The Malignant Cells in a Lennert’s Lymphoma Are T Lymphocytes With a Mature Helper Surface Phenotype. A Multiparameter Flow Cytometric Analysis

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The flow cytometric analysis of DNA content in cells obtained from a case of Lennert’s lymphoma demonstrated the presence of a discrete hypotetraploid cell population. Correlated multiparameter analysis of DNA, light scatter, and surface antigens by flow cytometry showed that the hypotetraploid cells were intermediate to large cells expressing T11, T3, and T4 antigens and lacking B1 and T8 antigens. These findings suggest that Lennert’s lymphoma represents a malignant neoplasm of T-helper lymphocytes.

In 1968 Lennert and Mestdagh described what they believed to be a variant of Hodgkin’s disease with a high content of epithelioid histiocytes and few Reed-Sternberg cells. In 1976, Burke and Butler speculated that so-called Lennert’s lymphoma was a distinct clinicopathologic entity and not a variant of Hodgkin’s disease. This idea was reinforced by Kim et al in 1978 and 1980. It is now believed that Lennert’s lymphoma is a distinct non-Hodgkin’s lymphoma with a multifocal epithelioid histiocytic reaction.

Using the multianalytical capabilities of flow cytometry, we studied the light scatter and antigenic expression of a hypotetraploid cell population detected by DNA measurements in a case of Lennert’s lymphoma. This approach permitted a direct analysis of relative size and surface immunophenotype of the presumably neoplastic aneuploid cells in this lymphoma.

CASE REPORT

A 40-year-old white male presented in July 1984 with arthralgias and loss of strength in hand muscles over a 4-month interval, with gradual development of cervical and inguinal adenopathy, an 8-lb weight loss, and generalized weakness. An inguinal lymph node biopsy performed elsewhere 10 months previously was diagnosed as nonspecific hyperplasia. Because of diffuse peripheral arthritis with swelling of the metacarpophalangeal and proximal intercarpal joints, reduction of wrist motion, and loss of shoulder motion various nonsteroidal anti-inflammatory drugs were prescribed, without benefit.

On admission, he had diffuse anterior and posterior cervical and inguinal adenopathy and diffuse swelling of metacarpophalangeal and proximal intercarpal joints with restricted motion in both wrists. Laboratory findings included a normal complete blood count and electrolyte values, elevated lactate dehydrogenase level, and depressed total protein level. Antinuclear antibody and rheumatoid factor were negative, serum immunoelectrophoresis was normal, and a chest film was normal. Computerized tomography of the abdomen showed splenomegaly and prominent paraaortic and retrocaval nodes. A biopsy specimen of an inguinal lymph node the day after admission was diagnosed as malignant lymphoma with a high content of epithelioid histiocytes (Lennert’s lymphoma). Lymphoma was also present in a bone marrow biopsy specimen. For the next 8 months he received 11 courses of combination chemotherapy and prednisone, and currently he has no peripheral adenopathy but does have residual lymphoma in the bone marrow.

MATERIALS AND METHODS

The first inguinal lymph node (Oct 1983) was fixed in formalin, and routine hematoxylin- and eosin-stained sections were made. The second inguinal lymph node biopsy specimen, taken in July 1984, was divided into two parts. One part was fixed in formalin, and routine hematoxylin- and eosin-stained sections were made. The other portion was minced, and the resulting monodispersed cell suspension was centrifuged through Ficoll-Hypaque (LSM, Litton Bionetics, Kensington, Md) to obtain viable mononuclear cells. An aliquot of cells was smeared onto a slide and stained with Giemsa stain for cytologic examination. Other aliquots were exposed to OKT11, OKT4, OKT8, OKT3 (Ortho Pharmaceutical Corp. Raritan, NJ), and B1 (Coulter Immunology, Hialeah, Fla) monoclonal antibodies followed by fluorescein isothiocyanate (FITC)-conjugated sheep antimouse antibody (Cappel Laboratories, Cochranville, Pa) and to FITC-conjugated goat antihuman IgM, IgG, and λ immunoglobulin IgG antibodies (Kallestad Laboratories, Inc, Austin, Tex). Normal mouse IgG and FITC-labeled goat IgG were used as controls. Other portions of the cell suspension were prepared for single-parameter DNA analysis, and for correlated multiparameter analysis of DNA, surface antigens, and forward-angle light scatter.

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MALIGNANT CELLS IN A LENNERT'S LYMPHOMA

Fig 2. DNA histogram produced from flow cytometric analysis of the inguinal lymph node cells. Increasing relative DNA content per cell, indicated by the flow cytometer’s channel number (x axis), is plotted vs the relative number of cells in each channel (y axis). Normal G1-phase cells produced the large peak at channel 65. Normal G2-phase and mitotic cells produced the small peak at channel 130. In between, a peak of abnormal cells with a hypotetraploid amount of DNA is seen (channel 110). This abnormal population represents 7% of the total cells analyzed.

RESULTS

Light microscopy. Sections of the lymph nodes biopsied in Oct 1983 and July 1984 demonstrated the features of Lennert’s lymphoma (Fig 1). The nodes contained large numbers of epithelioid histiocytes with a background of lymphoid cells. The epithelioid histiocytes had a diffuse arrangement and also formed several discrete cell clusters. The lymphoid component consisted of small, intermediate, and large lymphocytes with irregular, cleaved nuclei. Scattered atypical mononuclear cells were present that resembled Reed-Sternberg variants with large vesicular nuclei and prominent nucleoli. The Giemsa-stained smear of the cell suspension showed predominantly lymphocytes of varying size, occasional atypical large lymphocytes, and less than 1% epithelioid histiocytes.

Flow cytometry. Analysis of surface antigens on cells from the July 1984 lymph node demonstrated the following distribution: B1, 55%; IgG, 4%; IgM, 49%; κ, 31%; λ, 22%; OKT11, 50%; OKT4, 36%; OKT8, 8%; and OKT3, 35%. DNA analysis showed that the majority of these cells had a diploid DNA content. However, a relatively small number of cells (7% of all cells analyzed) formed a discrete peak located in a hypotetraploid position in relation to the diploid cells (Fig 2). By using correlated analysis of surface antigens and DNA this discrete population of hypotetraploid cells was shown to bear T11, T3, and T4 antigens (Fig 3). T8 and B1 antigens were absent on these cells (Fig 4). The diploid cells, most likely nonneoplastic, represented a mixture of cells expressing B1 (59%), T11 (32%), T3 (28%), T4 (27%), and T8 (4%) antigens. An insufficient number of cells precluded the analysis of other antigens. Forward-angle light scatter analysis, which is related to cell size, showed the hypotetraploid cells to be of intermediate-to-large size when compared with the rest of the cell population (Fig 5).

DISCUSSION

Reports of Lennert’s lymphoma progressing to a malignant lymphoma of the large cell, “histiocytic” type have been described. Klein et al in two cases demonstrated that the histiocytic lymphoma was cytoplasmic immunoglobulin-negative but was unable to perform T cell marker studies because of a lack of unfixed tissue. Miller et al described a case of histiocytic lymphoma with immunoglobulin production presumably originating from Lennert’s lymphoma. One
of four cases studied by Bedetti and Ollapally also demonstrated cytoplasmic staining for immunoglobulin.\textsuperscript{19}

A number of investigators have suggested that Lennert's lymphoma is a neoplasm of T cell origin.\textsuperscript{7,10-17} Lukes and Collins classified it as a T cell neoplasm in 1977.\textsuperscript{17} Palutke et al and Borowitz et al found E rosetting abnormal lymphocytes in cell suspensions prepared from Lennert's lymphoma.\textsuperscript{13,15} Bogomoletz and co-workers described a case in which OKT4 and OKT3 antibodies labeled 73% and 88% of the cells respectively.\textsuperscript{12} Knowles and Halpern demonstrated OKT3+, T10+ cells and a small number of OKT5+ and OKT8+ cells in a case of Lennert's lymphoma.\textsuperscript{14} None of these studies, however, demonstrated conclusively the neoplastic nature of these T cells.

Some authors have even stated that Lennert's lymphoma may be a reactive condition.\textsuperscript{8} The hypotetraploid cells detected in our sample of Lennert's lymphoma strongly suggested the presence of neoplastic cells. Ploidy abnormalities are indicative of neoplasia and hypotetraploid populations have never been observed in nonneoplastic conditions.\textsuperscript{6,18,19} Simultaneous measurement of DNA content and light scatter showed that the abnormal cells were of intermediate-to-large size when compared with the rest of the lymphoid population obtained. Correlated analysis of DNA content and surface antigens demonstrated that the hypotetraploid cells shared surface properties with the so-called helper/inducer T lymphocytes, thus resembling other lymphomas such as cutaneous T cell lymphomas,\textsuperscript{20} human T cell leukemia virus 1-associated leukemia-lymphomas\textsuperscript{21} and some peripheral T cell lymphomas.\textsuperscript{22}

The results of our analysis indicate that only a small percentage of the cells obtained in our final cell suspension had ploidy abnormalities. The remaining cells were presumable nonneoplastic since they were diploid elements expressing different surface antigens. More than half of the cells bore B cell antigens with a normal ratio of \( \kappa/\lambda \) immunoglobulin-

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**Fig 4.** Correlated two-parameter histogram produced from flow cytometric analysis of the inguinal lymph node cells. This plot is similar to that in Fig 3 except that T8 antigen expression is plotted against increasing relative DNA content per cell. The hypotetraploid cell population is lacking T8 antigen and is marked by the arrow.

**Fig 5.** Correlated two-parameter histogram produced from flow cytometric analysis of the inguinal lymph node cells. This plot is similar to Figs 3 and 4 except that increasing forward-angle light scatter, an indicator of cell size, is plotted against increasing relative DNA content per cell. The hypotetraploid cell population, marked by the arrow, produces an intermediate-to-large amount of light scatter when compared with the total cell population.
lin-bearing cells. Also, T4 and T8 antigen-bearing cells were present in proportions that are not different than those observed in reactive processes. Although we cannot exclude the possibility that in our case the neoplastic cells, like the epithelioid histiocytes, were preferentially lost during the cell suspension preparation, the morphologic changes suggested that normal elements outnumbered neoplastic cells. Large numbers of normal-appearing cells are usually recognized morphologically in conjunction with malignant cells in Hodgkin's disease. Also, numerous apparently normal T cells are often present in B cell neoplasms.\textsuperscript{23,24} It is likely that normal elements may also be present in T cell neoplasms, but this has not been well documented. Neoplastic T cells may produce factor(s) responsible for an active recruitment of normal cells at tumor sites. It is possible that normal lymphocytes immunoregulate the growth and/or differentiation of neoplastic cells as may happen in B cell lymphomas.\textsuperscript{25} Regardless of the mechanism responsible for their presence, the significance of large numbers of nonneoplastic cells admixed with relatively fewer tumor cells in these lymphomas is presently unknown.

Our findings do not permit us to make a general statement about the nature of the neoplastic cells in other cases of Lennert's lymphoma. Nevertheless, this study demonstrates the power of correlated multiparameter flow cytometric analysis which can identify and characterize neoplastic cells in a manner that cannot be easily attained by other techniques. This is of particular importance when, as in our case, neoplastic elements represent only a small percentage of the cells present in a tumor. The precise identification of tumor-associated surface antigens should allow a better delineation of the cell of origin of Lennert's lymphoma and may contribute to improve its therapy.

ACKNOWLEDGMENT

We would like to thank Janet McMillan for her excellent secretarial assistance.

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