Adult-Onset Cyclic Neutropenia Is Associated With Increased Large Granular Lymphocytes

By Thomas P. Loughran, Jr, Edward A. Clark, Thomas H. Price, and William P. Hammond

Human cyclic neutropenia occurs in adults as well as children. Clinical illness is similar in the childhood and adult diseases, but distinctly different modes of onset suggest heterogeneity in its pathophysiology. We studied seven patients with cyclic neutropenia, three with disease acquired in adulthood, and four with the childhood-onset disorder. All three patients with adult-onset cyclic neutropenia had increased numbers of circulating large granular lymphocytes (LGL), whereas the four children with cyclic neutropenia had normal LGL counts. LGL from patients with adult-onset cyclic neutropenia expressed cell surface antigens HNK-1 (three of three patients) and IgG Fc receptors (two of three patients), although natural killer activity was low. Two of these patients were treated with alternate-day steroids, resulting in decreased LGL counts and abrogation of neutrophil cycling. We suggest that adult-onset cyclic neutropenia may be distinguished from the childhood-onset form of the disease by increased numbers of LGL. Furthermore, increased LGL may identify a subset of patients with cyclic neutropenia who respond to steroid therapy.

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Clinical Research Center at the University Hospital on protocols approved by the Human Subjects Review Committee of the University of Washington. Patient 3 was recruited for study through the National Institutes of Health (NIH) Clinical Center: slides of bone marrow obtained prior to steroid therapy were obtained from the NIH, whereas blood for surface marker studies was obtained through her private physician.

All three patients with adult-onset cyclic neutropenia had recurrent symptoms of fever, malaise, aphthous stomatitis, and occasional local infections. Clinical onset of disease in patient 1 began in 1970 at the age of 59 when a complete blood cell count (CBC) showed neutropenia and absolute lymphocytosis. CBC with differential count had been normal in 1968 when she underwent distal pancreatectomy and splenectomy for chronic pancreatitis. The diagnosis of cyclic neutropenia was established in 1975. A trial of lithium carbonate in 1979 was unsuccessful, as described previously. She continues to experience periodic fevers, malaise, and aphthous stomatitis; she has declined treatment with alternate-day prednisone. Patient 2 was well until 1962 when at the age of 45 symptoms began to occur at approximately monthly intervals. A diagnosis of cyclic neutropenia was established in 1975, and a trial of alternate-day prednisone (50 mg) resulted in correction of neutrophil cycling. She currently remains well, receiving 20 mg prednisone every other day. Cyclic neutropenia was diagnosed in patient 3 at the age of 65. She has been the subject of previous studies of cyclic neutropenia performed at the NIH. A trial of alternate-day prednisone resulted in abrogation of neutrophil cycling and the clinical manifestations of her disease. She still receives prednisone, 10 mg daily, and has suffered multiple complications of steroid therapy including bleeding gastric ulcer, osteoporosis resulting in fracture of the right hip, and diabetes mellitus.

Patients 4 to 7 with childhood-onset cyclic neutropenia also had classic features of the disease: regularly recurring episodes of fever, malaise, aphthous stomatitis, and cervical lymphadenopathy. Patients 4 and 6 were previously reported by us; patient 4 died of necrotizing clostridial enterocolitis at age 11. Patient 5 presented with a lung abscess; she had severe periodontitis, as did patient 6. All four patients with childhood-onset cyclic neutropenia had cyclical neutropenia and the clinical manifestations of their disease. Three had cyclic fluctuations of either platelet or reticulocyte counts.

Patients 8 to 10 with chronic neutropenia had histories of recurrent infections similar to those of patients with cyclic neutropenia. Patients 8 and 10 were referred by their physicians because of the severity of their infections. They had otherwise normal blood counts and fit the diagnostic category of chronic idiopathic neutropenia. Patient 9 was also anemic and thrombocytopenic; he had had documented neutropenia and severe recurrent skin infections for at least 10 years. These patients served as one control group, whereas ten healthy volunteers served as the normal control group.

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ADULT-ONSET CYCLIC NEUTROPENIA

Blood counts. The diagnosis of cyclic neutropenia was established by performing blood counts a minimum of three times per week for at least 6 weeks as previously described. Patients with chronic neutropenia had serial blood counts similarly performed for a minimum of 4 weeks to document the absence of cycling. WBC counts were done on EDTA-anticoagulated specimens using a Coulter counter and total neutrophil counts (bands plus neutrophils) calculated from the WBC and a 100-cell differential count. LGL were identified by their characteristic morphology on Wright-Giemsa stains, ie, larger size and smaller nuclear cytoplasmic ratio than typical lymphocytes as well as prominent azurophilic granules. Total LGL counts were calculated by multiplying the total lymphocyte count by the percentage of LGL (of at least 100 cells counted) obtained in the differential lymphocyte count. Differential counts were performed by one observer (T.P.L.) on coded specimens to prevent biased reporting. Five hundred-cell bone marrow differential counts were performed by a single observer (T.H.P.).

Lymphocyte studies. Peripheral blood mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation and studied for expression of cell surface antigens using an Epics 5 flow cytometer with direct one-color and two-color analysis using a panel of fluorescein-conjugated or R-phycocerythrin-conjugated monoclonal antibodies (MoAb) as previously described. The antigens recognized by these MoAb are described using the current World Health Organization international nomenclature. The following MoAb, verified by International Workshop analyses, were kindly provided by Dr Jeff Ledbetter (Genetic Systems, Seattle): G19-4 anti-CD3 (T3) and G10-1 anti-CD8 (T8). Dr Toru Abe (University of Alabama, Birmingham) provided HNK-1. The Fc-1 MoAb recognizes IgGl Fc receptors (CD16) found on natural killer (NK) cells and does not recognize IgG Fc receptors expressed on monocytes. NK activity of peripheral blood mononuclear cells was determined using a 51Cr-labeled K562 erythroleukemia cell line as a target in a four-hour assay as previously described for our laboratory. Effector:target ratios were 6.25:1, 12.5:1, 25:1, and 50:1; lytic units for 30% killing of 104 target cells were derived from a graph of log10 effector/target ratio v the percent specific lysis plotted on semilog paper. Cytogenetic studies were done on peripheral blood mononuclear cells from patients 1 and 3 cultured either with or without phytohemagglutinin as previously described.

Neutrophil antibody studies. Tests for serum leukoagglutinating antibodies were performed according to the method of McCullogh et al. Neutrophil-associated immunoglobulin and serum or plasma neutrophil-binding immunoglobulin were determined by indirect immunofluorescence using a modification of the method of Verheught et al. Patient or normal target neutrophils were purified by Hypaque-Ficoll sedimentation and fixed with 1% paraformaldehyde. Neutrophil-associated immunoglobulin (direct test) was determined by flow microfluorometry after incubation of the cells with fluorescein isothiocyanate (FITC) goat antihuman IgG (or IgM) (Tago, Burlingame, Calif). Results were quantitated using peak fluorescence channel numbers and expressed as a multiple of average values for normal neutrophils. For determination of plasma/serum neutrophil-binding immunoglobulin (indirect test), normal neutrophils were incubated with test sera, washed, and surface immunoglobulin determined as before. For the indirect test, results are expressed as a multiple of values seen when target cells are incubated with autologous serum.

RESULTS

Cyclic neutropenia was well documented by frequent serial blood counts in seven patients. Of the patients with adult-onset cyclic neutropenia, patient 1 was studied on several occasions over a 10-year period; cycle length has remained constant at 20 days. Patient 3 has also been studied on several occasions over at least 14 years; cycle length has remained constant at 21 to 22 days. Blood counts obtained on patient 2 over a 10-month period before and after receiving prednisone therapy are shown in Fig 1. The cycle length was somewhat longer, with an average of 27 days. Alternate-day prednisone therapy eventually resulted in correction of cycling and stabilization of neutrophil counts at levels greater than 2,000/μL. Similar results of prednisone therapy have been reported previously for patient 3.

All three patients with adult-onset cyclic neutropenia had a marked increase in circulating LGL at the time of diagnosis (Table 1), values similar to those observed in patients with LGL leukemia. To determine whether an elevated LGL count was a nonspecific finding secondary to neutropenia and recurrent infections, we determined LGL counts in other groups of neutropenic patients. As shown in Table 1, three patients with chronic neutropenia and four children with cyclic neutropenia had LGL counts within the normal range. Clinical remission of adult-onset cyclic neutropenia was associated with a reduction in numbers of LGL in the two patients treated with alternate-day steroids (Table 1). Results of bone marrow differential counts in patients with adult-onset cyclic neutropenia are shown in Table 2. All three patients had increased numbers of mature-appearing marrow lymphocytes. In addition, characteristic findings of cyclic neutropenia were seen in serial marrow examinations from patient 1. During neutropenia, myeloid precursor cells predominated, whereas during the neutrophil recovery phase an expansion of the myeloid postmitotic pool was observed. Of interest, microscopic examination of the spleen of patient 1, obtained prior to clinical onset of cyclic neutropenia, revealed lymphoid infiltration of splenic red pulp cords, a finding similar to that reported in patients with LGL leukemia.

Figure 2 shows serial LGL, lymphocyte, and neutrophil counts obtained from patient 1 during a 26-day period.

![Graph showing neutrophil counts](image-url)
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obtained on cells from all three patients revealed some phenotypic char-

definite cycling in either LGL or total lymphocytes in patient

observed in the neutrophil counts, however, there was no

observed in LGL count. In contrast to the regular periodicity

showed that HNK-1

expressed IgG Fc receptors (Table 3). Two-color analysis

the patient’s total

24

periodogram analysis on this patient. Such an analysis

+ cells; HNK-1

in addition, two patients had LGL that

CD8 and lacked expression of IgG Fc receptors (CD 16).

1, although sufficient data were not available to perform a

patients except patient 9, whose hematocrit value was

PMN, polymorphonuclear cells.

Abbreviations: NA, not available; PMN, polymorphonuclear cells.

Results expressed as a percentage.23

Results of two-color analyses of peripheral blood mononu-

A 95% confidence range.23

Table 2. Bone Marrow Differential Counts

<table>
<thead>
<tr>
<th>Patients</th>
<th>Counts</th>
<th>1 (No. 1)</th>
<th>1 (No. 2)</th>
<th>2</th>
<th>3</th>
<th>Normal*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythroid</td>
<td></td>
<td>9.8</td>
<td>15.2</td>
<td>27.4</td>
<td>34.0</td>
<td>15.0–36.2</td>
</tr>
<tr>
<td>Myeloblast</td>
<td></td>
<td>0.4</td>
<td>0.2</td>
<td>1.3</td>
<td>1.9</td>
<td>0.9–1.7</td>
</tr>
<tr>
<td>Promyelocyte</td>
<td></td>
<td>7.8</td>
<td>0.9</td>
<td>1.7</td>
<td>3.5</td>
<td>1.9–4.7</td>
</tr>
<tr>
<td>Myelocyte</td>
<td></td>
<td>1.2</td>
<td>6.5</td>
<td>1.5</td>
<td>8.5</td>
<td>8.5–16.9</td>
</tr>
<tr>
<td>Metamyelocyte</td>
<td></td>
<td>0.9</td>
<td>4.3</td>
<td>0.9</td>
<td>4.4</td>
<td>7.1–24.7</td>
</tr>
<tr>
<td>Bands + segments</td>
<td></td>
<td>0.9</td>
<td>24.6</td>
<td>6.3</td>
<td>8.5</td>
<td>13.2–26.4</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td></td>
<td>61.9</td>
<td>39.9</td>
<td>46.5</td>
<td>29.4</td>
<td>8.6–23.8</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>17.1</td>
<td>8.4</td>
<td>14.4</td>
<td>9.8</td>
<td>9.8–23.8</td>
</tr>
<tr>
<td>Blood PMN</td>
<td></td>
<td>0</td>
<td>1,540</td>
<td>900</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not available; PMN, polymorphonuclear cells.

* A 95% confidence range.23

Results expressed as a percentage, 500 cells counted.
patients. There was no evidence for circulating antineutrophil antibodies in either patient 1 or 3; in contrast, both IgG and IgM antibodies were demonstrated in the serum of patient 2. High levels of neutrophil-associated IgM were documented in patient 2; this patient also had elevated serum levels of IgM (808 mg/dL). In addition, there was a slight increase in neutrophil-associated IgG in patient 3. Of note, patient 3 had previously been shown to have a normal neutrophil survival.24

**DISCUSSION**

This study suggests that adult-onset cyclic neutropenia may be distinguished morphologically from the more common childhood-onset cyclic neutropenia by the presence of increased numbers of circulating LGL. All three patients with the adult-onset disorder met clinical and hematologic criteria for cyclic neutropenia: (1) recurrent symptoms of fever, malaise, aphthous stomatitis, and occasional superficial infections and (2) periodic episodes of profound neutropenia associated with reciprocal mononcytosis. In patients 1 and 3 the cycle length was within the 20- to 22-day cycle period seen in 85% of patients with cyclic neutropenia,1 whereas the cycle length in patient 2 was a bit longer (27 days). Furthermore, the cycle lengths of individual patients were very stable when studied at intervals of several years, a feature of classic cyclic neutropenia.1,2

Results of lymphocyte surface antigen phenotyping in these patients with acquired cyclic neutropenia revealed some heterogeneity. Mononuclear cells from the only patient studied in an untreated state (patient 1) were CD3+, CD8+, HNK-1+, CD16−. The other two patients also had increased numbers of HNK-1+ cells; approximately 40% of these cells also expressed IgG Fc receptors, a surface antigen normally associated with active NK cells.34–37 Of note, NK activity of peripheral blood mononuclear cells from all three patients was very low, a finding previously noted in most patients with large granular lymphocytosis and chronic neutropenia.22,33

No prior studies of LGL in adult-onset cyclic neutropenia have been reported. Lymphocyte subpopulations have been studied in three children with cyclic neutropenia,12,38 but LGL were examined in only one of these patients.38 In that report, LGL (defined as HNK-1+ cells) fluctuated from 0.7% to 7.5% of the total blood lymphocytes; using this data, we estimate that absolute LGL numbers were always below 300/µL, consistent with our finding of normal LGL counts in the childhood disorder.

The syndrome of chronic neutropenia associated with excess LGL was first described in 1977 and has recently been reviewed.33 We showed previously that in at least some of these patients this disorder represents a leukemia of LGL that is characterized by autoimmune manifestations and infiltration of splenic red pulp, hepatic sinusoids, and bone marrow by immature NK cells.23 Although cycling of neutrophil counts did not occur in patients with LGL leukemia (unpublished observations), the three patients with adult-onset cyclic neutropenia we report here do share features of that disease: (1) excess LGL that express some NK surface antigens yet have little NK activity and (2) lymphocytic infiltration of splenic red pulp cords and possibly bone marrow. In contrast, our patients with adult-onset cyclic neutropenia did not have arthritis or positive tests for antinuclear antibody or rheumatoid factor, common features of LGL leukemia.23,39 Furthermore, they have had extraordinarily prolonged illnesses ranging from 8 to 14 years, demonstrating a remarkable stability of the disorder.

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**Table 3. Expression of NK Cell–Associated Antigens on Peripheral Blood Mononuclear Cells of Patients With Adult-Onset Cyclic Neutropenia (Percentage Positive)**

<table>
<thead>
<tr>
<th>Antigens</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Normal*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNK-1+ total</td>
<td>46</td>
<td>54</td>
<td>60</td>
<td>13±3.9</td>
</tr>
<tr>
<td>HNK-1+,CD3(T3)+</td>
<td>35</td>
<td>11</td>
<td>16</td>
<td>2±0.8</td>
</tr>
<tr>
<td>HNK-1+,CD8(8T)+</td>
<td>41</td>
<td>14</td>
<td>17</td>
<td>3±1.6</td>
</tr>
<tr>
<td>HNK-1+,CD16(IgG FcR)+</td>
<td>1</td>
<td>23</td>
<td>20</td>
<td>4±2.4</td>
</tr>
<tr>
<td>CD16+ total</td>
<td>5</td>
<td>34</td>
<td>30</td>
<td>13±6.4</td>
</tr>
</tbody>
</table>

*Normal values shown represent the mean ± SD obtained from four normal controls.

**Table 4. Neutrophil Antibody Results**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Lag</th>
<th>Direct*</th>
<th>Indirect†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgG</td>
<td>IgM</td>
</tr>
<tr>
<td>1</td>
<td>negative</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>negative</td>
<td>1.5</td>
<td>5.9</td>
</tr>
<tr>
<td>3</td>
<td>negative</td>
<td>2.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Normal‡</td>
<td>—</td>
<td>0–1.9</td>
<td>0–1.4</td>
</tr>
</tbody>
</table>

Abbreviation: Lag, leukoagglutination; ND, not determined.

*Results expressed as multiples of values for control cells.
†Results expressed as multiples of values for autologous serum.
‡A 95% confidence limit.

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**Fig 2.** The top graph depicts serial total lymphocyte counts (black circles) and LGL counts (white circles) obtained from patient 1 during one period of neutrophil cycling (bottom graph). Although there were fluctuations in both total lymphocytes and LGL, a regular periodicity similar to that of neutrophils was not observed.
The etiology of cyclic neutropenia is not known. Previous
marrow transplantation studies have suggested that this
disorder is a result of a periodic failure of myelopoiesis
occurring at the stem cell level. However, it may not be
possible to generalize these studies performed in the autosomal
recessive model disease of grey collie dogs or in human
childhood-onset cyclic neutropenia to the adult-onset disease
in humans. Kinetic studies, though, have shown normal
neutrophil survival, fluctuating neutrophil reserves, and vir-
tually absent myelocyte incorporation of H-thymidine dur-
ing neutrophilia in both childhood-onset and adult-onset
disease in humans.3,24 strongly suggesting a common final
pathway for the neutropenia, namely periodic failure of
production. In patients with LGL leukemia, on the other
hand, there is some evidence that neutropenia is secondary to
autoimmune peripheral destruction.23 In contrast to these
findings, not all of our patients with adult-onset cyclic
neutropenia had antineutrophil antibodies. Furthermore,
normal neutrophil half-life was demonstrated previously in
patients with LGL leukemia. Thus, it seems likely that increased LGL are
elevated neutrophils and suggest that the pathogenesis of this
form of the disease may be different from the childhood-
onset disorder. Furthermore, this form of the disease may be
amenable to steroid or other therapy. Future studies on
similar patients may provide further insight into the patho-
gensis of cyclic neutropenia and the regulatory role of LGL
in myelopoiesis.

Perhaps of most interest was the dramatic clinical
response to alternate-day steroid therapy. Both patients 2
and 3 had correction of neutrophil cycling; the other patient
depressed such treatment. Therapy was associated with stabi-
lization of bone marrow neutrophil precursors and neutrophil
marrow reserves in patient 3.15 Although LGL counts still
remained greater than normal, clinical remission induced by
prednisone was also associated with a reduction in numbers of
LGL. Thus, it seems likely that increased LGL are
involved in adult-onset cyclic neutropenia, either as a causa-
tive factor or as still another phenomenological component of
cyclic fluctuations of stem cells. Whether LGL cycle in all
untreated patients would be interesting to know but can not
be determined from this study; in any event, the marked
elevation of LGL levels in patients with adult-onset disease is
in striking contrast to the normal levels in childhood-onset
disease. Also of note, a lymphoid infiltration of splenic red
pulp cords was documented in patient 1, 2 years before the
clinical and hematologic onset of cyclic neutropenia. Fur-
thermore, LGL are known to produce multiple regulatory
factors and it has been emphasized that cyclic neutrope-
nia may be secondary to an abnormality in feedback regula-
tion of hematopoiesis.43

Two other patients with cyclic neutropenia have been
reported to respond to steroid therapy.16,17 Another patient
took a temporary response to plasmapheresis. Documented
improvement in neutrophil counts was also observed in at
least three patients following splenectomy,5,7 in one patient
undergoing splenectomy and receiving adrenocorticotropin
hormone injections,8 in two patients receiving androgen
therapy,9 and in one patient receiving lithium.10 All of these
patients had the adult-onset form of cyclic neutropenia;
lymphocyte morphology was not noted. In contrast, no
successful therapy for childhood-onset cyclic neutropenia has
been reported. The results of our study indicate that adult-
onset cyclic neutropenia may be characterized by markedly
increased LGL and suggest that the pathogenesis of this
form of the disease may be different from the childhood-
onset disorder. Furthermore, this form of the disease may be
amenable to steroid or other therapy. Future studies on
similar patients may provide further insight into the patho-
gensis of cyclic neutropenia and the regulatory role of LGL
in myelopoiesis.

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