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.

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, Fl) monoclonal antibodies followed by fluorescein isothiocyanate (FITC)-conjugated sheep antimouse antibody (Cappel Laboratories, Cochranville, Pa) and to FITC-conjugated goat antihuman IgM, IgG, and IgA 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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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.

Fig 3. Correlated two-parameter histogram produced from flow cytometric analysis of the inguinal lymph node cells. One axis represents increasing relative DNA content per cell, whereas the other represents increasing expression of the T4 antigen. The number of cells is plotted vertically. The hypotetraploid cell population bearing T4 antigen is marked by the arrow.

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: Bl, 55%; IgG, 4%; IgM, 49%; x, 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 Bl antigens were absent on these cells (Fig 4). The diploid cells, most likely nonneoplastic, represented a mixture of cells expressing Bl (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. 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.
of four cases studied by Bedetti and Ollapally also demonstrated cytoplasmic staining for immunoglobulin. A number of investigators have suggested that Lennert’s lymphoma is a neoplasm of T cell origin. Lukes and Collins classified it as a T cell neoplasm in 1977. Palutke et al and Borowitz et al found E rosetting abnormal lymphocytes in cell suspensions prepared from Lennert’s lymphoma. Bogomoletz and co-workers described a case in which OKT4 and OKT3 antibodies labeled 73% and 88% of the cells respectively. Knowles and Halper demonstrated OKT3+, T10+ cells and a small number of OKT5+ and OKT8+ cells in a case of Lennert’s lymphoma. 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. 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. 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, human T cell leukemia virus 1–associated leukemia-lymphomas and some peripheral T cell lymphomas.

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 presumably 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 κ/λ immunoglobulins.
MALIGNANT CELLS IN A LENNERT’S LYMPHOMA

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