

ADULT-ONSET CYCLIC NEUTROPENIA IS A BENIGN
NEOPLASM ASSOCIATED WITH CLONAL PROLIFERATION
OF LARGE GRANULAR LYMPHOCYTES

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Human cyclic neutropenia is a rare disease of unknown etiology characterized by periodic oscillations of the blood neutrophil count. Onset of illness usually begins in childhood, although in ~25% of patients the first symptoms occur after age 20 (1, 2). These distinctly different modes of onset suggest heterogeneity in the pathophysiology of cyclic neutropenia. Recently we determined (3) that adult-onset cyclic neutropenia could be distinguished morphologically from the childhood-onset form of the disease by the presence of increased numbers of circulating large granular lymphocytes (LGL).

In this report we investigated the possibility that cyclic neutropenia could be associated with a clonal proliferation of lymphocytes. We assessed clonality by analyzing genomic DNA for evidence of rearrangement of the T cell receptor β chain gene. Our results show that adult-onset cyclic neutropenic patients with increased LGL had clonal populations of lymphocytes, whereas those patients with childhood-onset cyclic neutropenia had no evidence for clonal rearrangement of the T_{β} gene.

Materials and Methods

Patients. Clinical details concerning patients 1–5 have been published previously (3). Patients 1–3 had adult-onset disease; patients 4 and 5 had childhood-onset cyclic neutropenia. The diagnosis of cyclic neutropenia was established by performing blood counts a minimum of three times per week for at least 6 wk, as previously described (1–4). Cycle lengths of patients 1, 3, 4, and 5 were within the 19–22-d cycle period seen in 85% of patients with cyclic neutropenia (1), whereas the cycle length in patient 2 was a bit longer at 27 d.

Patients 1–3 with adult-onset cyclic neutropenia had markedly increased LGL counts at time of diagnosis, ranging from 2,170–6,143/mm³ (normals in our laboratory: 223 ± 99, $n = 10$), whereas the two patients with childhood-onset disease had normal LGL counts (3). At the time of this study, patients 2 and 3 were in clinical remission from neutrophil cycling on alternate-day steroid therapy. Such therapy resulted in reduction of numbers of LGL, although LGL counts remained greater than normal (3).

This work was supported by Public Health Service grants HL36444 (formerly CA-30924) and AM-18951 from the National Institutes of Health (Bethesda, MD), and by the Leukemia Research Foundation. Dr. Loughran is a Fellow of the Leukemia Society of America. Parts of this work were presented at the American Society of Hematology meeting, December 1985, and were published in abstract form (38). Address correspondence to T. P. Loughran, Jr., M.D., Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, WA 98104.

Blot Hybridization Analysis. Genomic DNA was extracted from PBMC as previously described (5). 75–90% of these PBMC were LGL in the patients with adult-onset cyclic neutropenia. The DNA samples were then digested with restriction enzymes Bam HI, Eco RI, or Hind III. Digested DNA was separated on 1.1% agarose gels and transferred onto nitrocellulose filter by the method of Southern (6). Filters were then hybridized to DNA probes that had been ^{32}P -labeled by nick translation and visualized by autoradiography as previously described (5). The cDNA clone Jurkat B₂ containing the C and J regions of the T_{β} gene (7) was kindly provided by Dr. Tak Mak (Ontario Cancer Institute, Toronto, Canada). A fragment representing nucleotides 100–870 (7) was isolated on agarose gels and used as the hybridization probe.

Results

The human T_{β} gene locus has two constant region genes designated $C_{\beta 1}$ and $C_{\beta 2}$ (8). Digestion of non-T cell DNA with restriction enzyme Bam HI produces a 23-kb germline fragment containing both constant region genes. Therefore, rearrangements at either C_{β} gene locus may be detected after digestion with this enzyme by the appearance of a smaller Bam HI fragment containing the C_{β} gene. Eco RI cleaves within the T_{β} gene locus and produces two germline fragments of 11 and 4 kb, containing $C_{\beta 1}$ and $C_{\beta 2}$, respectively. Since the $C_{\beta 2}$ rearrangements are not detected by constant region probe when DNA is digested with this enzyme (9), any nongermline band observed represents rearrangement of the $C_{\beta 1}$ gene. Digestion of non-T cell DNA with restriction enzyme Hind III produces two germline $C_{\beta 2}$ containing fragments of 8 and 6.5 kb, and one 3.5-kb fragment containing the $C_{\beta 1}$ gene. These patterns of somatic rearrangement of T cell receptor gene can be detected in clonal populations of T cells, and therefore can be used to demonstrate clonality of various T cell malignancies (10, 11).

Results of Southern blot hybridization analyses using the T_{β} gene probe are shown in Fig. 1. Analysis of DNA after digestion with Bam HI or Eco RI showed clonal rearrangement of T_{β} gene in patients 1–3. In contrast, we saw a germline pattern in DNA from patients 4 and 5 after digestion with these enzymes. Hind III digestions showed germline pattern in all five patients (data not shown). As indicated by Eco RI analysis, rearrangement in patients 1 and 3 involved the $C_{\beta 1}$ gene. In patient 2, it was not possible to determine by Eco RI or Hind III digestion whether the rearrangement indicated by Bam HI analysis involved the $C_{\beta 1}$ or $C_{\beta 2}$ gene. The intensity of signal of the rearranged bands indicates that the vast majority of PBMC were clonal. Therefore, it is not possible that T cells with a non-LGL morphology represented the clonal proliferation, since they were only a minor population of the PBMC.

Discussion

These results show that all three patients with adult-onset cyclic neutropenia had a clonal proliferation of LGL, as indicated by somatic rearrangement of the T_{β} gene. In contrast, both patients with childhood-onset cyclic neutropenia had no evidence for a clonal lymphocyte proliferation. These data suggest that adult-onset cyclic neutropenia can be distinguished from the childhood-onset form of the disease by the presence of a clonal proliferation of LGL.

Having shown clonality in these patients with acquired cyclic neutropenia, the

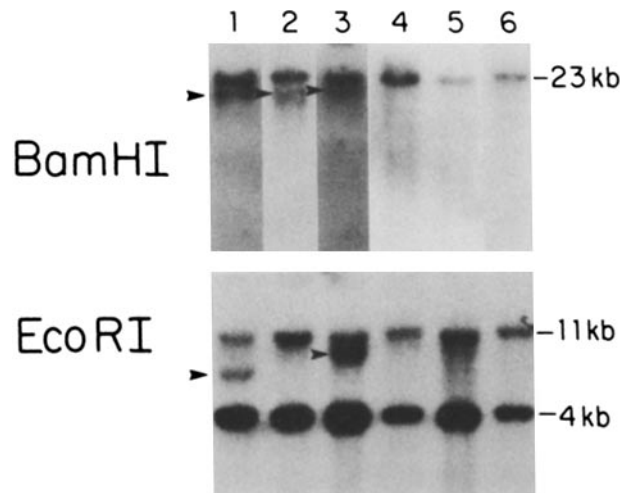


FIGURE 1. Results of Southern blot hybridization analyses using T_{β} gene probe showing rearrangement pattern after digestion with Bam HI or Eco RI. Lanes 1–3 represent patients 1–3 with adult-onset cyclic neutropenia, lanes 4–5 represent patients 4–5 with childhood-onset cyclic neutropenia. Lane 6 represents the germline pattern obtained when analyzing DNA extracted from neutrophils from a normal volunteer. Positions of rearranged bands are indicated by arrowheads.

question remains whether this disease is actually malignant. The clinical course of these patients has been remarkably stable, with cyclical illnesses having occurred over 8–14 yr of duration. These patients with adult-onset cyclic neutropenia share several features of patients with LGL leukemia, who also have a clonal proliferation of LGL (12–15). Both groups have excess LGL that express some NK cell surface antigens. These LGL have little NK cell activity in vitro (3, 13); however, cytotoxicity can be induced by treatment with anti-CD3 mAb or IL-2 (16). Furthermore, lymphocytic infiltration of splenic red pulp cords and bone marrow has been documented in both groups (3, 13). Most patients with LGL leukemia also have a chronic clinical course, with morbidity and mortality generally resulting from infections acquired during severe neutropenia rather than from tissue infiltration by abnormal lymphocytes (17). Thus it would appear that in most instances an abnormal clone remains under partial immunoregulatory control, as occurs, for example, in benign monoclonal gammopathy.

The etiology of cyclic neutropenia is not certain, although marrow transplantation studies have shown that the defect originates at the stem cell level in both dogs and man (18–21). In both childhood-onset and adult-onset disease in man, a final common pathway for the neutropenia, namely a periodic failure of production, has been demonstrated by kinetic studies (1, 22). Furthermore, mathematical models have stressed that stable oscillations almost certainly result from an abnormality in feedback regulation of hematopoiesis (23–27). Although several investigators (28–32) have attempted to document a causative role for specific feedback abnormalities in patients with this disease, considerable differences in interpretation of the data exist and no consensus for the mechanism has come forth. The data we report here strongly implicate a role for a population of LGL in the etiology of cyclic hematopoiesis. LGL have been reported to

produce multiple regulatory factors including colony-stimulating factor (33, 34), as well as cause inhibition of granulocyte/macrophage colony formation (35–37). The clonal expansion of LGL could also conceivably decrease the number or function of cells (such as other lymphocytes or monocytes) crucial to the regulatory feedback loop. The demonstration of a clonal expansion of LGL in this subset of patients with cyclic hematopoiesis provides an opportunity to examine one component of the homeostatic control mechanisms for granulopoiesis.

Summary

Human cyclic neutropenia occurs in children and adults. Adult-onset cyclic neutropenia is an acquired disease characterized by increased numbers of large granular lymphocytes (LGL), in contrast to childhood-onset cyclic neutropenia in which LGL counts are normal. We investigated the clonality of lymphocytes in these two groups of patients by assessing the rearrangement status of the T cell receptor β chain gene. Patients with adult-onset cyclic neutropenia showed clonal rearrangement of the T_β gene whereas the children did not. Since LGL are known to have multiple regulatory effects on normal hematopoiesis, the finding of a clonal proliferation of this lymphocyte population implicates these cells in the pathogenesis of cyclic neutropenia.

We thank Dr. Paul Neiman for helpful discussions.

Received for publication 14 August 1986 and in revised form 29 September 1986.

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