Leu M1 and Peanut Agglutinin Stain the Neoplastic Cells of Hodgkin's Disease

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It has been suggested that the malignant cells of Hodgkin's disease (HD), Reed–Sternberg cells, and their mononuclear variants may be related to cells of the monocyte–histiocyte system. To test this hypothesis, 20 cases of HD were tested with nine antibodies, monoclonal or polyclonal, that normally react with cells of monocytic/histiocytic/granulocytic lineages, as well as PNA, which binds to histiocytes directly. Only two reagents, Leu M1 and PNA bound to the neoplastic cells in 20/22 and 13/22 cases tested, respectively. Leu M1 was the most sensitive reagent and was negative in only two cases of lymphocyte predominant HD. Leu M1 also could be employed in routinely fixed and processed formalin or B5-fixed tissue. This antibody, which was negative in 27 cases of non-Hodgkin's lymphoma, including 12 peripheral T-cell lymphomas, should prove to be of value in differential diagnosis of HD and morphologically similar reactive and neoplastic lymphoid lesions. (Key words: Hodgkin's disease; Peanut agglutinin; Immunohistochemistry) Am J Clin Pathol 1984; 82: 29–32

HODGKIN'S DISEASE is a unique clinical and morphologic disorder with both neoplastic and reactive components. The reactive component consists of lymphocytes, histiocytes, polymorphonuclear leukocytes, plasma cells, and fibroblasts in varying proportions. The uncertain nature of the neoplastic cells, Hodgkin's, and Reed–Sternberg (H-RS) cells has stimulated decades of research, and this question still remains unanswered. Proposed cells of origin have included histiocytes, transformed T- or B-cells, and reticulum cells.2122

A B-cell origin of Hodgkin's tumor cells was supported by demonstration of surface or cytoplasmic IgG in tumor cells by immunohistochemical or immunofluorescence staining.7 However, the polyclonal nature of the immunoglobulin suggested that it was not a product of cell synthesis but resulted from the internalization of exogenous IgG and/or phagocytosis of immune complexes.2,11

The ability of the Hodgkin's tumor cells to ingest immune complexes as well as latex particles, red blood cells, and lymphocytes has raised the possibility of a histiocytic origin for the tumor cells.11–13

Recently, the antigenic profile of H-RS cells from cases of Hodgkin's disease, and a Hodgkin's disease-derived cell line (L-428-KS) has been analyzed using a panel of monoclonal antibodies reactive with cells of lymphoid and hematopoietic origin.15,17,20 It was found that H-RS cells consistently lack markers found on null cells, B-cells, and T-cells. In contrast, the majority of typical and lacunar type H-RS cells contained the granulocyte-related antigens detected by monoclonal antibodies, Tü 5, Tü 6, Tü 9, and 3C4, which do not react with monocytes/macrophages. The results might suggest that H-RS cells are related to cells of the granulocytic cell lineage but do not necessarily allow the conclusion that Hodgkin's disease is a granulocytic neoplasm.17

In this article, we expand the search for the antigenic profile of H-RS cells using several monoclonal antibodies directed against monocytes/histiocytes/granulocytes. Among nine antibodies tested, anti-Leu M1 consistently stained H-RS cells. Based on this finding, we further explored the specificity of Leu M1 in normal human lymphoid as well as nonlymphoid tissues. Additionally, the investigation of binding of peanut agglutinin to H-RS cells was also pursued, based on the finding of its reactivity with normal histiocytes.16

Materials and Methods

Tissues

Lymph nodes and/or spleens containing pathologically proven involvement by Hodgkin's disease and non-Hodgkin's lymphomas (Table 1) were obtained for study. All tissues had been embedded in OCT (Miles Laboratories), snap frozen in a mixture of dry ice-2-methyl butane (−75°C), and stored at −70°C. Five-micron sections were cut and placed on gelatinized slides. Routinely prepared B5-fixed and in some cases formalin-fixed paraffin-embedded tissues also were obtained for immunohistochemical staining.

In order to establish the specificity of the antibody (Leu M1) reactions, the following tissues also were included for testing: (1) Five each of normal human lymphoid tissues, including bone marrow, thymus, tonsils, spleen,
Table 1. Staining of Leu M1 and Peanut Agglutinin in Hodgkin’s Disease and Non-Hodgkin’s Lymphoma

<table>
<thead>
<tr>
<th>Histologic Diagnosis</th>
<th>Leu M1 (No. Positive/No. Studied)</th>
<th>Peanut Agglutinin (No. Positive/No. Studied)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hodgkin’s disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodular sclerosis</td>
<td>15/15</td>
<td>8/15</td>
</tr>
<tr>
<td>Lymphocyte predominant</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>Mixed cellularity</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>Lymphocyte depletion</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Non-Hodgkin’s lymphomas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral T-cell lymphoma</td>
<td>0/12</td>
<td>0/12</td>
</tr>
<tr>
<td>Large cell lymphoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B or null cell type)</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Hairy cell leukemia</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Cutaneous T-cell lymphoma</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Reactive nonneoplastic lymphoid</td>
<td>Granulocytes</td>
<td>Scattered histiocyte</td>
</tr>
<tr>
<td>tissues</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results

Hodgkin’s Disease

In frozen sections, H-RS cells were negative for muramidase and negative with all anti-monocyte/histiocyte antibodies except for Leu M1. Anti-Leu M1 stained a majority of tumor cells in each case with a primary membranous and juxtanuclear (golgi?) localization of the reaction product. In addition to tumor cells, anti-Leu M1 also reacted with granulocytes. The staining (cytoplasmic reaction) usually was very intense so that the nucleus in some cases was masked by the diaminobenzidine precipitation. In contrast, the membranous staining of tumor cells did not obscure the nuclei, which usually contained large, typical refractile nucleoli. Thus, the distinction of tumor cells and granulocytes was not a problem. This differentiation was further assisted by comparison of two serial sections, which were stained by either muramidase or anti-Leu M1. Granulocytes, predominately eosinophils, were positive for both Leu M1 and muramidase, while neoplastic cells were positive for only Leu M1. Furthermore, granulocytes were distributed randomly, whereas Leu M1-positive tumor cells usually formed aggregates with a patchy distribution.

In formalin- or B5-fixed paraffin sections, the staining patterns of muramidase and Leu M1 were almost identical.

Staining Procedures

Paraffin sections were deparaffinized through xylene and graded alcohol and rehydrated with TRIS-buffered saline (0.05 M, pH 7.6), as in routine processing. Frozen sections or cytocentrifuge smears were fixed in acetone, at room temperature, for 5 minutes prior to immunostaining.

The staining procedure using monoclonal antibodies has been described elsewhere in detail. Briefly, the sections were washed with TRIS-buffered saline, 0.05 M, pH 7.4, and then immersed in 1% normal horse serum in buffer for 5 minutes. The primary antibodies were used at 1–2 μg/mL, followed by biotin-labeled secondary antibody (1:400). After extensive washing, the sections were immersed in methanol-H2O2 solution for 30 minutes to minimize staining diffusion and block endogenous peroxidase activity. Avidin–biotin–peroxidase complex then was applied, followed by developing in DAB-Ni-H2O2 solution. The sections were counterstained with methyl green or hemtoxylin, dehydrated, and cleared as in routine processing. The staining procedures with biotin-labeled lectin in paraffin sections were performed as previously reported.

Controls for method specificity were performed by omission of primary antibody or replacement of primary antibody with normal nonimmune serum, BALB/c mouse serum, or ascites fluid. Sugar absorption control was used to confirm the specificity of PNA reaction.

Reagents

Rabbit anti-human muramidase antibody was obtained from Dakopatts® (Accurate, New York). Monoclonal antibodies to monocytes/granulocytes/histiocytes/macrophages used in this study included Mac (Hybritech, La Jolla, CA); OKM1 (Ortho® Diagnostic, Inc., Raritan, NJ); Mo-2 (Coulter, Hialeah, FL); anti-Leu M1, anti-Leu M2, anti-Leu M3, anti-Leu M4 (Becton, Dickinson and Co., Mountain View, CA); Mo-1 (Bethesda Research, Gaithersburg, MD); and Mo-2 (Bethesda Research Lab). The specificities of these monoclonal antibodies have been reported previously.

Labeling reagents, including biotin-conjugated horse antimouse IgG or goat antirabbit IgG and avidin–biotin–peroxidase complex (Vectastain, ABC Kit, PK 4005), were obtained from Vector Laboratories (Burlingame, CA).

For peanut agglutinin reaction, biotin-labeled PNA (Vector Laboratories) was used.

and lymph nodes, in frozen or paraffin sections. (2) One each of normal human nonlymphoid tissues, including lung, heart, gastrointestinal tract, pancreas, liver, kidney, adrenal gland, thyroid, brain, uterus, fallopian tube, testis, and prostate. (3) Mononuclear cell fractions of normal human peripheral blood obtained after separation on a Ficoll–Hypaque gradient (PBM).

Cytochemical Identification of Monocytes

The α-naphthyl acetate esterase (ANAE) reaction was used to quantitate monocytes in cytocentrifuge smears prepared from peripheral blood mononuclear cells. The smears were fixed for 30 seconds in formol-calcium in cacodylate buffer and then incubated in a mixture containing hexazotized pararosanilin and α-naphthyl acetate for 3 hours at 37°C as previously described. Rabbit antimuramidase antibody was obtained from Dakopatts® (Accurate, New York). Monoclonal antibodies to monocytes/granulocytes/histiocytes/macrophages used in this study included Mac (Hybritech, La Jolla, CA); OKM1 (Ortho® Diagnostic, Inc., Raritan, NJ); Mo-2 (Coulter, Hialeah, FL); anti-Leu M1, anti-Leu M2, anti-Leu M3, anti-Leu M4 (Becton, Dickinson and Co., Mountain View, CA); Mo-1 (Bethesda Research, Gaithersburg, MD); and Mo-2 (Bethesda Research Lab). The specificities of these monoclonal antibodies have been reported previously.

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FIG. 1. Hodgkin’s disease, nodular sclerosis type, stained with: (A, left) Leu M1; (B, center) Leu M1; (C, right) PNA. The Hodgkin’s cells and Reed–Sternberg (arrows) cells are positive for Leu M1 and PNA. A, C: (X100). B: (X400).

to those in frozen tissues (Table 1). The staining was predominately membranous; in some cases the pattern was diffuse or multigranular, whereas in others (4/22) a globular reaction product was seen (Fig. 1). In paraffin sections, PNA bound to H-RS cells in 13/22 cases. PNA in general reacted with fewer cells than anti-Leu M1, and the intensity of the staining was less (Fig. 1). PNA reacted with histiocytes and did not stain granulocytes. The reaction pattern of PNA in H-RS was mainly membranous; in some cases (7/13), a juxtanuclear (golgi?) without a membranous reaction product was seen.

Peripheral T-Cell Lymphomas and Other Non-Hodgkin’s Lymphomas

Both PNA and anti-Leu M1 failed to stain the neoplastic cells in 12 peripheral T-cell lymphomas, five cutaneous T-cell lymphomas, five diffuse large cell lymphomas of B or “null” cell origin, and five hairy cell leukemias.

Reactivity of Leu M1 in Normal Lymphoid Tissues and PBM

Monocytes in cytocentrifuge smears obtained from PBM were identified by positivity for ANAE in 16 normal volunteers and ranged from 10 to 30%. Anti-Leu M1 reacted with only 10–20% of monocytes, showing weak, intracytoplasmic punctate staining. Anti-Leu M1 stained granulocytes intensely. In frozen sections of normal thymus, tonsil, lymph node, and spleen, anti-Leu M1 reacted with granulocytes and rare (<0.1%) mononuclear cells. In paraffin sections, anti-Leu M1 reacted with only granulocytes but no mononuclear cells.

In bone marrow, anti-Leu M1 stained a great majority of cells, with the exception of normoblasts and megakaryocytes. The stained cells had heavy, intracytoplasmic diaminobenzidine precipitation that did not allow specific identification of cell type. The majority of cells, however, appeared to be granulocyte and myeloid precursors.

Reactivity of Anti-Leu M1 in Nonlymphoid Tissues

Wide reactivity of anti-Leu M1 was seen in virtually all normal tissues, including squamous epithelium, ducts of salivary glands, pancreatic acini, gastrointestinal mucosa, and epithelial elements of prostate, urethra, renal tubules, endometrium, gallbladder, and breast. Anti-Leu M1 did not react with macrophages or histiocytes in lung or the foamy histiocytes in colon.

Both frozen and paraffin sections showed identical distributions.

Discussion

Our findings indicate that anti-Leu M1, an antibody primarily reactive with cells of granulocytic lineage, also reacts with H-RS cells of Hodgkin’s disease. Anti-Leu M1 appears to be similar to the antibodies of the Tü series, especially Tü 9 in its reactivity with both normal and neoplastic cells. Both anti-Leu M1 and Tü 9 react with granulocytic cells but not with macrophages/histiocytes, interdigitating cells, or B- or T-cells. Both antibodies can be used in formalin-fixed paraffin-embedded sections, a rare phenomenon among more than 35 monoclonal lymphoid antibodies tested in our laboratory.

Although the reactivity of Tü 9 with nonlymphoid tissues is not known, this study indicates that anti-Leu M1 reacts with antigenic determinants broadly distributed
in epithelial cells of multiple organs. This finding, plus other evidence (see below), would make us reluctant to conclude that H-RS cells and granulocytic cells are of common or related lineages. Although anti-Leu M1 and Tu 9 both stain granulocytes at a late stage of maturation, H-RS cells do not exhibit the morphologic and/or cytochemical characteristics of granulocytes and lack both peroxidase and chloroacetate esterase activities. The presence of lysozyme or α1-antitrypsin in H-RS cells after long-term culture 12 is a nonspecific finding, certainly not restricted to cells of granulocytic lineage.

Anti-Leu M1 antigens are well preserved in formalin-fixed paraffin-embedded sections, which is, in general, a characteristic of carbohydrates. 9 Furthermore, because the Leu M1 reaction pattern and distribution resembled those of a galactose-binding lectin, peanut agglutinin (PNA), 9,10 we undertook an investigation of PNA binding in H-RS cells. In the majority (60%) of cases tested, PNA bound to H-RS cells in most histologic subtypes of Hodgkin's disease. PNA binds to normal histiocytes, 16 and thus this is another feature that H-RS cells share with cells of the histiocytic series. The cytoplasmic and often perinuclear pattern of anti-Leu M1 and PNA stainings suggest reactivity with glycoconjugates in the Golgi region. 9

Both anti-Leu M1 staining and PNA binding can be detected in routinely fixed and processed lymphoid tissues, as clearly demonstrated in this retrospective study. Thus, these reagents should prove to be extremely useful in differential diagnosis. HD is often difficult to differentiate from certain non-Hodgkin's lymphomas (NHL), particularly peripheral T-cell lymphomas. This study demonstrated that while 13/22 cases of HD bound both Leu M1 and PNA, all cases of NHL tested including 12 cases of peripheral T-cell lymphoma were negative. These reagents also would be useful in the differential diagnosis of HD from atypical lymphoid hyperplasia. In normal lymphoid tissues, both histiocytes and immunoblasts were consistently negative for Leu M1, although it should be noted that PNA binds to some of the histiocytes. 16

The clinical and epidemiologic differences between nodular sclerosing HD and the other histologic subtypes have suggested that these might be different diseases. In this study, malignant cells in nodular sclerosis (15 cases), mixed cellularity (2 cases), and lymphocytic depletion (3 cases) were all similar in their staining profiles, whereas the three cases of lymphocytic predominance tested failed to show significant Leu M1 and PNA binding. Whether this difference relates to a stage in evolution of the disease process or to a difference in the cytogeneology of the neoplastic cell remains to be determined.

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References