Chronic Natural Killer Cell Lymphocytosis: A Descriptive Clinical Study

By Ayalew Tefferi, Chin-Yang Li, Thomas E. Witzig, Madav V. Dhodapkar, Scott H. Okuno, and Robert L. Phyliky

We review the clinical manifestations and long-term outlook of patients with chronic natural killer (NK) cell lymphocytosis. After reviewing more than 1,500 peripheral blood lymphoid flow cytometry reports and molecular genetics data from patients with suspected large granular lymphocytes (LGL) proliferation, we identified 10 patients (median age at diagnosis, 60 years; range, 35 to 76 years; male:female ratio, 3:2) with persistent (greater than 6 months) increase in phenotypically determined NK cells (CD3-CD16+). Southern blot analysis performed on 9 patients showed no clonal T-cell receptor gene rearrangements. Disease duration was measured from time of initial recognition of LGL or NK cell excess (greater than 40% of the lymphocyte fraction). Clinical data from these 10 patients were compared with those from 68 patients with T-cell LGL (T-LGL) leukemia. Currently, all patients are alive (median disease duration, 5 years; range, 0.8 to 8 years). Associated disease manifestations included pure red blood cell aplasia, recurrent neutropenia, recurrent neutropenic sepsis, and vasculitic syndromes, all of which were responsive to immunosuppressive therapy. No patient had palpable lymphadenopathy or splenomegaly. Compared with the patients with T-LGL leukemia, patients with chronic NK cell leukemia had similar lymphocyte counts, associated conditions, treatment responses, and survival but had less neutropenia and anemia.

© 1994 by The American Society of Hematology.

Material and Methods

Patients were identified from several sources including a review of more than 1,500 peripheral blood (PB) lymphoid flow cytometry reports obtained at our institution during the last 5 years and a review of molecular genetics data obtained from patients with suspected proliferations of LGLs. Patients were considered to have chronic NK cell lymphocytosis after demonstration of persistent (greater than 6 months) NK cell excess (evaluated with lymphoid flow cytometry) or LGL excess (evaluated with PB smear and subsequently phenotyped as NK cell excess by lymphoid flow cytometry). In addition, eligibility for this study included the absence of viral infections and medications known to influence lymphocyte subset distribution and number.

From the Division of Hematology and Internal Medicine and the Division of Hematopathology, Mayo Clinic and Mayo Foundation, Rochester, MN.

Submitted March 31, 1994; accepted June 23, 1994.
Address reprint requests to Ayalew Tefferi, MD, Mayo Clinic, 200 First Street SW, Rochester, MN 55905.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1994 by The American Society of Hematology.
molecular genetic studies were performed in all but 1 patient and showed no clonal T-cell antigen receptor gene rearrangements.

**Patient characteristics and physical findings.** The median age of the patients was 60 years (range, 35 to 76 years), and the male-to-female ratio was 3:2. The median duration of disease was 5 years (range, 0.8 to 8 years; see Table 1). None of the patients had palpable lymphadenopathy or splenomegaly at diagnosis, and palpable splenomegally developed in only 1 patient during the course of the disease.

**Associated disease manifestations and symptoms.** At the time of initial immunophenotypic analysis, associated disease manifestations included pure red blood cell (RBC) aplasia (1 patient), recurrent neutropenia, recurrent neutropenic fever sometimes associated with pneumonia or bacterial cellulitis (2 patients), and vasculitic syndromes (3 patients; see Table 1). Of the 4 other patients, 3 were asymptomatic and 1 had severe constitutional symptoms, including arthralgias, myalgias, night sweats, and low-grade fever (patient no. 3). The patient with pure RBC aplasia was RBC transfusion-dependent, with a reticulocyte count of 2722 \( \times 10^3 \) /μL, and a leukocyte count of 2.9 \( \times 10^3 \) /μL, and a leukocyte count of 2.9 \( \times 10^3 \) /μL.

**Laboratory findings.** In addition to the required CD3 CD16" phenotype, the NK cells displayed a CD2 CD8" phenotype in all cases. HLA-DR was expressed in all but 1 patient (patient no. 9); CD7 was expressed in 8 of the 10 patients (patients no. 1 and 10; CD7"; see Table 1). A total of 2 patients had anemia attributable to chronic NK cell lymphocytosis, and 1 was transfusion-dependent (patient no. 6; see Table 1). Two patients had mild progressive thrombocytopenia (patients no. 1 and 9). Neutropenia (absolute neutrophil count less than 1,500/μL) was observed in 3 patients, and normal findings on liver function tests were noted in 2 patients with CD57 (Leu-7) was useful in visualizing the BM specimens with CD57 (Leu-7) was useful in visualizing the BM specimens.
tests in all patients. Rheumatoid factor assay and antinuclear antibody test were performed in 5 and 6 patients, respectively, and the results were positive only in the patient with rheumatoid arthritis (patient no. 10).

Cytotoxicity assays were performed in 2 patients (patients no. 2 and 3) and showed marked activity in both direct NK cell-mediated cytotoxicity and antibody-dependent cellular cytotoxicity. In addition, the NK cells from 1 of these 2 patients (patient no. 2) had altered in vitro growth requirements, suggesting that these cells did not represent a polyclonally expanded population of normal NK cells. Subsequent X-linked DNA analysis in this patient showed a monoclonal pattern of X-chromosome inactivation.13

Treatment outcome. Six patients required immunosuppressive therapy for vasculitic syndromes (2 patients), symptomatic neutropenia (2 patients), pure RBC aplasia (1 patient), or constitutional symptoms (1 patient; see Table 1). In the 2 patients with vasculitic syndromes, corticosteroid therapy resulted in amelioration of symptoms and signs of vasculitis. In addition, both patients had documented partial hematologic remissions. Treatment with corticosteroids failed to resolve the symptomatic neutropenia in patient no. 2, but hematologic complete remission was achieved with cyclophosphamide administered orally. Patient no. 10 was receiving treatment with prednisone for rheumatoid arthritis when recurrent neutropenic fevers developed. The patient had partial remission with cyclophosphamide administered orally. Similarly, the patient with pure RBC aplasia had partial remission with corticosteroid treatment and subsequently achieved a durable complete remission with azathioprine. Despite treatment that lasted for only 1 to 11 months, cytopenia has not recurred in these 3 patients, who have been followed up for 1, 4, and more than 5 years. Neither the symptoms nor the laboratory abnormalities responded to either corticosteroids or cyclophosphamide in the patient with constitutional symptoms.

Comparison with patients with T-LGL leukemia. During the study period, we also saw a series of 68 consecutive patients with T-LGL leukemia.16 We compared several clinical variables, including presenting clinical features, laboratory findings, treatment outcome, and survival, between patients with chronic NK cell lymphocytosis and those with T-LGL leukemia. No significant differences were observed with regard to age, sex, symptoms, white blood cell count (P = .14), absolute lymphocyte counts (P = .37), treatment responses, or survival. In contrast, patients with chronic NK cell lymphocytosis showed less neutropenia (P = .03) and anemia (P = .06).

DISCUSSION

NK cells are defined operationally as a subpopulation of lymphocytes carrying the membrane phenotype, CD3−
CD16`, and expressing nonmajor histocompatibility-restricted cytotoxicity without previous sensitization. Normally, NK cells constitute approximately 15% of the PB mononuclear cell fraction. Morphologically, they appear similar to T-suppressor lymphocytes, with abundant cytoplasm and azurophilic cytoplasmic granules (Fig 1A). Because of this morphologic similarity, disorders of NK cell proliferation are categorized as a subset of LGL proliferative disorders, which also include T-cell disorders.3

However, as shown by 1 of our patients and previously appreciated by others,6–7 NK cells are not always discernible as LGLs. Therefore, it might be more appropriate to use a less restrictive terminology such as "NK cell proliferative disorders." This has direct clinical relevance, because suspected NK cell disorders may need to be evaluated with both PB smear examinations and immunophenotypic analysis.

The amount of information available about the spectrum of clinical conditions associated with NK cell proliferations is limited.7 Similarly, the clonal nature of persistent NK cell proliferations is not always evident. Nevertheless, at least two clinical disorders characterized by persistent NK cell proliferations have been confirmed.8 The first, operationally defined as "NK-LGL leukemia/lymphoma," affects relatively younger patients and is characterized by an acute systemic disease with multiorgan involvement, severe constitutional symptoms, and short survival.10 The clonal nature of this disease has been confirmed by the demonstration of clonal cytogenetic abnormalities2 or single episomal form of Epstein-Barr virus DNA in the leukemic cells.19 A causative role for Epstein-Barr virus in disease pathogenesis or transformation has been suggested.19,20

The second NK cell disorder, which we refer to as "chronic NK cell lymphocytosis," has a more indolent disease course similar to that of T-LGL leukemia.8,11 Unlike NK-LGL leukemia/lymphoma, the results of cytogenetic studies are only occasionally abnormal.8,11 Similarly, T-cell antigen receptor gene rearrangement studies are not helpful in the clonal determination of chronic NK cell lymphocytosis.3 We showed monoclonality with X-linked DNA analysis in 1 of our patients,12 an observation supported by some13 but not by others.6 Regardless, in the majority of patients with chronic NK cell lymphocytosis, the clonal nature of the disorder is uncertain.

An overview of our experience with 10 patients with chronic NK cell lymphocytosis described herein indicates that patients with this disorder can expect prolonged survival but that their disease may be associated with life-threatening cytopenia or severe vasculitic syndrome. These complications were usually responsive to immunosuppressive therapy; cyclophosphamide administered orally was the most useful agent. Similar to earlier reports,11 1 of our patients had severe constitutional symptoms (fatigue, arthralgias, night sweats, weight loss, fever) unresponsive to therapy. The heterogeneity in clinical manifestations may be related partly to the phenotypic and possibly functional diversity of the excess NK cell populations.21 Regardless, it may be clinically helpful to include chronic NK cell lymphocytosis in the differential diagnosis of unexplained cytopenias, vasculitic syndromes, and persistent constitutional symptoms.

The observed clinical remissions were associated with a significant decrease in the number of NK cells, suggesting a causative role in disease manifestation. NK cells have been shown to have a negative regulatory control on erythropoiesis22 and, thus, could suppress in vitro erythroid colony formation.23 Similarly, the associated neutropenia may be secondary to an interleukin-2-induced cell-mediated suppression of myeloid progenitors possibly involving γ-interferon.24 Alternatively, it may involve a functional deficiency of myeloid colony-stimulating factors mediated by humoral mechanisms.25 The latter supposition is supported by reports of successful treatment of T-LGL leukemia-associated neutropenia with colony-stimulating factors.26,27 Although abnormal B-cell function has not been studied in chronic NK cell lymphocytosis, their abnormal function that results in autoantibody production has been implicated in T-LGL leukemia as the pathogenetic basis for associated autoimmune diseases.28

The durable remissions observed with cyclophosphamide taken orally are similar to those reported in patients with T-LGL leukemia.8,15,16 We previously reported on a successful immunosuppressive therapy with corticosteroids and azathioprine in a patient with pure RBC aplasia associated with chronic NK cell lymphocytosis.29 Although some patients responded to corticosteroid therapy, remission depended on continued administration of high doses of the drug.

Comparative clinical data between patients with chronic NK cell lymphocytosis and those with T-LGL leukemia showed similar epidemiologic and clinical features, including the spectrum of associated disease manifestations and survival. The only differences were lesser incidences of anemia and neutropenia in patients with chronic NK cell lymphocytosis and a higher incidence (26%) of rheumatoid arthritis in patients with T-LGL leukemia.16 Although acute transformation into a clinically more aggressive disease was not observed in our patients, it has been reported with chronic NK cell lymphocytosis.30

REFERENCES
2. Loughran TP: Clonal diseases of large granular lymphocytes. Blood 82:1, 1993
7. Loughran TP, Starkebaum GL: Large granular lymphocyte leu-


Chronic natural killer cell lymphocytosis: a descriptive clinical study

A Tefferi, CY Li, TE Witzig, MV Dhodapkar, SH Okuno and RL Phyliky