

**BRIEF REPORT: LYMPHOMA WITH  
RECURRENT CYCLES OF SPONTANEOUS  
REMISSION AND RELAPSE — POSSIBLE  
ROLE OF APOPTOSIS**

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**W**E describe a patient with a unique mantle-cell lymphoma, with cycles of acute illness alternating with spontaneous remissions. During the acute phase the patient had fever, generalized lymphadenopathy, hepatosplenomegaly, increased liver-enzyme concentrations, leukocytosis, and thrombocytopenia. These bouts remitted after two to three weeks without treatment. Fifteen such cycles were documented over a period of 50 months.

During the acute phase blast-like B lymphocytes in the blood expressed surface IgM, IgD, and CD5, whereas during clinical remission a small population of IgM and CD5+ lymphocytes persisted. Two B-cell clones with differently rearranged heavy-chain genes consistently appeared in the blood during the recurrent acute phases. During the spontaneous remissions, one clone (termed the "acute clone") regressed while the second clone remained relatively stable. These findings suggest that proliferation and death of the acute-clone cells determined the cyclic nature of the disease. The lymphoma cells of the acute phase, but not those of remission, were shown to undergo spontaneous apoptosis *in vitro*. This process was enhanced by inhibitors of messenger RNA and protein synthesis, suggesting that it was regulated by preexisting factors with short half-lives. No molecular alterations were demonstrated in *bcl-2* and *p53* genes, which are known to regulate apoptosis.

**CASE REPORT**

A 61-year-old man was hospitalized in March 1990 because of a high fever, perspiration, abdominal pain, and vomiting of one week's duration. Physical examination revealed generalized lymphadenopathy, splenomegaly (spleen palpable 3 cm below the costal margin), and hepatomegaly (liver tip palpable 5 cm below the costal margin). Laboratory tests revealed a hemoglobin level of 14 g per deciliter, leukocytosis (68,000 leukocytes per cubic millimeter), and a platelet count of 44,000 per cubic millimeter. The differential white-cell count showed 12 percent segmented and 88 percent mononuclear cells, 70 percent of which were blast-like, with large cleaved nuclei and prominent nucleoli. Examination of the bone marrow showed heavy infil-

tration by the lymphoid cells with the same morphologic features. A lymph-node biopsy revealed intermediate-grade malignant lymphoma of the diffuse mixed, predominantly small-cleaved-cell type. Cell-surface markers of peripheral mononuclear cells showed that 80 to 90 percent were B cells expressing IgM- $\kappa$  and CD5 but not CD10. The diagnosis of mantle-cell lymphoma was established. The t(11;14) translocation, common in mantle-cell lymphoma,<sup>1</sup> was not detected.

The bilirubin concentration was 2.8 mg per deciliter (48  $\mu$ mol per liter), the serum uric acid concentration was 9.6 mg per deciliter (571  $\mu$ mol per liter), the blood urea nitrogen concentration was 56 mg per deciliter (20 mmol per liter), and liver-enzyme levels were substantially increased. Serologic tests for Epstein-Barr virus, cytomegalovirus, human immunodeficiency virus, human T-cell lymphotropic virus type I, herpesviruses 1 and 6, and hepatitis A, B, and C were negative.

A few days after the leukocytosis reached its peak, the patient became afebrile and the number of white cells and percentage of immature lymphocytes decreased. The differential count showed 70 to 80 percent atypical lymphocytes. Two weeks later, without any therapy, the patient felt well and the lymphadenopathy, splenomegaly, and hepatomegaly had disappeared. All liver-enzyme, uric acid, and blood urea nitrogen concentrations returned to normal. The leukocyte count was normal, but 30 to 40 percent of the mononuclear cells were IgM- $\kappa$ , CD5+ B cells. The spontaneous regression of the acute phase prompted one of us to withhold therapy.

During the following 50 months 14 additional such cycles occurred. The intervals between the attacks, termed remissions, ranged from three weeks to six months (Fig. 1). The last five exacerbations were characterized by severe thrombocytopenia (with counts as low as 9000 per cubic millimeter), preceding the peak leukocytosis by 48 hours. Even though there was no bleeding, prednisone at a dose of 30 mg per day was instituted, and a week later the platelet count became normal. The attacks that occurred during prednisone therapy (episodes 11, 12, 13, 14, and 15), were shorter, and the lymphadenopathy and hepatosplenomegaly were less prominent.

**METHODS**

Mononuclear cells from heparin-treated blood were separated by Ficoll-Hypaque density-gradient centrifugation and used either fresh or after cryopreservation. Immunophenotyping was performed with a fluorescence-activated cell sorter.<sup>2</sup> Intracytoplasmic antigens were identified by the alkaline phosphatase technique.<sup>3</sup> Gene rearrangements were identified by Southern blot analyses.<sup>4</sup> DNA samples were digested with the *Hind*III restriction enzyme, and DNA probes were labeled with phosphorus-32 by multipriming. The JH and J $\kappa$  probes, which detect rearrangements in the genes for immunoglobulin heavy chains and kappa light chain, respectively, were used.<sup>5</sup> A *bcl-2* probe that detects rearrangements of the mbr region of *bcl-2* (Pr-1 probe, Oncogene Science) was used. Analysis for p53 mutations was performed by examination of single-strand conformation polymorphisms of DNA together with denaturation gradient gel electrophoresis.<sup>6</sup> Exons 5 through 8 were examined after amplification with the polymerase chain reaction. For the DNA fragmentation assay, samples of 2 million cells were examined. Intact DNA was removed from fragmented DNA before electrophoresis.<sup>7</sup>

**RESULTS**

About 80 to 90 percent of the patient's peripheral-blood lymphocytes expressed cell-surface IgM- $\kappa$  and CD5 but not CD10 during an acute phase (Table 1). Low levels of IgD were also detected on 40 percent of the cells, which is typical of immature lymphocytes in mantle-cell lymphoma. During remissions, the IgM- $\kappa$  cells constituted 20 to 40 percent of the lymphocytes, and almost no surface IgD was detected on the cells. The analysis was repeated with cytocentrifuged blood cells, which allows the determination of both cytoplasmic and surface proteins. During the leukemic phase, both IgD and IgM were expressed in 80 to 90 percent

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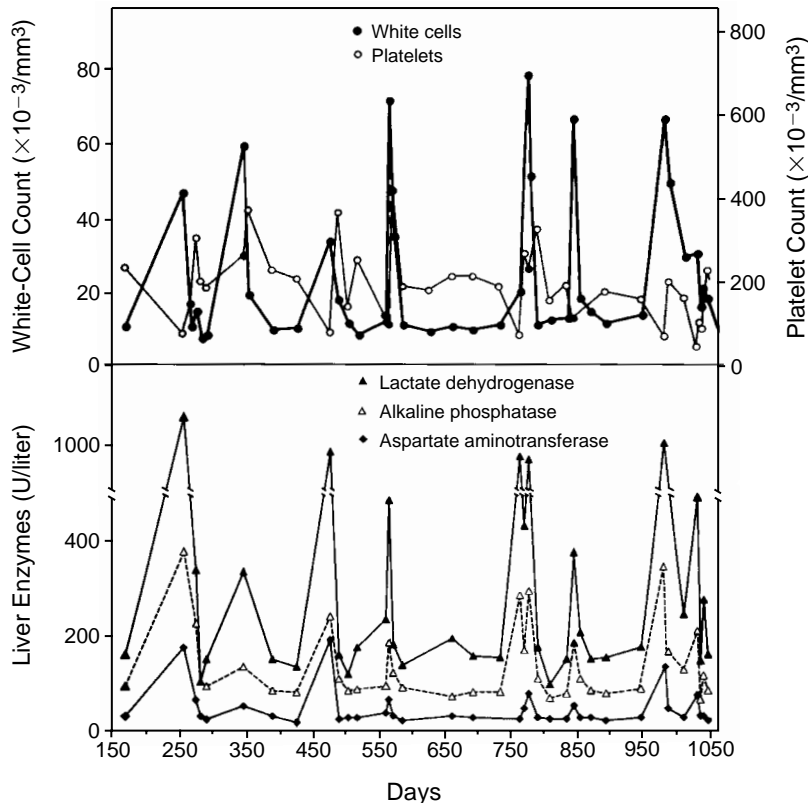


Figure 1. Blood Counts and Serum Enzyme Levels during Seven Cycles of Remission and Relapse.

The values are shown for cycles 2 through 8 (September 17, 1990, to February 3, 1993). The initial presentation was considered to have occurred on day 1. The normal ranges for liver-enzyme levels are as follows: lactate dehydrogenase, 100 to 260 U per liter; alkaline phosphatase, 45 to 115 U per liter; and aspartate aminotransferase, 7 to 40 U per liter.

of the lymphocytes. In contrast, IgD was not detected in lymphocytes during the initiation of remission.

#### The Identification of Two Clones

Figure 2 shows a representative Southern blot analysis of immunoglobulin heavy-chain and light-chain genes. DNA samples extracted from the patient's blood cells at different stages of the disease were examined with probes for the J regions of the genes for immunoglobulin kappa light chain and heavy chains and a probe for the mbr region of *bcl-2*. The analysis demonstrated that the *bcl-2* gene was in its germ-line configuration, a finding in agreement with the negative cytogenetic analysis for the t(14;18) translocation. A single rearranged kappa-light-chain band (HP) was found

in samples obtained during acute phases and remissions. The intensity of this band correlated with the level of the lymphoma cells in the blood during recurrent cycles. The heavy-chain probe revealed two bands in addition to the germ-line band. One band, designated ST, had a similar intensity in all cell preparations. By contrast, the second band, AC, predominated during acute phases and persisted at very low levels during remissions. The changes in the relative levels of the two rearranged immunoglobulin heavy-chain genes during different phases of the disease suggest shifts in the bclonal population of malignant B cells in the patient's blood. The pattern shown in Figure 2 was seen over a period of two years in five subsequent cycles.

#### Spontaneous Apoptosis of the Lymphoma Cells Correlating with the Repeated Regressions of the Acute Phase

To determine the mechanism of remissions we examined the patient's blood cells for spontaneous apoptosis. DNA fragmentation was not detected in fresh cells at any phase. However, when cells obtained during the acute phase were cultured for 16 hours, most of the cellular DNA was cleaved into nucleosomal fragments of 180 base pairs and multimers typical of apoptosis. In contrast, under the same conditions, cells obtained during remissions, containing 30 to 40 percent lymphoma cells, did not show substantial DNA fragmentation (Fig. 3). As estimated by trypan blue staining and DNA staining with propidium iodide, 50 to 60 percent of the lymphocytes obtained during acute phases died in culture within 16 hours, whereas 90 percent of lymphocytes obtained during remission were viable after three days. To characterize the mechanism regulating the death of lymphoma cells, we examined the effect of macromolecular-synthesis inhibitors. Lymphocytes obtained during an acute phase were cultured for five or seven hours, during which time apoptosis was limited.

Table 1. Typical Expression of Immunologic Cell-Surface Markers by Peripheral-Blood Lymphocytes during Acute Phases and Remissions in the Study Patient.\*

PHASE†	WBC	IgM	IgG	IgA	IgD	$\kappa$	$\lambda$	CD20	CD19	CD40	CD5	CD10	DR	CD2	CD3	CD4	CD8
	$\times 10^{-3}/\text{mm}^3$							% of cells expressing marker									
Acute	73.0	86	4	1	40	87	2	80	90	90	95	0	92	6	7	2	6
Remission	7.7	30	3	2	2	34	3	27	38	NT	79	1	38	55	46	23	28

\*WBC denotes white-cell count, and NT not tested.

†Representative cell samples were obtained during the sixth acute episode and the ninth remission.

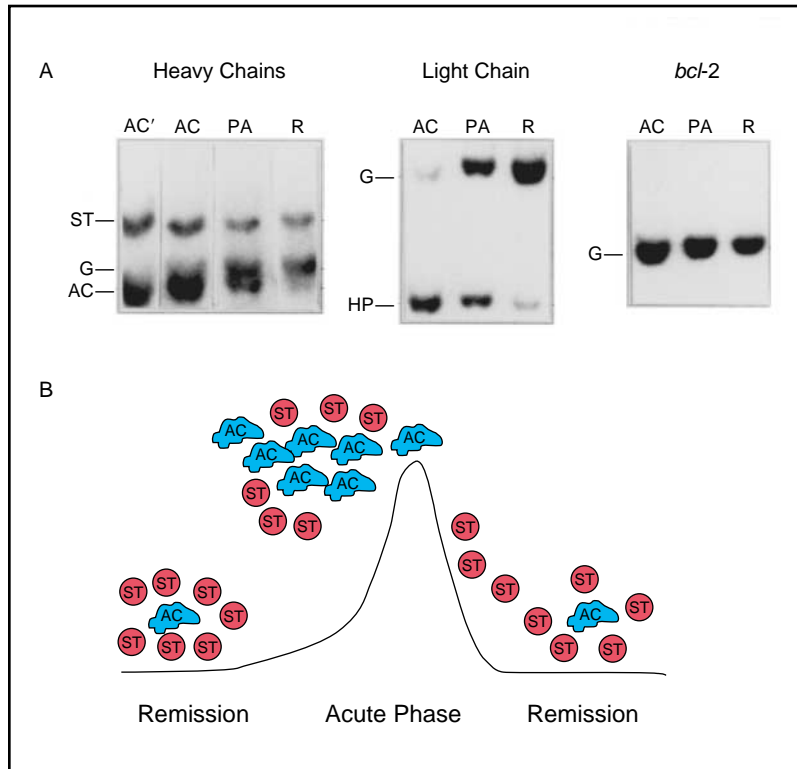


Figure 2. Southern Blot Analysis to Identify Gene Rearrangements of Immunoglobulin Heavy and Kappa Light Chains and *bcl-2*.

The patient's cells were obtained at different stages and contained the following percentage of B4+ (CD19) cells: 88 percent and 82 percent during the acute phases of the second episode (AC') and fourth episode (AC), respectively; 65 percent after the acute phase of the fourth episode (PA); and 38 percent during remission after the fourth episode (R). Panel A shows representative hybridization patterns with *Hind*III-digested cellular DNA and radioactively labeled probes for the J regions of the genes for immunoglobulin heavy and kappa light chains and *bcl-2*, which detect germ-line bands at 11, 5.4, and 4.2 kb, respectively. G denotes germ-line bands, AC and ST rearranged heavy-chain bands, and HP a rearranged kappa-light-chain band. In Panel B, a model representing one cycle suggests that the immunoglobulin heavy chains ST and AC, each of which is associated with the same light chain (HP), are distinct clones. The stable ST clone exists during all phases, whereas AC dominates the acute phase and declines during remission. The line shows the leukocyte levels.

The presence of either cycloheximide or dactinomycin in the culture dramatically increased apoptotic DNA fragmentation (data not shown).

Analyses of the p53 tumor-suppressor gene in the lymphoma cells did not reveal mutations in exons 5 through 8, in which the majority of p53 mutations have been localized.

#### DISCUSSION

In this unusual case of lymphoma we observed 15 spontaneous exacerbations and remissions over a period of 50 months. The clinical features, histologic pattern of the lymphoma, and immunophenotype of the neoplastic cells were those of mantle-cell lymphoma. A leukemic phase is common in low-grade lymphomas, either at the onset or during the course of the disease. The studies of mantle-cell lymphomas published to date usually involve treated patients, so the natural history of the disease is unknown. In a few patients therapy was deferred anywhere from 4 months to more

than 13 years,<sup>8</sup> but to our knowledge, no cases with repeated spontaneous remissions have been reported. The unusual feature in our patient is an intrinsic mechanism signaling remission that is consistently activated at the peak of the leukemic phase.

This pattern of the disease could reflect a preferential response of one of the malignant clones (which we termed the "acute clone") to both proliferative and cell-death signals. Such a mechanism could explain why two B-cell clones were observed during acute phases but only one clone was detected during remission. Moreover, the Southern gel band that defined the cells of the acute clone was undetectable during five subsequent remissions but became the main band during the following acute phases. Thus, the clone defined by the acute-phase band constitutes the majority of the lymphoma cells that proliferated during the leukemic phases. The cells of this same clone were preferentially eliminated on spontaneous entry into clinical remission. We do not know what makes the cells of one clone susceptible to death and the cells of the second clone relatively stable. The finding that the two clones share the same kappa light chain suggests that both originated from a single malignant clone. Indeed, in the majority of lymphoproliferative disorders, biclonality is due to somatic mutations within the malignant clone.<sup>4,9</sup>

When studied at the peak of acute phases, the lymphoma cells died spontaneously in vitro within 16 hours as a result of apoptosis. This suggests that programmed cell death, activated in the neoplastic cells in vivo at the peak of the acute phase, eliminated most of the lymphoma cells from the blood. The finding that inhibiting RNA and protein synthesis in the lymphoma cells enhanced apoptosis is surprising, because the induction of apoptosis usually requires RNA and protein synthesis.<sup>10</sup> Nevertheless, in some cell lines either dactinomycin or cycloheximide can induce apoptosis.<sup>11,12</sup> It is possible that the suicide program of the lymphoma cells is activated on initiation of the acute phase and is actively suppressed by unstable protective proteins. We found no indication of molecular alterations in p53 and *bcl-2* genes.

What causes the repeated regression of the acute phases? Autoantibodies with antiidiotypic activity<sup>13,14</sup> are candidates. Indeed, the patient's circulating B cells constitutively secreted IgM (data not shown), although

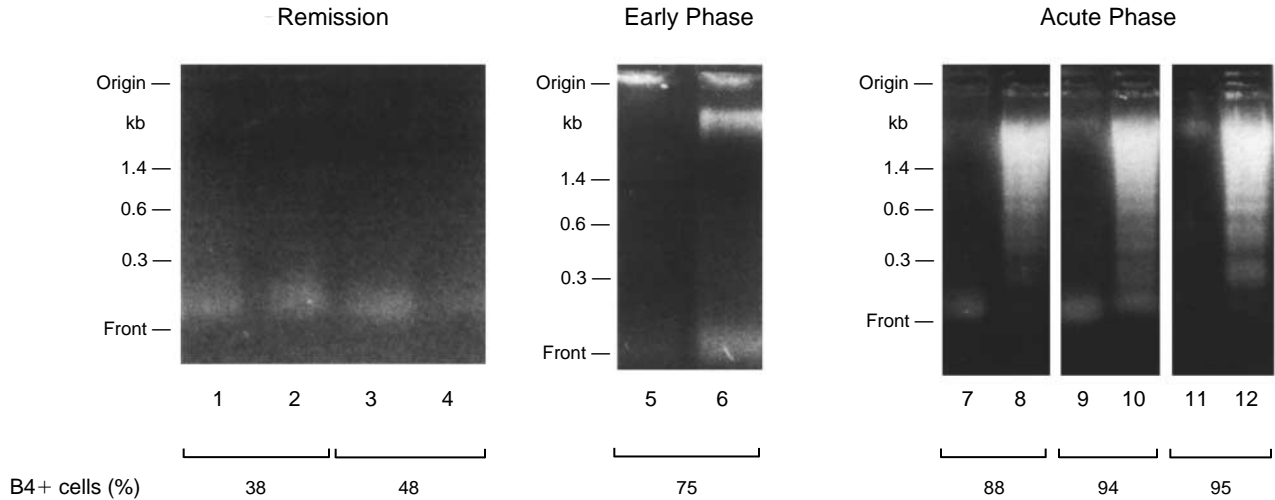


Figure 3. Patterns of Fragmented DNA from Cells Obtained from the Patient during Different Phases of the Disease Cycle.

Fragmented DNA was isolated from fresh cells (lanes 1, 3, 5, 7, 9, and 11) or cells incubated for 16 hours at 37°C in RPMI medium containing 10 percent fetal-calf serum (lanes 2, 4, 6, 8, 10, and 12). The DNA was separated by agarose-gel electrophoresis. Cells were obtained during remission after the third and eighth episodes (Remission), soon after the onset of the sixth episode (Early Phase), and during the acute phases of the sixth, seventh, and eighth episodes (Acute Phase). The percentage of B4+ (CD19) cells in each preparation is indicated. An *HaeIII* digest of OX174 DNA was used to provide the molecular size markers. Origin indicates where the samples were loaded onto the gel, and front the farthest point of migration of the sample.

the specificity of these antibodies has not yet been examined. What causes the induction of the leukemic phases? Expansion and blastogenesis of the lymphoma cells and induction of IgD, CD23, and intercellular adhesion molecule 1 on initiation of the acute phase suggest that the neoplastic cells have responded to an activation signal. A chronic viral infection could provide such a signal.

We speculate that activation of the lymphoma cells, possibly mediated by T cells, enables their escape from a regulatory mechanism and thereby initiates the leukemic phase. An example of such a regulatory mechanism is the interaction of CD40 molecules on the lymphoma cells, with CD40 ligands on activated T cells.<sup>15</sup> An alternative mechanism may involve cytokines. The imbalance in the ratio of lymphoma cells to T cells at the peak of the acute phase may result in the loss of the T-cell-mediated survival signal, followed by apoptosis and clinical remission.

#### REFERENCES

- Weisenburger DD, Sanger WG, Armitage JO, Purtilo DT. Intermediate lymphocytic lymphoma: immunophenotypic and cytogenetic findings. *Blood* 1987;69:1617-21.
- Kaufmann Y, Levanon M, Davidsohn J, Ramot B. Interleukin 2 induces human acute lymphocytic leukemia cells to manifest lymphokine-activated-killer (LAK) cytotoxicity. *J Immunol* 1987;139:977-82.
- Buccheri V, Matutes E, Dyer MJS, Catovsky D. Lineage commitment in bi-phenotypic acute leukemia. *Leukemia* 1993;7:919-27.
- Hakim I, Rechavi G, Brok-Simoni F, et al. Analysis of rearranged immunoglobulin genes indicating a process of clonal evolution in chronic lymphocytic leukemia. *Br J Haematol* 1993;84:436-42.
- Korsmeyer SJ, Arnold A, Bakhshi A, et al. Immunoglobulin gene rearrangement and cell surface antigen expression in acute lymphocytic leukemias of T cell and B cell precursor origins. *J Clin Invest* 1983;71:301-13.
- Mor O, Hakim I, Stiebel H, et al. Application of DGGE and SSCP to the analysis of point mutations in the p53 tumor suppressor gene. *Mol Biol (Life Sci Adv)* 1994;13:53-61.
- Sellins KS, Cohen JJ. Gene induction by gamma-irradiation leads to DNA fragmentation in lymphocytes. *J Immunol* 1987;139:3199-206.
- Bookman MA, Lardelli P, Jaffe ES, Duffey PL, Longo DL. Lymphocytic lymphoma of intermediate differentiation: morphologic, immunophenotypic, and prognostic factors. *J Natl Cancer Inst* 1990;82:742-8.
- Kon S, Levy S, Levy R. Retention of an idiotype determinant in a human B-cell lymphoma undergoing immunoglobulin variable-region mutation. *Proc Natl Acad Sci U S A* 1987;84:5053-7.
- Cohen JJ. Apoptosis. *Immunol Today* 1993;14:126-30.
- Martin SJ, Lennon SV, Bonham AM, Cotter TG. Induction of apoptosis (programmed cell death) in human leukemic HL-60 cells by inhibition of RNA or protein synthesis. *J Immunol* 1990;145:1859-67.
- Cuende E, Ales-Martinez JE, Ding L, Gonzalez-Garcia M, Martinez C, Nunez G. Programmed cell death by bcl-2-dependent and independent mechanisms in B lymphoma cells. *EMBO J* 1993;12:1555-60.
- van Enderd PM, Heilig B, Hammerling GJ, Moldenhauer G. Monoclonal antibodies to idiotype inhibit in vitro growth of human B-cell lymphomas. *Blood* 1992;79:129-37.
- Friedman DF, Cho EA, Goldman J, et al. The role of clonal selection in the pathogenesis of an autoreactive human B cell lymphoma. *J Exp Med* 1991; 174:525-37.
- Tsubata T, Wu J, Honjo T. B-cell apoptosis induced by antigen receptor crosslinking is blocked by a T-cell signal through CD40. *Nature* 1993;364: 645-8.