Malignant Lymphoma of Small Cleaved Lymphocytes of the Follicular Mantle Zone

By Margarita Palutke, Leopoldo Eisenberg, Ila Mirchandani, Pamela Tabaczka, and Mujtaba Husain

We describe a highly unusual lymphocytic lymphoma. It appeared to originate in the mantle zones of hyperplastic follicles that had large reactive centers. The tumor cells in the mantle zone were small lymphocytes with cleaved or very irregular nuclei. They had coarse and abundant IgM, kappa surface immunoglobulin markers, and receptors for complement. The tumor involvement was generalized at the time of discovery. The diagnosis of a malignant lymphoma was initially made with difficulty because of the presence of reactive follicular centers as well as a polyclonal hypergammaglobulinemia and large numbers of interfollicular plasma cells and plasmacytoid lymphocytes containing all classes of immunoglobulin. However, 2 yr later, the follicular centers were replaced by tumor nodules composed of lymphocytes identical in appearance and immunologic type to those seen originally. This case illustrates that not all nodular lymphomas are follicular center cell (FCC) neoplasms and that morphological transformation from small round to small cleaved lymphocytes and a corresponding increase in surface immunoglobulins may take place in the follicular mantle zone. The patient had a high titer of antibody to Epstein-Barr virus (EBV) and a poor lymphocyte response to concanavalin A, but neither the tumor cells nor the plasmacytoid lymphocytes contained EBV DNA.

EXTENSIVE INVESTIGATION of a recently encountered lymphoproliferative disorder suggested origin of the neoplasm in the follicular mantle zone. A diagnosis of malignant lymphoma was difficult to establish because of concomitant marked follicular center hyperplasia and polyclonal gammopathy. The monoclonal surface markers and abnormal morphology of the cells in the mantle zone, small lymphocytes with cleaved and irregular nuclei identical to those in the blood during the hemi phases, the progressive expansion of the mantle zone, and the patient's clinical course established the diagnosis of malignant lymphoma of the follicular mantle zone.

This diagnosis was confirmed 2 yr later when another lymph node and tonsil exhibited complete replacement of the follicular centers by tumor. The entity, "malignant lymphocytic lymphoma of intermediate differentiation," described by Mann et al., has been suspected of arising from follicular mantle cells. The lesion we report may be related to that reported by these workers and supports the concept of the existence of such lymphomas.

CASE REPORT

A 61-yr-old white woman presented with diffuse massive lymphadenopathy, splenomegaly, fever, and dyspnea of 2-wk duration. Eight months previously she had papular urticaria and an elevated serum IgE concentration. Pertinent laboratory findings on admission were: hemoglobin 12 g/dl and WBC 22,400/cumm with 9% band forms, 7% PMNs, 13% eosinophils, 69% lymphocytes, and 2% monocytes. Of the lymphocytes, half were plasmacytoid and a large number had cleaved or irregular nuclei (Table 1). Serum proteins were 9.9 g/dl. All classes of immunoglobulins were increased, especially IgE, which was 29,800 U/ml (normal, up to 30 U). Other abnormalities included uric acid 13.0 mg/dl (normal 2.1–6.4), alkaline phosphatase 253 mU/ml (normal, 30–85), and lactate dehydrogenase 510 mU/ml (normal, 100–225). Epstein-Barr virus (EBV) antibody titer was elevated, but EBV DNA could not be detected in either plasmacytoid lymphocytes or cleaved lymphocytes.' Serial antibody determinations to a battery of viruses showed no elevations except for a stable titer of 1:64 to Herpes simplex.

Four lymph nodes (2 cervical, 1 axillary, and 1 abdominal) and a 1645-g spleen were removed and a diagnosis of "atypical follicular hyperplasia" was made on each. A liver biopsy revealed "reactive follicles" in the portal areas. Bone marrow was similarly involved. Numerous immunologic studies were performed (Table 2). The most striking finding in all tissues examined was the increased number of lymphocytes with IgM, kappa surface markers, which were abundant and coarse, indicating a monoclonal origin. The patient was discharged without therapy. Six months later further enlargement of lymph nodes including those in the mediastinum occurred. A submental lymph node showed "follicular hyperplasia." Prednisone therapy caused a dramatic reduction in size of lymph nodes, but at the same time, the leukocyte count rose to 59,600/cumm due to a marked increase in lymphocytes with cleaved and irregular nuclei (Table 1) bearing the abundant coarse single class (monoclonal) surface immunoglobulin markers, IgM, kappa. At the same time, serum IgG and IgM levels fell. Chlorambucil therapy was initiated. Lymph nodes further decreased in size and the leukocyte count fell to 10,000/cumm, with rare atypical lymphocytes. Complications of chemotherapy resulted in periodic discontinuance of the drugs, (which later included cytoxan and vincristine) by the patient herself. Such periods were marked by increasing lymphadenopathy and a leukemic blood picture and finally, involve-

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Table 1. Peripheral Blood Leukocyte Counts and Differentials

<table>
<thead>
<tr>
<th>Date</th>
<th>Leukocyte Count (x 10^9/µl)</th>
<th>Absolute Lymphocyte Count/µl</th>
<th>Morphological Variants of Lymphocytes (%)*</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Usual Lymphs</td>
</tr>
<tr>
<td>10/19/78</td>
<td>22.4</td>
<td>15,460</td>
<td>27</td>
</tr>
<tr>
<td>10/25/78</td>
<td>10.0</td>
<td>5,600</td>
<td>40</td>
</tr>
<tr>
<td>10/31/78</td>
<td>8.9</td>
<td>4,980</td>
<td>45</td>
</tr>
<tr>
<td>11/09/78</td>
<td>10.0</td>
<td>4,000</td>
<td>43</td>
</tr>
<tr>
<td>11/16/78</td>
<td>10.4</td>
<td>4,680</td>
<td>41</td>
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<td>01/16/79</td>
<td>16.6</td>
<td>8,800</td>
<td>66</td>
</tr>
<tr>
<td>03/29/79</td>
<td>20.3</td>
<td>8,120</td>
<td>68</td>
</tr>
<tr>
<td>04/05/79†</td>
<td>18.8</td>
<td>7,710</td>
<td>83</td>
</tr>
<tr>
<td>04/24/79</td>
<td>59.6</td>
<td>47,080</td>
<td>38</td>
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<tr>
<td>05/15/79</td>
<td>87.3</td>
<td>78,570</td>
<td>42</td>
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<tr>
<td>06/31/79</td>
<td>63.4</td>
<td>56,430</td>
<td>60</td>
</tr>
<tr>
<td>06/14/79†</td>
<td>66.4</td>
<td>55,780</td>
<td>61</td>
</tr>
<tr>
<td>09/12/79</td>
<td>9.1</td>
<td>2,730</td>
<td>64</td>
</tr>
<tr>
<td>11/02/79</td>
<td>13.4</td>
<td>5,230</td>
<td>72</td>
</tr>
<tr>
<td>10/15/80</td>
<td>28.5</td>
<td>7,695</td>
<td>76</td>
</tr>
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</table>

*Differential of 100 lymphocytes.
†Steroid therapy initiated (4/10/79).
‡Chlorambucil therapy initiated (6/14/79).

Table 2. Immunologic Studies

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Date</th>
<th>E 4°C</th>
<th>E 37°C</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
<th>IgD</th>
<th>Kappa</th>
<th>Lambda</th>
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<tr>
<td>Blood</td>
<td>10/19/78</td>
<td>21%</td>
<td>2%</td>
<td>4%</td>
<td>13%</td>
<td>16%</td>
<td>1%</td>
<td>2%</td>
<td>40%</td>
</tr>
<tr>
<td>4/05/79†</td>
<td>3,250*</td>
<td>310</td>
<td>618</td>
<td>2,010</td>
<td>13%</td>
<td>16%</td>
<td>1%</td>
<td>2%</td>
<td>40%</td>
</tr>
<tr>
<td>5/15/79‡</td>
<td>5,240</td>
<td>230</td>
<td>848</td>
<td>1,234</td>
<td>13%</td>
<td>16%</td>
<td>1%</td>
<td>2%</td>
<td>40%</td>
</tr>
<tr>
<td>11/15/80</td>
<td>11,790</td>
<td>30</td>
<td>54,999</td>
<td>4,714</td>
<td>13%</td>
<td>16%</td>
<td>1%</td>
<td>2%</td>
<td>40%</td>
</tr>
<tr>
<td>Reactive lymph nodes (27)</td>
<td>23%</td>
<td>0%–9%</td>
<td>1%–16%</td>
<td>12%–41%</td>
<td>1%–14%</td>
<td>0%–6%</td>
<td>1%–3%</td>
<td>0%–7%</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>11/21/78</td>
<td>25%</td>
<td>2%</td>
<td>19%</td>
<td>22%</td>
<td>1%</td>
<td>7%</td>
<td>4%</td>
<td>42%</td>
</tr>
</tbody>
</table>

*Absolute numbers per cu mm.
†Steroid therapy initiated (4/10/79).
‡Chlorambucil therapy initiated (6/14/79).

MATERIALS AND METHODS

Bone marrow, lymph nodes, spleen, and numerous blood samples were studied morphologically and immunologically.

Morphology

Light microscopy. Blood and bone marrow smears and touch preparations of freshly cut lymph node and spleen surfaces were stained with Leishman’s stain. Tissue was fixed in B5 fixative, cut at 4 µ, and stained with hematoxylin and eosin, periodic acid Schiff and methyl green pyronine.

Electron microscopy. Material was prepared for electron microscopy as described previously and examined with a Zeiss EM10A microscope.

Immunology

The methods for preparing mononuclear cell suspensions and evaluation of surface immunoglobulin markers and receptors for unsensitized sheep red cells, complement, immunoglobulin (Fc portion), and mitogen-stimulated lymphocyte cultures have been described.
FOLLICULAR MANTLE ZONE LYMPHOMA

Intracytoplasmic immunoglobulins (heavy chains of IgG, IgM, IgA, and IgD, and kappa and lambda light chains) and cytoplasmic muramidase were evaluated in B5-fixed paraffin-embedded sections of lymph nodes using an immunoperoxidase method, and in fixed mononuclear cell preparations using fluorescent antisera to the various immunoglobulins. Antisera were obtained from DAKO-Immunoglobulins (Accurate Chemical and Scientific Co., Hicksville, NY) and Meloy Laboratories, Inc. (Biological Products Division, Springfield, Va.). Subpopulations of T lymphocytes were enumerated by indirect immunofluorescence in blood, bone marrow, and lymph node using Orthoclone antibodies OKT3, 4, 6, and 8, obtained from Ortho Pharmaceutical Corporation (Raritan, N.J).

Histochemistry and Cytochemistry

Imprints and sections of spleen stored at −70°C were examined for alkaline phosphatase activity according to the method described by Nanba et al.

RESULTS

Light Microscopy

Blood. Differential counts of all leukocytes and morphological types of lymphocytes in peripheral blood are given in Table 1. Initially, plasmacytoid lymphocytes predominated, but cleaved lymphocytes were present from the beginning and became the predominant cells during the frankly leukemic phase following steroid therapy (Fig. 1).

Initial lymph nodes and spleen. All four lymph nodes and spleen contained essentially the same features. There was marked follicular hyperplasia (Fig. 2). The large follicles were present throughout the lymph nodes and white pulp of the spleen. The mantle zone was large and prominent. It appeared to enlarge with time so that the last biopsy showed confluence of some mantle zone areas and slight infiltration of follicular centers by cells from the mantle zone. The cells in the mantle zone in all samples studied were distinguished by prominent nuclear irregularity, consisting of deep clefts or several folds (Fig. 3). They were small to medium-sized lymphocytes and appeared identical to those seen in the blood (Fig. 1). A few mitoses were seen in the mantle zone. The follicular centers contained the usual mixture of lymphoid cells in various stages of transformation from small to large cleaved and large round cells, numerous phagocytic histiocytes, and many mitotic figures. In the interfollicular area, a mixture of mature lymphocytes, plasma cells, large transformed lymphocytes, and histiocytes were seen.

Bone marrow. Many large lymphoid nodules, some directly adjacent to bone spicules, were seen. Occasional nodules had reaction centers. In smears, cleaved and plasmacytoid lymphocytes were abundant.

Liver. In portal areas, a prominent increase in lymphoid tissue, frequently with reaction centers, was noted. Many lymphocytes were seen in sinusoids.

Subsequent biopsies of lymph node, tonsil, and colon. In the lymph nodes and tonsil, vague nodules

Fig. 1. Blood smear from 4/24/79 when absolute lymphocyte count was 47,000/cumm, showing marked irregularity of nuclei. Leishman’s stain, ×713; insert, ×1226.

Fig. 2. Lymph node from 11/21/78 showing large follicle centers and prominent mantle zone. Hematoxylin and eosin, ×53.

Fig. 3. Higher power of mantle zone near follicle center (lighter area). Note irregularity of nuclei in mantle zone. Hematoxylin and eosin, ×758.
and sheets of tumor cells with the same morphological features as described above were replacing all normal structures (Fig. 4). Mitotic figures were rare, and except for an occasional very small remnant, no reaction centers were seen. In some areas the nodules were still surrounded by many plasma cells and histiocytes, but in many areas the nodules were becoming confluent, presenting a diffuse pattern of tumor growth. The colon biopsy was too small to evaluate growth pattern adequately.

Electron Microscopy

Electron microscopic examination of lymph nodes confirmed the irregularity of the nuclei of the mantle zone lymphocytes (Fig. 5). These lymphocytes lacked rough endoplasmic reticulum.

Immunologic Studies

Immunologic studies are listed in Table 2. Numerous cells bearing single class, IgM, kappa immunoglobulin markers were noted in the blood from the beginning (Table 2) and were roughly proportional to the numbers of cleaved lymphocytes. All four lymph nodes, spleen, and bone marrow contained a similar predominant population of lymphocytes with IgM, kappa markers.

Cytocentrifuge preparations demonstrated that the cells with very irregular nuclei formed rosettes with IgGEA- and IgMEAC-coated ox cells. However, the percentage of cells with these receptors was different in blood from that in lymph nodes and spleen. In the lymph nodes a much larger percentage of cells contained receptors for complement, and in the blood a larger percentage contained receptors for the Fc portion of immunoglobulin. Since some of these tissues were studied simultaneously, technical error is an unlikely possibility and changes in the expression of these receptors must be considered as an explanation for the apparent discrepancy. The absolute number of blood T lymphocytes varied from normal to increased. In the lymph nodes, initially they were in the low normal range and then lower than normal. Their percentage in the spleen was within our normal range. In the lymph nodes and spleen, many T cells formed stable E rosettes at 37°C as well as at 4°C, a finding previously described in various lymphomas and in reactive lymph nodes. 

The peripheral lymphocyte response to mitogens was of interest in that there was a consistently decreased response to concanavalin A (Con-A). The response was adequate to phytohemagglutinin (PHA) and variable to pokeweed (PW).

The patient’s ratio of helper (H) to suppressor (S) T cells differed from 16 normal controls. In the controls the normal H:S ratios were approximately 2:1, whereas in the patient the ratio was almost 1:1, indicating an adequate number of not necessarily functionally normal, suppressor T cells. The patient’s bone marrow T cells did not react with either suppressor or helper T-cell antibodies.

The persistent hyperproteinemia correlated well with the presence of numerous plasmacytoid lymphocytes in blood, bone marrow, lymph nodes, and spleen containing immunoglobulins of all classes, including IgE, and both light chains by the cytoplasmic fluores-
cent technique on cell suspension and by the immunoperoxidase technique in fixed tissues. In the latter they were found primarily in interfollicular areas. A few were seen within follicular centers. The follicular mantle zone lymphocytes did not contain cytoplasmic immunoglobulin.

Cytochemistry and Histochemistry

In imprints, numerous lymphocytes showed surface alkaline phosphatase activity. In frozen sections, strong activity was seen in all of the mantle zones but none in the follicular centers.

DISCUSSION

Lukes and Collins have proposed that most B-cell lymphomas arise from the secondary follicles. The morphological stages of cellular transformation within the follicular centers in response to antigen, from small cleaved lymphocytes to large noncleaved lymphocytes, can be correlated with lymphomas composed of these cell types. Surrounding these follicles are mantle zones, composed of small lymphocytes with round, mature-appearing nuclei. Malignant lymphoma, well differentiated type according to the Rappaport classification, and chronic lymphocytic leukemia are thought to represent neoplasms of these cells.

Our example of malignant lymphoma demonstrates that the mantle zone may also give rise to a nodular lymphoma composed of small cleaved lymphocytes. It may be related to the lymphocytic lymphoma of intermediate differentiation described by Mann et al., who speculated that this lymphoma arose from the mantle zone or primary follicles. It is said to be composed of a mixture of small cleaved and small round lymphocytes, has small remnants of follicular centers, and a vague nodularity. The malignant cells are further distinguished by having surface immunoglobulin markers of intermediate quantity between chronic lymphocytic leukemia and poorly differentiated lymphocytic lymphoma and also by containing the enzyme leukocyte alkaline phosphatase normally found in mantle zone and primary follicles. However, this enzyme is also found in other B-cell lymphomas.

In our case the initial morphological expression of the mantle zone lymphoma occurred around greatly enlarged follicular centers that we considered to be reactive rather than malignant components. The reactive nature of the centers was suggested by the presence of lymphocytes in various stages of transformation, numerous phagocytic histiocytes, absence of single class immunoglobulin in the large transformed lymphocytes, and the presence of plasma cells containing multiple classes of immunoglobulins within the follicles and in great abundance in the interfollicular areas. Strong alkaline phosphatase reactivity was seen in membranes of lymphocytes in the mantle zones but not on those in the reaction centers. Within 2 yr of the presentation, the follicular centers were replaced by a vaguely nodular and diffuse lymphocytic lymphoma composed almost entirely of small lymphocytes with cleaved and irregular nuclei identical to those seen in mantle zones previously. The abundant, coarse, single class surface immunoglobulins and receptors for complement on the malignant cells were consistent in all biopsies and tissues examined and resembled those seen on cleaved lymphocytes of FCC origin or similar irregular lymphocytes of poorly differentiated lymphocytic lymphomas (Rappaport classification) rather than those of well differentiated or intermediate types.

This neoplasm of small, cleaved lymphocytes of mantle zone origin thus seems to represent an exception to the usual type of cleaved lymphocytic lymphoma, which originates in the follicular centers, and further suggests that changes in nuclear shape and increase in surface immunoglobulins may take place outside the follicular center. The possibility that the tumor cells migrated from the follicular centers to the mantle zone or that they “homed” to this site from an obscure source was considered but thought to be unlikely.

The specific cause of the prominent follicular center hyperplasia, polyclonal plasmacytoid lymphocyte proliferation, and marked increase in all, but especially the IgE portion of gamma globulins as well as the monoclonal B-cell proliferation is unknown. Interestingly, Lennert et al. have reported increased IgE levels in lymphomas. A persistent EB virus infection was initially considered, however, viral DNA could not be demonstrated within the lymphoid cells. A T-cell abnormality was suggested by the persistently poor response to concanavalin A. Suppressor T cells were not decreased numerically but might be functionally abnormal.

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REFERENCES


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