one possible smoldering leukemia). The 16 patients with chronic neutropenia of uncertain etiology are presented here. Eight of these had had cell surface marker studies performed for various reasons, usually related to peripheral blood or marrow lymphocytosis or the prevalence of large granular lymphocytes (see below).

Bone Marrow Biopsy Examination

A trephine biopsy and an aspirate anticoagulated with EDTA were taken from a posterior iliac crest of each patient. They were processed for morphologic examination as described by Brynes and associates. The bone marrow cellularity was estimated from the trephine biopsy. A diagnosis of hypercellularity or hypocellularity was made based on the values established by Hartsock and associates. Granulocyte cellularity was estimated from the narrow cellularity and the differential count, as well as from the appearance of the histologic sections. The granulocyte series was considered to show maturation arrest if the number of myelocytes and their precursors was five times that of the later cells. A cellular composition of greater than 23.8% lymphocytes (two standard deviations above the mean) in a normocellular marrow constituted marrow lymphocytosis.

Cytochemical Studies

Acid phosphatase, and alpha-naphthyl acetate esterase activities were determined and the periodic acid-Schiff (PAS) reaction was performed as described previously.

Laboratory Studies

Blood counts were performed using a Coulter S-Plus® counter and a differential count was performed on a peripheral film. Serum immunoglobulin levels were assessed by serum protein electrophoresis on cellulose acetate membranes. Quantitative immunoglobulin levels were measured using rate nephelometry (Beckman Instruments Inc., Fullerton, CA). Rheumatoid factor titers were determined by measuring agglutination of IgG-coated latex particles (Rheuma-Welco Test; Wellcomb Research Lab, Beckenham, England), and antinuclear antibody titers were evaluated by indirect immunofluorescence on freshly prepared frozen sections of mouse liver. Peripheral blood T cells were enumerated by counting cells forming rosettes with sheep erythrocytes (E-rosettes), and surface immunoglobulin-positive B cells were detected using peroxidase-conjugated antibody to human immunoglobulins (Cappel, Cochranville, PA). T-cell subsets were determined using an indirect immunoperoxidase technic and monoclonal antibodies against cells with the Th phenotype (OKT4) and cells with the Ts phenotype (OKT8) (Ortho Pharmaceutical Co., Raritan, NJ) as described previously.

Fc Gamma Assay

Ox erythrocytes were sensitized with rabbit IgG antibody to ox erythrocytes (Pel-Freez Biologicals, Rogers, AK) at an optimal subagglutinating concentration. 20 μL of a 0.5% suspension of these cells were mixed with an equal volume of mononuclear cells (3 × 10⁶/mL in RPMI-1640 with 20% fetal calf serum), centrifuged at 200 × g for 5 minutes at 4°C, and incubated for 5 minutes at 20°C. The supernatant was removed and the pellet was resuspended gently in 50 μL of methylene blue. The assays were performed in duplicate, and the proportion of rosetting cells was calculated from counts of 200 cells.

Cytotoxic Cell Assays

Antibody-dependent cell-mediated cytotoxic (ADCC) and natural killer (NK) cell activities were assayed by measuring radioactivity released by 51Cr-labeled target cells. Target cells were labeled with 51Cr as described previously. One times 10⁵ chicken erythrocytes (CRBC) sensitized with a previously determined optimal concentration of rabbit antibody to CRBC were used in the ADCC assay and 10⁵ K562 cells in the NK assay. Assays were performed in duplicate in V-bottomed 96 well microtiter plates (Linbro). Various numbers of effector cells were added, and the plates were incubated for 4 hours at 37°C. Controls for spontaneous release of radioactivity were obtained for the ADCC assay by incubating target cells with effector cells but without antibody and for the NK assay by incubating without effector cells. Maximum releasable radioactivity was obtained by freeze-thawing the target cells. Radioactivity released into the supernatants was counted and the percentage of cytotoxicity calculated according to the formula:

\[
\text{cpm (test wells)} - \frac{100 \times \text{cpm (spontaneous release)}}{\text{cpm (maximum release)}} - \text{cpm (spontaneous release)}
\]

Results

Lymphocyte surface marker studies were performed on peripheral blood specimens from eight patients with chronic neutropenia (Table 1). None of the patients had any major infections or flare up of their arthritis at the time of the immunologic studies. There was a decrease in the ratio of Th to Ts cells in five of these patients and a borderline decrease in a sixth patient. Three of these five had Ts lymphocytosis (greater than 3 sd above the mean), and all five had decreased numbers of Th cells. Patient 7 had blood lymphocytosis during the initial workup but a normal lymphocyte count when the cellular studies were performed.
Serial cellular studies were performed in two patients. Patient 3 was studied twice, the examinations being 18 months apart. Neither his clinical status nor the Th/Ts ratio (0.08 vs. 0.05) changed significantly. Patient 2 was studied four times. The first Th/Ts ratio was 0.01 and was measured two months after the initial diagnosis. The Th/Ts gradually increased to 0.06 four months later, 0.7 after nine months, and 1.5 after 13 months. At these times his hematocrit was 23.9, 21.0, 31.6, and 39.4; the absolute granulocyte counts were 413, 510, 648, and 1,312 cells per mm$^3$; and the absolute lymphocyte counts were 6,408, 2,686, 1,755, and 1,560 cells per mm$^3$. The patient had been treated with transfusions but not with steroids or cytotoxic drugs. A leukapheresis had been performed approximately four months after the first Th/Ts cell studies.

The morphology of the lymphocytes in all three patients with Ts lymphocytosis was similar (Fig. 1), and patients 4 and 5 also had large proportions of these distinctive cells (Table 1). The cells were of medium size with a small slightly eccentric nucleus and abundant pale blue cytoplasm containing many azurophilic granules that were also acid-phosphatase positive. None of these cells showed the focal dot-like acid phosphatase activity usually described for T-lymphocytes. However, cytocentrifuged preparations of the E-rosetting cells showed that most of the cells containing azurophilic granules were T cells. Most of the cells did not show alpha naphthyl acetate esterase activity and were PAS negative. We subsequently will refer to these cells as large granular lymphocytes.

Since cells with similar morphology from normal individuals have been shown to express ADCC and NK activity, we measured ADCC and NK cell cytotoxic functions of cells from three of the patients with Ts lymphocytosis. Their peripheral blood mononuclear cells showed strong ADCC (Fig. 2) but minimal NK activity (Fig. 3). Since ADCC activity involves Fc receptors, we assayed the lymphocytes from these three patients for Fc gamma receptors. All showed an increase in cells with this receptor (30, 78, and 29%, respectively, for patients 1, 2, and 3).

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*The morphologic criteria for large granular lymphocytes are described in the results section. It is expressed as a percentage of total lymphocytes. Patients 2 and 5 had 38% and 30% smaller lymphocytes with prominent azurophilic granules, and the numbers in parentheses indicate the total number of large and small granular lymphocytes. The correlation coefficient between the granular (large and small) lymphocyte and Ts percentages was 0.91 (linear regression analysis), with a $P$ value of <0.001 (F test). The correlation between large granular lymphocytes and Ts percentages was 0.77 ($P < 0.03$).
The salient clinical features and the bone marrow findings of all 16 patients with chronic neutropenia who were evaluated by bone marrow biopsy during the period of this study are presented in Table 2. Lymphocyte surface marker studies had been performed on the first eight. The characteristics of the five patients (patients 1 to 5) with T-cell imbalance will be discussed in the following sections.

Two of these patients had clinically apparent rheumatoid arthritis, and, although all had other medical problems, they were all different. Splenomegaly, when present was only slight and there was no lymphadenopathy or hepatomegaly. Skin infections were common, and there were two instances of serious systemic infection (pneumonitis, septicemia). Two patients had been treated with prednisone with no sustained improvement.

Bone marrow cellularity ranged from slightly decreased to increased and in two of the five patients with T-cell imbalance the granulocyte series was decreased. Granulocyte maturation was abnormal in all but one patient. All patients with a T-cell imbalance had marrow lymphocytosis. The cellularity of the myeloid series in the marrow could be normal in the face of severe peripheral neutropenia, and the degree of marrow myeloid cellularity did not correlate well with the severity of neutropenia.

Table 3 summarizes the hematologic, serum immunoglobulin, and autoantibody data. All patients had normal platelet counts. Two patients had lymphocytosis. In patient 2, however, the lymphocyte count decreased after 4 months. Two patients with Ts imbalance had moderately severe anemia (Hgb < 10 g/dL). Initially, patient 2 had a transfusion requirement. Serum immunoglobulin levels were normal in two patients and elevated in two. High titers of autoantibodies were present in the two patients with clinically apparent rheumatoid arthritis, and lower levels of antinuclear antibodies were detected in the other patient studied.

Because T-cell studies were not performed in patients 9 through 16, we cannot compare directly the clinical features of these patients with those with documented T-cell imbalance. Since the patients were selected for T-cell studies partially because of the higher percentages of large granular lymphocytes, we suspect that the prevalence of T-cell imbalance in the patients who were not studied may be lower. Nevertheless, most of the patients with a T-cell imbalance had marrow lymphocytosis and an increased percentage of large granular lymphocytes in the
Table 2. Clinical Features and Bone Marrow Biopsy Studies of 16 Patients with Chronic Neutropenia

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age</th>
<th>Duration† (yrs)</th>
<th>Other Problems‡</th>
<th>Infections</th>
<th>Bone Marrow Cellularity§</th>
<th>Granulocyte Series Cellularity</th>
<th>Granulocyte Maturation</th>
<th>Bone Marrow Lymphocytes (% Nucleated Cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>F</td>
<td>68</td>
<td>2.0</td>
<td>Rheumatoid arthritis, Corneal keratolysis, Leg Ulcer</td>
<td>None</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>2*</td>
<td>M</td>
<td>43</td>
<td>1.5</td>
<td>Apthous ulcers, Warts</td>
<td>Septicemia</td>
<td>Increased</td>
<td>Normal</td>
<td>Left shift</td>
<td>37</td>
</tr>
<tr>
<td>3*</td>
<td>M</td>
<td>28</td>
<td>1.5</td>
<td>Alopecia</td>
<td>Dermal sepsis</td>
<td>Slightly decreased</td>
<td>Moderately decreased</td>
<td>Arrested</td>
<td>33</td>
</tr>
<tr>
<td>4*</td>
<td>M</td>
<td>30</td>
<td>7.0</td>
<td>Hematuria</td>
<td>Dermal sepsis</td>
<td>Normal</td>
<td>Moderately decreased</td>
<td>Left shift</td>
<td>34</td>
</tr>
<tr>
<td>5*</td>
<td>F</td>
<td>75</td>
<td>1.8</td>
<td>Rheumatoid arthritis, Diabetes mellitus, Fever, weight loss</td>
<td>Dermal sepsis</td>
<td>Increased</td>
<td>Normal</td>
<td>Arrested</td>
<td>35</td>
</tr>
<tr>
<td>6*</td>
<td>M</td>
<td>43</td>
<td>1.0</td>
<td>Probable rheumatoid arthritis, No specific symptoms</td>
<td>None</td>
<td>Normal</td>
<td>Slightly decreased</td>
<td>Left shift</td>
<td>25</td>
</tr>
<tr>
<td>7*</td>
<td>F</td>
<td>48</td>
<td>2.5</td>
<td>Hypertension</td>
<td>Dermal sepsis</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>3</td>
</tr>
<tr>
<td>8*</td>
<td>F</td>
<td>39</td>
<td>1.0</td>
<td>Fever, abdominal pain,</td>
<td>None</td>
<td>Slightly decreased</td>
<td>Normal</td>
<td>Normal</td>
<td>15</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>65</td>
<td>1.5</td>
<td>Rheumatoid arthritis, Fever</td>
<td>Dermal sepsis</td>
<td>Increased</td>
<td>Arrested</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>63</td>
<td>1.0</td>
<td>Rheumatoid arthritis,</td>
<td>None</td>
<td>Increased</td>
<td>Increased</td>
<td>Arrested</td>
<td>5</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>48</td>
<td>1.0</td>
<td>None</td>
<td>Pharyngitis</td>
<td>Increased</td>
<td>Increased</td>
<td>Normal</td>
<td>17</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>39</td>
<td>3.5</td>
<td>None</td>
<td>Pneumonia</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>4</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>44</td>
<td>7.0</td>
<td>Pyoderma gangrenosum, Warts</td>
<td>Septicemia</td>
<td>Increased</td>
<td>Normal</td>
<td>Greater than 23</td>
<td>17</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>43</td>
<td>1.0</td>
<td>None</td>
<td>None</td>
<td>Moderately decreased</td>
<td>Normal</td>
<td>Markedly decreased</td>
<td>15</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>35</td>
<td>1.0</td>
<td>Allergy</td>
<td>Cellulitis</td>
<td>Normal</td>
<td>Normal</td>
<td>Left shift</td>
<td>10</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>52</td>
<td>6.0</td>
<td>Depressive disorder, Cervical spondylosis</td>
<td>None</td>
<td>Slightly increased</td>
<td>Normal</td>
<td>Left shift</td>
<td>6</td>
</tr>
</tbody>
</table>

* Patients in whom T-cell analysis was performed, as summarized in Table 1.
† Patients 3, 5, 8, 12, and 13 had been treated with prednisone; patients 8 and 13 had also received azathioprine; patient 12 had also received lithium carbonate; patient 9 had a splenectomy. Patient 2 showed significant spontaneous improvement of the cell counts; patients 9 and 12 had transient improvement and patient 8 had uncertain improvement; none of the other patients showed improvement.
‡ None of the patients had lymphadenopathy or hepatosplenomegaly; patients 2, 5, and 6 had slight splenomegaly and patient 9 had moderate splenomegaly.
§ The criteria for assessment of marrow and granulocyte cellularity and granulocyte maturation are described in the “Materials and Methods” section.
¶ Lymphocytes were markedly increased. The exact percentage could not be obtained because of marrow fibrosis.

Table 3. Peripheral Blood Parameters of 16 Patients with Chronic Neutropenia

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Hemoglobin* (g/dL)</th>
<th>Granulocytes† (cells/mm³)</th>
<th>Lymphocytes‡ (cells/mm³)</th>
<th>Platelets* (cells/mm³)</th>
<th>Immunoglobulin Level</th>
<th>Rheumatoid Factor Titer</th>
<th>Antinuclear Antibody Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.0</td>
<td>530</td>
<td>8,480</td>
<td>268,000</td>
<td>High IgA</td>
<td>1,280</td>
<td>160</td>
</tr>
<tr>
<td>2</td>
<td>8.7</td>
<td>413</td>
<td>6,408</td>
<td>204,000</td>
<td>Normal</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>13.5</td>
<td>135</td>
<td>3,780</td>
<td>261,000</td>
<td>Not done</td>
<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>4</td>
<td>13.6</td>
<td>110</td>
<td>952</td>
<td>248,000</td>
<td>Not done</td>
<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>5</td>
<td>8.8</td>
<td>208</td>
<td>936</td>
<td>182,500</td>
<td>Normal</td>
<td>5,120</td>
<td>1,280</td>
</tr>
<tr>
<td>6</td>
<td>13.8</td>
<td>780</td>
<td>820</td>
<td>161,000</td>
<td>Not done</td>
<td>2,560</td>
<td>160</td>
</tr>
<tr>
<td>7</td>
<td>13.1</td>
<td>892</td>
<td>1,716</td>
<td>161,000</td>
<td>Normal</td>
<td>2,560</td>
<td>160</td>
</tr>
<tr>
<td>8</td>
<td>12.8</td>
<td>168</td>
<td>2,190</td>
<td>256,000</td>
<td>Normal</td>
<td>5,120</td>
<td>1,280</td>
</tr>
<tr>
<td>9</td>
<td>11.4</td>
<td>23</td>
<td>2,112</td>
<td>153,000</td>
<td>Normal</td>
<td>5,120</td>
<td>1,280</td>
</tr>
<tr>
<td>10</td>
<td>13.9</td>
<td>1,656</td>
<td>648</td>
<td>183,000</td>
<td>Normal</td>
<td>0</td>
<td>640</td>
</tr>
<tr>
<td>11</td>
<td>14.4</td>
<td>600</td>
<td>1,675</td>
<td>237,000</td>
<td>Normal</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>14.4</td>
<td>696</td>
<td>1,152</td>
<td>203,000</td>
<td>Normal</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>9.4</td>
<td>0</td>
<td>3,696</td>
<td>388,000</td>
<td>Normal</td>
<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>14</td>
<td>15.2</td>
<td>598</td>
<td>1,740</td>
<td>226,000</td>
<td>Normal</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>11.8</td>
<td>360</td>
<td>1,584</td>
<td>341,000</td>
<td>Not done</td>
<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>1,378</td>
<td>1,066</td>
<td>257,000</td>
<td>Normal</td>
<td>Not done</td>
<td>Not done</td>
</tr>
</tbody>
</table>

* Hemoglobin and platelet counts values at time of initial diagnosis.
† Neutrophil counts fluctuated significantly in some patients. These values represent the minimal count observed before any therapy was administered.
‡ Lymphocyte counts are expressed as a percentage of white blood cells. The lymphocyte count was determined by direct counting. The values were not significantly different from the initial values except in patient 7 whose lymphocyte count dropped from an initial value of 8,880 to 1,716 at the time of cell surface marker studies a year later.
blood, suggesting that these features can identify patients within this group.

Discussion

In our patients with chronic neutropenia, T-cell imbalance was observed frequently. Five of eight patients tested had increased proportions of Ts cells, and all five had Th lymphopenia. The incidence of this disorder was therefore at least 31% in our population, since only a total of 16 consecutive patients had been evaluated for their cytopenia by means of bone marrow examination during the period of this study. While a number of patients with Ts lymphocytosis and neutropenia have been reported by others, the prevalence of this T-cell disturbance, the associated Th lymphopenia, and the serologic studies have not been investigated systematically.

Decreased, rather than increased, proportions of Ts cells are common in patients with autoimmune disorders such as rheumatoid arthritis and systemic lupus erythematosus. Rheumatoid arthritis or serum autoantibodies occurred in 63% of our patients, including at least three of the five patients who had increased proportions of Ts cells. Of further interest, none of our patients, and none of those previously reported, had hypogammaglobulinemia, an abnormality that has been associated with increased Ts cells. Thus, the Ts cells in these patients do not appear to function primarily to suppress immunoglobulin synthesis but may represent a subset of Ts cells that regulates hematopoiesis.

The lymphocytes from all three of our patients with Ts lymphocytosis were uniform populations of large granular lymphocytes. In two other patients with increased proportions of Ts cells, the proportions of these distinctive cells also were increased greatly. In normal peripheral blood, cells with similar morphology have been shown to be the major population responsible for ADCC and NK activities. Ortaldo and associates recently showed that only a minor subset of peripheral blood large granular lymphocytes expresses the Ts phenotype. In our patient, most of the large granular lymphocytes had the Ts phenotype and probably represent an expansion of a normally minor subpopulation of large granular lymphocytes. Since an apparently high proportion of patients with neutropenia have excess Ts cells with this morphology, and since the cells have cytotoxic activity, we suspect that they may be involved in the pathogenesis of the disorder. The concomitant decrease in Ts cells in the one patient whose neutropenia spontaneously improved also supports the hypothesis that the Ts excess and the neutropenia are related. In patients with an overall decrease in the granulocyte series in the bone marrow, the Ts cells might kill the precursor cells. In other patients with maturation arrest, the Ts cells might be cytotoxic for the more mature cells.

There is evidence that human T cells interact with the hematopoietic system and may be involved in the pathogenesis of some cytopenias. For example, a subgroup of patients with aplastic anemia responds to therapy with antithymocyte globulin. In addition, immunosuppression of several patients with severe aplastic anemia has been required in order to permit engraftment of marrow from an identical twin, suggesting that failure of the initial engraftment was immunologically mediated.

Several in vitro observations further suggest an interaction of the immune system with hematopoietic cells. Factors elaborated by T cells enhance proliferation of immature erythroid colony forming cells (burst-forming units). T cells also produce factors that stimulate colony formation by granulocyte progenitor cells. T cells that suppress marrow cell colony forming units can be generated by stimulating normal peripheral blood lymphocytes with pokeweed mitogen in vitro.

Despite the strong association of T-cell lymphocytosis and T-cell imbalances with neutropenia, the role of the various T-lymphocyte subsets in the pathogenesis of the cytopenia remains to be established. Recently, Bagby reported that two neutropic patients had Ts cells in their marrow that inhibited granulocyte colony formation in vitro. When remission was induced with azathioprine in one of these patients, the inhibition was no longer demonstrable. He did not state whether these patients had peripheral blood Ts lymphocytosis, but Ts cells were not proportionately increased in the marrow. In contrast, Linch and associates reported two patients with Ts lymphocytosis, but could not demonstrate suppression of granulocyte colony forming units (CFU-G). Similar studies of the function of Th cells in granulocyte colony forming assays have been inconclusive. The prevalence of Th lymphocytopenia in our patients with neutropenia, however, suggests that these cells also may be important in this disorder. The spontaneous remission in patient 2 accompanied by the normalization of the Th/Ts ratio is provocative. Clearly, further studies on the effects of Ts and Th cells on in vitro granulopoiesis, and abrogation of such effects following successful treatment, are necessary to establish their role in the pathogenesis of neutropenia.

Several authors have considered their cases of T lymphocytosis with neutropenia to be chronic T lymphocytic leukemia. The lymphocytes appeared to be uniform populations of cells, both by morphologic and surface marker studies. Those cases that were studied using monoclonal antibodies had the phenotype of Ts cells.

Since the antigens characteristic of the T-cell subsets are not clonal markers, as are the light chains present on
B lymphocytes, this test did not confirm or rule out a clonal neoplasia. In our patients, the Ts lymphocytosis was modest and marrow infiltration was not extensive. None of the patients had lymphadenopathy, hepatomegaly, marked splenomegaly, or involvement of the skin or central nervous system. The patients have not been followed long enough to determine whether the lymphocytosis will progress, and in one of our patients it appears to have resolved. In three other reports of T lymphocytosis with neutropenia, the condition was stable or at most slowly progressive. None of the patients died of the direct consequences of lymphoproliferation.

In this series, 5 of 8 patients had an abnormally high proportion of Ts cells, but three of them had normal lymphocyte counts. There was a continuum with respect to the numbers of Ts cells. Thus, Ts lymphocytosis may represent the extreme end of a spectrum of T-cell immunoregulatory disturbances. We agree with Aisenberg that a descriptive term, such as Ts lymphocytosis with neutropenia, be used to classify these patients unless the lymphocytes unequivocally can be demonstrated to be clonal in origin.

Most of our neutropenic patients had skin infections, two developed life-threatening infections, and one eventually died. Only one of the patients showed significant improvement of the neutropenia during the period of observation. Therapy for neutropenia is largely empiric, frequently toxic, and generally unsuccessful. A better understanding of the pathogenesis of this disorder is essential for the development of rational treatment. If a particular subset of the lymphocytes could be shown to be important in the pathogenesis of the neutropenia, then therapy directed at altering the specific subset might be warranted.


References