CONCISE REPORT

Cytogenetic Evidence for the Clonal Nature of Richter’s Syndrome

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Sequential chromosome studies were done on a patient who developed diffuse histiocytic lymphoma (DHL) after a long history of untreated chronic T-cell leukemia. During the indolent phase of her disease, a pseudodiploid lymphocyte population with 3q- and 14q- chromosome markers gradually replaced the originally diploid tumor cells. The karyotype of the lymphoma was hypertriploid (70–74 chromosomes) with the same 3q- and 14q- markers. The findings indicate that DHL in this patient evolved from the leukemic T-cell clone.

A SMALL proportion of patients with chronic lymphocytic leukemia (CLL) ultimately develops a diffuse “histiocytic” lymphoma (DHL). This clinicopathologic entity has been termed Richter’s syndrome.1 There has been debate as to whether the lymphoma represents a second neoplasm or evolves from the CLL. Although certain morphological and immunologic studies have supported the latter explanation, direct evidence has been lacking that the DHL arises as the result of clonal evolution within the original population of CLL cells.2-5

In this article, we describe a patient with untreated T-cell CLL who ultimately developed subcutaneous and abdominal diffuse histiocytic lymphoma. Sequential chromosome studies during the early phase of her disease had demonstrated cytogenetic evolution correlated with changes in the characteristics of her T cells, but no clinical progression.6 The present report provides additional cytogenetic data on this patient, indicating that the terminal Richter’s syndrome represented further evolution of the neoplastic clone.

CASE REPORT

The patient was a 52-yr-old woman in whom an absolute lymphocytosis (9520/cu mm) was observed incidently in 1970. She was first evaluated hematologically in October 1975, and the diagnosis of chronic lymphocytic leukemia (Ria stage I) was made on the basis of her prolonged and unexplained lymphocytosis. During 3 yr of follow-up, she had no symptoms referable to her lymphoproliferative disorder and had neither pathologic adenopathy, hepatosplenomegaly, nor skin infiltration. She received no therapy. The total leukocyte count varied from 15,200 to 23,400/cu mm, without major change in the absolute lymphocyte count (12,900 ± 3600 SD). Hematocrit, reticulocyte count, and platelet count remained within normal limits. The bone marrow was not studied. Quantitative immunoglobulins, immunoelectrophoresis, and serum protein electrophoresis were normal on three occasions (1975, 1977, 1978) and the Coombs’ test was negative.

In June 1980, she presented with weight loss, abdominal pain, and multiple abdominal masses. Biopsy of a subcutaneous nodule showed diffuse histiocytic lymphoma, consistent with a T-cell immunoblastic lymphoma (Lukes-Collins classification). An abdominal CAT scan showed massive intra- and retroperitoneal tumor. White blood count was 14,000 with 49% normal-appearing lymphocytes. Despite an initial partial response to chemotherapy (cyclophosphamide, doxorubicin, vincristine, prednisone, bleomycin) she developed progressive, drug-resistant disease and died in December 1980. A limited autopsy revealed extensive involvement of the abdominal viscera with lymphoma, and no evidence of either DHL or CLL in the bone marrow.

Venous blood for cytogenetic, membrane marker, and proliferation studies on the patient’s lymphocytes was drawn on 11 occasions between October 1975 and July 1980. Biopsy material from the lymphoma was obtained in July 1980.

MATERIALS AND METHODS

Cytogenetics

Chromosome studies were done on 8 occasions between October 1975 and July 1980 on lymphocytes from the peripheral blood cultured and processed as previously reported.1 The mitogens used were either phytohemagglutinin (PHA-M, Difco), concanavalin A (Calbiochem), the calcium ionophore, A23187, or the tumor promoter, tetradecanoyl-0-phorbol-13-acetate (TPA, Chemical Carcinogenesis). There were no mitoses in cultures grown without mitogen. The preparations were examined with routine Giemsa staining and with G-banding.7

The biopsy of the lymphoma was finally minced to produce a single cell suspension, and these cells were then cultured in the same fashion as lymphocytes separated from blood.

Membrane Markers and Proliferation

For sheep erythrocyte (E) rosettes, complement (EAC) rosettes, and immunofluorescence studies of surface immunoglobulins (slg), the patient’s lymphocytes were processed using procedures previously described.6 Proliferation was assessed6 with the same mitogens as were used for the cytogenetic studies.

RESULTS

Cytogenetic, membrane marker, and proliferation studies done on this patient between October 1975 and April 1978, during the stable phase of her disease, have previously been reported in detail.6 When first studied, 77% of the lymphocytes formed E rosettes and only 8% formed EAC rosettes. The cells gave a normal proliferative response to PHA, Con-A, and A23187.

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On the basis of these findings, the patient's disease was considered a T-cell neoplasm. The cells proliferating in the mitogen-stimulated cultures did not initially show chromosome changes.

Over the next 2.5 yr, despite clinical stability, a clone of cells with multiple chromosome changes (Fig. 1A) gradually replaced the original diploid neoplastic population. The frequency of abnormal cells varied with different mitogens, and by April 1978, constituted as much as 92% of the dividing metaphases in cultures stimulated with A23187.

The abnormal clone was characterized by a pseudodiploid karyotype, with an easily recognizable large marker chromosome. Banding demonstrated that the marker was a 3q- chromosome, resulting from translocation of most of the long arm of a number 2 chromosome to the long arm of a number 3. There was also a translocation from a number 18 to the distal end of the long arm of a number 14 (band q24–32), producing a 14q+ chromosome, a common abnormality in lymphoid tumors. One number 17 appeared to have an extra band, of undetermined origin, in the long arm (Fig. 1A).

During the period of emergence of this cytogenetically aberrant clone, there was a roughly parallel alteration in the T-cell characteristics of the circulating lymphocytes. The percentage of cells forming E rosettes fell as low as 16% (without an increase in EAC rosettes), and there was an associated partial loss in responsiveness to all T-cell mitogens.

Throughout this period, the surface immunoglobulin (slg) patterns of the patient's lymphocytes were unusual. A high proportion of the cells (48%–83%) were positive for slg when stained with polyclonal antiserum, and there was consistently a high percentage of cells positive for different Ig classes, with a sum greater than 100%. The staining was much more intense than is typical for B-CLL.6 On three occasions, more than half of the slg positive cells became negative after exposure to trypsin or pronase, and surface Ig was only minimally restored after overnight culture in serum-free medium.

Studies subsequent to those previously reported6 were done in October 1978, September 1979, and July 1980. The findings in October 1978 did not differ significantly from 6 mo previously, but in September 1979, fewer than 10% of cells from the peripheral blood were chromosomally abnormal, with the same aberrant karyotype, and the membrane marker and proliferation studies suggested that many of the circulating lymphocytes at this time were non-neoplastic.

In July 1980, shortly after the patient presented with an abdominal lymphoma, the findings in the peripheral blood again showed fewer than 10% chromosomally abnormal metaphases, but the rosette and slg data, as well as the proliferative responses to T-cell mitogens, were similar to those previously obtained with the neoplastic cells.

At this time, 25 metaphases were examined from cultures of the lymphoma grown for 24 hr without mitogen, and 20 cells from 72-hr PHA cultures. All were grossly abnormal, with a predominant chromosome number in the range of 70–74 chromosomes (hypertriploid). The predominant karyotype showed triploidy for most chromosomes, with tetraploidy for the F and G groups and diploidy for number 2. Aberrant chromosomes included: the large 3q+ marker chromosome previously observed in the pseudodiploid clone (this marker was recognizable in nearly all of the lymphoma metaphases examined) (Fig. 1B); the 14q+ chromosome also previously seen; and two isochromosomes for the long arm of number 17 (this abnormality was seen as an extra chromosome in one cell of the pseudodiploid clone in October 1978).

The cells from the lymphoma had “null” membrane characteristics, failing to form either E rosettes or EAC rosettes, or to demonstrate surface immunoglobulin. They did give a moderate proliferative response to both PHA and TPA, as compared to cultures without mitogen, suggesting that some T-cell characteristics may have been retained.

**DISCUSSION**

Although some patients with CLL show clinical progression to more aggressive variants of the disease, there has not been complete agreement that Richter's syndrome represents one such evolutionary process.2,5 Morphological comparisons have been of limited value, and the few attempts to relate the terminal histiocytic lymphoma to the preexisting CLL by immunologic means have produced conflicting results. Brouet et al.,9 for instance, reported a patient with Waldenstrom's macroglobulinemia who had apparently monoclonal surface immunoglobulin (IgM, kappa) on marrow lymphocytes that was the same as that subsequently observed on the DHL cells. Splinter et al.,10 however, found the slg of the CLL and lymphoma cells of a patient with Richter's syndrome to be of differing light chain classes (IgM, lambda versus IgM, kappa).

The cytogenetic findings in the present patient support the view that, at least in this case, the DHL arising in a patient with preexisting CLL represents clonal evolution of the original neoplasm. The cells of the lymphoma (Fig. 1B) showed a marked increase in chromosome number (a common finding in tumor progression).11 However, the same large marker chro-
Fig. 1. (A) Representative G-banded karyotype of the patient's leukemic T-cell clone, interpreted as 46,XX,-3,+t(2q;3), 2q-,14,+t(14;18q). 18q., 17q-. Translocation from a no. 2 chromosome to a no. 3 produced a large 3q' marker. Insert illustrates normal and abnormal no. 3 chromosomes from another cell. There is also a translocation from no. 18 to the long arm of no. 14, producing a 14q' and an extra band in the long arm of a no. 17 (arrows). (From Nowell et al.) (B) Representative G-banded metaphase, with 70 chromosomes, from the patient's diffuse histiocytic lymphoma. Note same 3q' and 14q' markers (large arrows) as illustrated in (A), indicating derivation of the DHL cells from the original leukemia. Two isochromosomes for the long arm of no. 17 are also present (small arrows).

This patient provides striking correlations between biologic progression and cytogenetic evolution within the tumor cell population in both phases of her disease. During the long stable period, it seems probable that the gradual decline of certain specialized T-cell characteristics resulted from the first chromosomally altered subline gradually replacing the original diploid neoplastic cells in the circulation. The ability to form E rosettes and to respond to T-cell mitogens both appear to depend on specific membrane receptors. In this instance, the chromosome changes in the mutant subpopulation may have caused alterations in these receptors without any important change in growth

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foundings in the original CLL are probably irrelevant to the present considerations. The nonclonal nature of the slg, its intensity of staining, and its partial removal by enzymes, all suggest that it was not being synthesized by the neoplastic cells, and instead represented circulating antibodies or immune complexes attached to the surface of the leukemic T cells via Fc receptors or other mechanisms.6

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regulatory pathways. The subsequent stage, on the other hand, appears to be an example of clinical progression to a highly aggressive neoplasm as a result of additional major karyotypic change. This fits with a general view of clonal evolution, which suggests that tumor progression results from genetic instability in neoplastic populations, permitting sequential selection, over time, of increasingly mutated subpopulations within the original clone. This patient had never received therapy prior to the appearance of Richter's syndrome, and there is no obvious explanation for the apparent genetic lability underlying the sequential cytogenetic changes.

The growth characteristics of her neoplastic cells in the two phases of her disorder appear also to fit with previous observations on clonal evolution. In some instances, a clone may have only very minimal selective growth advantage over normal cells, and this appeared to be the case both in her diploid tumor cells and in the original pseudodiploid population. The situation may be analogous to that seen in patients with ataxia-telangiectasia, where slowly expanding T-cell clones with cytogenetic abnormalities may persist in the peripheral blood for a number of years, only progressing to frank leukemia when additional chromosome changes occur. It is not clear why in the final two studies in this patient (September 1979; July 1980), the frequency of chromosomally aberrant neoplastic cells in the circulation appeared to be greatly reduced from the previous several years. A fall in leukocyte count has been reported in most patients with Richter's syndrome, but treatment of the preexisting CLL appeared to be the causative factor. Perhaps in the present case, the expanding DHL overgrew the sites of production of the pseudodiploid CLL cells, or sufficiently altered immune regulation to inhibit their production or recirculation.

REFERENCES
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