Leu-M1—A Marker for Reed–Sternberg Cells in Hodgkin's Disease

An Immunoperoxidase Study of Paraffin-Embedded Tissues

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Monoclonal antibody to Leu-M1, a granulocyte-related differentiation antigen, represents a highly effective reagent for detection of diagnostic Reed–Sternberg (R-S) cells and variants in paraffin-embedded tissues of Hodgkin's disease. In 69 of 73 cases of Hodgkin's disease (41 nodular sclerosis, 25 mixed cellularity, 4 lymphocyte predominance, and 3 lymphocyte depletion types), R-S cells were strongly immunoreactive for Leu-M1. Four cases of lymphocyte predominance Hodgkin's disease (nodular) were uniformly nonreactive for Leu-M1. In most of the positive cases (57/69, 83%), the majority (60–90%) of R-S cells and variants exhibited immunoreactivity for Leu-M1. A characteristic staining pattern included granular and/or vesicular cytoplasmic immunoreactivity, often with a prominent globular paranuclear reaction product, and membrane staining with highly irregular cytoplasmic borders. Evaluation of B-cell (37 specimens), T-cell (20 specimens), and true histiocytic (3 specimens) neoplasms and a case of mastocytosis revealed immunoreactivity for Leu-M1 only in 1 B-cell and 4 T-cell malignancies. The staining patterns in these cases, however, clearly differed from that observed for R-S cells. Studies of nonneoplastic lymphoid tissues (38 total) demonstrated that lymphoid cells were typically nonreactive; histiocytes revealed variable reactivity for Leu-M1. Occasional histiocytes of the sinusoidal network of lymph nodes, particularly in toxoplasmic lymphadenitis, exhibited a staining pattern (membranous/cytoplasmic/paranuclear) similar to that observed for R-S cells. Leu-M1 represents a potentially helpful diagnostic discriminant in the assessment of Hodgkin's disease and its distinction from non-Hodgkin's lymphomas and other lymphoid proliferations. (Am J Pathol 1985, 119:244–252)

DESPITE extensive advances in the therapy and management of Hodgkin's disease, considerable controversy still exists regarding the derivation of this neoplasm and the origin of the Reed–Sternberg (R-S) cell. Precise characterization of the neoplastic cells has been hampered by difficulties in establishing cell cultures from R-S cells. Conflicting data have proposed an origin from macrophages, T or B-lymphocytes, interdigitating reticulum cells, dendritic reticulum cells, and a unique, as yet undefined, cell population of the lymphoid system.[1–12] *In vitro* cell cultures by Diehl et al have suggested that R-S cells and variants represent an early myelomonocytic progenitor cell.[13] In further support of that hypothesis, Stein et al,[11,14] using monoclonal antibodies TÜ9 and C34, recently reported that R-S cells reveal granulocyte-related antigens, though other granulocyte-specific markers such as peroxidase, chloroacetate esterase, lysozyme, cationic leukocyte antigen, and OKM1 were consistently lacking.

Anti-Leu-M1, a monoclonal antibody which detects granulocytic cells and monocytes,[15] is known to react with antigens which are preserved after fixation and paraffin embedding.[16] Preliminary studies in our laboratory using this antibody revealed not only the anticipated strong immunoreactivity for granulocytic cells but, in cases of Hodgkin's disease, also demonstrated strong staining for R-S cells. The possibility that Leu-M1 represented a diagnostic discriminant for Hodgkin's

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disease, permitting its distinction from other neoplastic and nonneoplastic lymphoid proliferations, was therefore further evaluated.

The aims of this study were 1) to evaluate the consistency of immunoreactivity of diagnostic R-S cells and R-S variants for Leu-M1 in paraffin sections of a large series of Hodgkin's disease of all histologic types, 2) to determine the specificity of this staining pattern, as compared with a large group of non-Hodgkin's lymphomas; and 3) to define the staining pattern of Leu-M1 in a variety of nonneoplastic lymphoid proliferations.

Materials and Methods

Cases were retrieved from the surgical pathology files of Cedars Sinai Medical Center, Los Angeles, California (76 specimens) and Brigham and Women's Hospital, Boston, Massachusetts (96 specimens). One case represented autopsy material. Tissues were fixed in formalin or B5 solution in most cases; a few were fixed in Zenker's, Bayley's, or Bouin's solution or in multiple fixatives.

Cases of Hodgkin's disease were classified according to the Rye modification of the Lukes and Butler classification.[17,18] Non-Hodgkin's lymphomas were classified according to the Lukes and Collins classification.[19] Lymphomas were classified as B- or T-cell type on the basis of cell suspension and/or cryostat section studies as previously described.[20-22] Cryostat section studies included a large panel of monoclonal antibodies (Leu-1, 2a, and 3a; Becton Dickinson Monoclonal Center, Inc., Mountain View, Calif; OKT11, OKT4, and OKT8, Ortho Diagnostic Systems, Inc., Raritan, NJ; MO-2, Bethesda Research Laboratories, Inc., Gaithersberg, Maryland; B1, B2, kappa and lambda light chains, IgM, IgD, IgG heavy chains kindly provided by Dr. Lee Nadler, Harvard Medical School and Dana Farber Cancer Institute, Boston, Mass, and others as appropriate) and polyclonal (rabbit) antibodies to kappa and lambda light chains (Dako Corp, Santa Barbara, Calif). Monoclonal antibody Leu-M1 was obtained from Becton Dickinson, and is a monoclonal IgM kappa antibody derived by hybridization of mouse P3-X63-Ag8.653 cells (Ig nonsecreting myeloma) with spleen cells of BALB/c mice immunized with U-937 histiocytic cell line. This antibody has been shown to identify a differentiation antigen on human myelomonocytic cells.[15]

For immunoperoxidase studies, paraffin sections were mounted on slides coated with glue (Elmer's Glue-All, Borden Inc., Columbus, Ohio, or LePage Bondfast Glue, LePage's Limited, Bramalea, Ontario, Canada). Slides were deparaffinized, rehydrated, then placed in methanolic peroxide. (0.3–0.5% hydrogen peroxide in methanol) for 30 minutes at room temperature. The slides were then washed and placed in Tris buffer (0.05 M, pH 7.6) or in phosphate-buffered saline (PBS, 0.01 M, pH 7.4). Formalin-fixed material was evaluated with and without trypsinization prior to immunoperoxidase studies. Sections were incubated in a solution of trypsin (Type 3 bovine, 1.2 mg/ml, or Type 2 porcine, 0.2 mg/ml, Sigma Chemical Co., St. Louis, Missouri) for 20 minutes at 37 C. Though immunoreactivity for Leu-M1 was enhanced by trypsinization, good results were obtained without proteolytic digestion. Following trypsinization, the slides were washed and placed in Tris buffer or PBS, supplemented with 1–2% normal swine serum, and were sequentially incubated with Leu-M1 monoclonal antibodies (1/30 or 1/50 dilutions) for 1 hour, peroxidase-conjugated rabbit antibodies to mouse immunoglobulin (adsorbed, 1/40 dilution; Dako Corporation, Santa Barbara, Calif) for 30–45 minutes, and peroxidase-conjugated swine antibodies to rabbit immunoglobulin (1/40 dilution; Dako Corp.). The latter two reagents were diluted with PBS or Tris buffer supplemented with 2.5% human AB serum (to eliminate any cross-reactivity with human immunoglobulin) 30–60 minutes prior to use. Sections were washed with PBS or Tris-saline (1 part 0.5 M Tris buffer, pH 7.6, and 9 parts normal saline) after each incubation and placed in PBS or Tris buffer supplemented with 1–2% normal swine serum.

Antibody localization was effected by incubation with a solution containing 3,3′-diaminobenzidine tetrahydrochloride (6–20 mg, quantity dependent on reagent lot; Aldrich Chemical Co., Milwaukee, Wis) in 10 ml of PBS or Tris buffer, to which 0.1 ml of 3% hydrogen peroxide was added immediately prior to use, giving a brown reaction product. Sections were counterstained with Harris's hematoxylin or methyl green solution and mounted in Permount. Negative controls consisted of duplicate sections, with PBS or Tris buffer substituted for the primary antibody, and consistently revealed no staining.

Results

Preliminary studies of paraffin sections usig Leu-M1 monoclonal antibody demonstrated excellent staining of granulocytic cells. In Zenker's-fixed bone-marrow sections, the strongest immunoreactivity was observed in the most mature myeloid cells, there being relatively less intense staining in immature forms. Segmented myeloid cells were immunoreactive even at dilutions of 1/500 and 1/1000. Cytoplasmic staining was generally strong and diffuse, often obscuring the nuclei in these

Table 1—Leu-M1 Immunoreactivity for Reed–Sternberg Cells in Different Types of Hodgkin's Disease

Histologic type	Number of cases	Number immunoreactive for Leu-M1
Lymphocyte predominance*	4	0
Nodular sclerosis	41	41†
Mixed cellularity	25	25‡
Lymphocyte depletion	3	3§
Total	73	69

Tissues were fixed in B5 solution (35 specimens), formalin (29 specimens), Bouin's solution (1 specimen), Bayley's solution (1 specimen), and multiple fixatives (7 specimens). In all cases, granulocytic cells were strongly immunoreactive, providing internal positive controls.

* Nodular subtype.

† Thirty-five cases with 60–90% of R-S cells and variants immunoreactive; 6 cases with 15–25% of cells positive.

‡ Twenty cases with 60–90% of R-S cells and variants immunoreactive; 5 cases with less than 25% of cells positive.

§ Two cases with 60–90% of R-S cells and variants immunoreactive; 1 case with less than 50% of cells positive.

cells. With increased dilution of antibody, weaker diffuse cytoplasmic staining was observed, but membrane staining was better delineated. Erythroid precursors, megakaryocytes, and plasma cells were distinctly negative. Occasional mononuclear cells of probable monocytic or histiocytic type exhibited weak, granular cytoplasmic staining and/or delicate membrane staining.

Hodgkin's Disease

Results of Leu-M1 immunoreactivity in 73 cases of Hodgkin's disease, including lymphocyte predominance, nodular (4 cases), nodular sclerosis (41 cases), mixed cellularity (25 cases), and lymphocytic depletion types (3 cases), are summarized in Table 1. In all 69 cases of Hodgkin's disease of nodular sclerosis, mixed cellularity, or lymphocyte depletion types, R-S cells and R-S variants, eg, lacunar, mononuclear, and multinucleated/multilobated types were strongly immunoreactive for Leu-M1 (Figures 1–3). In 57 of the 69 cases, most or nearly all (60–90%) of the cells were stained. The most characteristic staining pattern consisted of diffuse granular and/or vesicular cytoplasmic staining, often with an intense globular reaction product, usually paranuclear (Golgi zone?), associated with membrane staining (Figures 1B and 2B). Cytoplasmic borders were often irregular and appeared to reflect areas of impingement related to immediately contiguous lymphoid cells, with areas of cytoplasmic attentu-

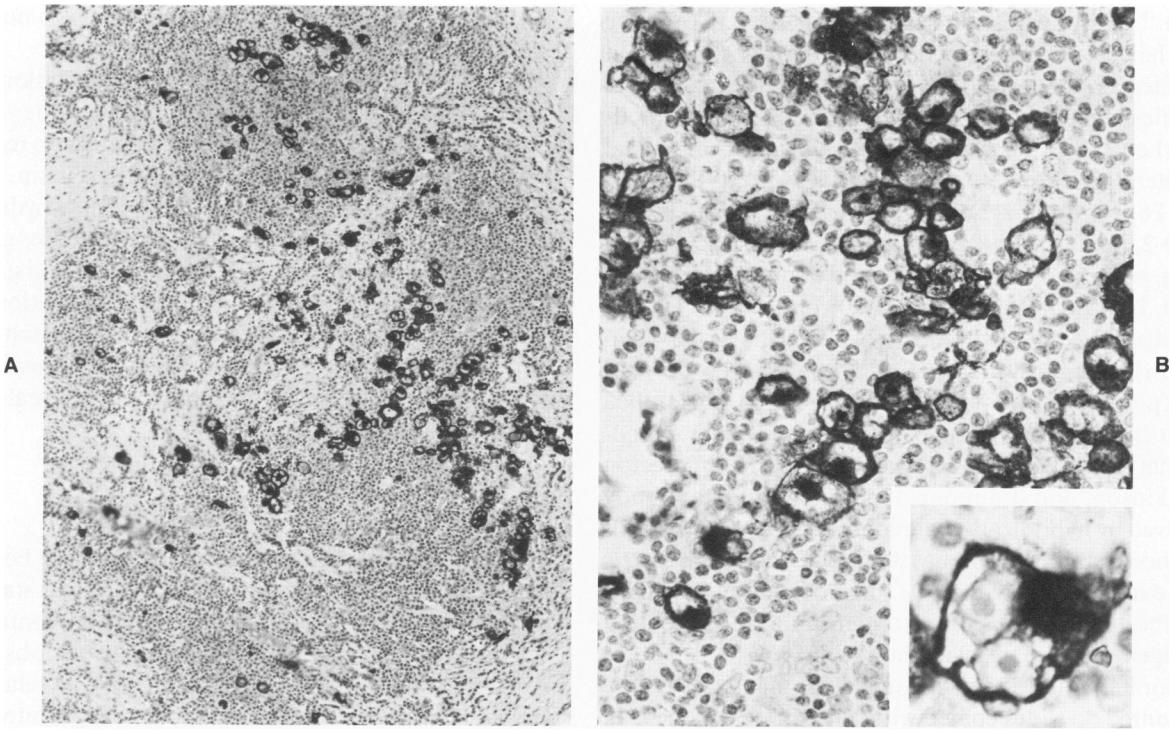

Figure 1—Hodgkin's disease, nodular sclerosis type, B5 fixation. **A**—Essentially all R-S cells and variants, mainly lacunar forms, exhibit strong immunoreactivity for Leu-M1. Small lymphocytes, blood vessels, and sclerotic foci are nonreactive. **B**—Higher magnification illustrates strong membrane staining with irregular cytoplasmic margins and cytoplasmic staining with focal paranuclear (Golgi?) globular reactivity for R-S cells and variants. The **inset** exemplifies this characteristic staining pattern in a diagnostic R-S cell. (Immunoperoxidase technique, hematoxylin counterstain, **A**, ×85; **B**, ×300; **inset**, ×820)

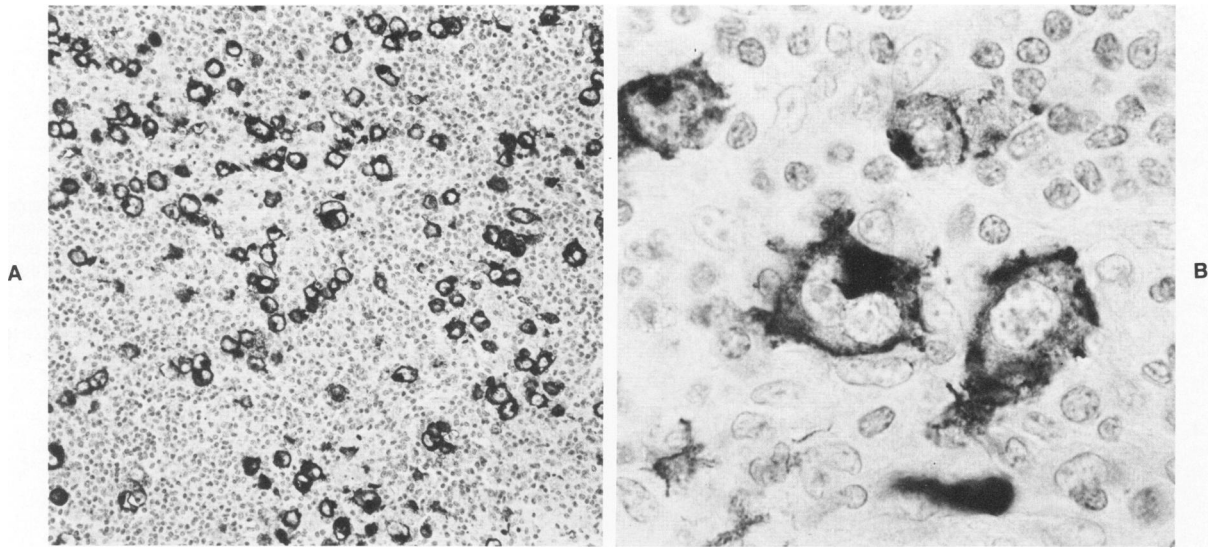

Figure 2—Hodgkin's disease, mixed cellularity type, B5 fixation. **A**—Many R-S cells and variants are present in the field, with essentially all demonstrating intense immunoreactivity for Leu-M1. Small lymphocytes are negative. **B**—Higher magnification illustrates the staining pattern in a diagnostic R-S cell and mononuclear variants. Note membrane staining with irregular, focally scalloped cell borders (related to contiguous lymphoid cells?) and areas of attenuation, suggesting cell processes or extensions. Granular and vesicular cytoplasmic staining, with paranuclear globular positivity (in the diagnostic form) is also apparent. (Immunoperoxidase technique, hematoxylin counterstain, **A**, ×150; **B**, ×730)

ation, producing the appearance of cell processes or extensions (Figure 2B). Some R-S cells had highly distorted cell membranes or contours, as defined by Leu-M1 immunoreactivity. Occasional R-S cells exhibited only cytoplasmic or membranous staining. Nuclei were nonimmunoreactive. In the 4 cases of Hodgkin's disease of lymphocyte predominance type, nodular, neither the L&H variants of R-S cells nor the rare diagnostic forms were immunoreactive for Leu-M1, representing a distinct divergence from the patterns observed for R-S cells in other types of Hodgkin's disease. Two cases of lymphocyte predominance Hodgkin's disease in which frozen tissue was available were also nonreactive for Leu-M1 in cryostat sections. In all cases of Hodgkin's disease, eosinophilic and/or neutrophilic myeloid cells were strongly positive, providing an internal positive control.

Occasional histiocytes identified within the infiltrates revealed focal granular cytoplasmic staining for Leu-M1, though the majority were negative. Plasma cells were nonreactive. Rare lymphoid cells of small to intermediate size exhibited focal granular cytoplasmic staining. In some nodes, vascular channels contained abundant cells, some representing identifiable granulocytic cells, which were strongly positive for Leu-M1. However, occasional R-S variants could also be discerned, as well as mononuclear cells of indeterminate type.

Immunoreactivity of R-S cells for Leu-M1 was well preserved in tissues, regardless of fixative, though formalin fixation did yield decreased staining intensity as compared with B5 solution. Proteolytic digestion with trypsin prior to immunoperoxidase studies was effective in enhancing staining, but generally satisfactory results were obtained without this treatment.

B- and T-Cell Malignancies and Other Disorders

Immunoreactivity for Leu-M1 in a variety of B and T cell malignancies (all immunologically phenotyped) and other disorders is summarized in Table 2.

Thirty-seven B-cell neoplasms were evaluated, including lymphomas of all follicular center cell types (21 specimens), small lymphocytic cell type (4 specimens), and immunoblastic sarcomas (7 specimens); hairy-cell leukemia (3 spleens); and multiple myeloma (2 bone marrows). Neoplastic cells were completely devoid of immunoreactivity for Leu-M1, except for 1 case of immunoblastic sarcoma which revealed membrane staining for most lymphoid cells (Figure 4). Granulocytic cells were consistently strongly stained in all cases. Histiocytes present in the proliferation were variable. Most were nonreactive, with a minor proportion exhibiting finely granular and/or focal localized cytoplasmic staining.

Of 20 T-cell neoplasms, including acute (3) and chronic (2) lymphocytic leukemia, and lymphoma of convoluted cell (lymphoblastic) type (3), immunoblastic sarcoma (7), mycosis fungoides (1), lymphoepithelioid cell (3) and unclassified (1) types, neo-

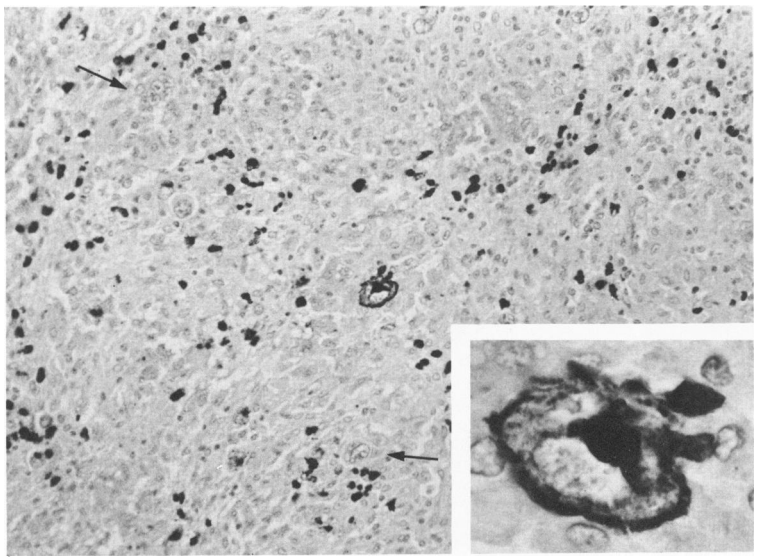

Figure 3—Hodgkin's disease, lymphocyte depletion type, B5 fixation. Some R-S cells are immunoreactive for Leu-M1, and others are negative (*arrows*). Smaller Leu-M1–positive cells are of myeloid type. Fibroblasts, histiocytes, and scattered lymphoid cells appear nonreactive. The **inset** illustrates an immunoreactive R-S cell at higher magnification. (Immunoperoxidase technique, hematoxylin counterstain, ×170; **inset**, ×840)

plastic cells in 4 cases were immunoreactive for Leu-M1. In 3 immunoblastic sarcomas, finely granular cytoplasmic staining was observed. Most neoplastic cells were positive in 1 case (Figure 5), with 10–25% reactive in the other 2 cases. In 1 convoluted T-cell lymphoma, about 10% of the neoplastic cells revealed fine granular cytoplasmic staining. In another case of convoluted T-cell lymphoma involving breast tissue, neoplastic cells were nonreactive, but mammary epithelium was immunoreactive for Leu-M1.

Table 2—Immunoreactivity for Leu-M1 in B- and T-Cell Malignancies and Other Disorders

Diagnosis	Number of cases evaluated	Number with neoplastic cells immunoreactive for Leu-M1
B-cell malignancies		
Non-Hodgkin's lymphoma		
Small lymphocytic	4	0
Follicular center cell*	21	0
Immunoblastic sarcoma	7	1†
Hairy-cell leukemia	3	0
Myeloma	2	0
T-cell malignancies		
Lymphocytic leukemia		
Chronic	2	0
Acute	3	0
Convoluted-cell (lymphoblastic)	3	1‡
Immunoblastic sarcoma	7	3§
Mycosis fungoides	1	0
Lymphoepithelioid cell type	3	0
Unclassified	1	0
Other disorders		
True histiocytic lymphoma	3	0‖
Mastocytosis	1	0
Total	61	5

Tissues were fixed in B5 solution (34 specimens), formalin (13 specimens), Zenker's solution (6 specimens; bone marrow biopsies), Bayley's solution (5 specimens), and multiple fixatives (3 specimens).

* Small cleaved (7 cases), large cleaved (7 cases), large noncleaved (6 cases), and small noncleaved (1 case) follicular center cell types.

† Membrane staining for the majority of cells.

‡ Finely granular staining in about 10% of cells.

§ Finely granular staining in 90% of cells in 1 case and 10–25% of cells in 2 cases.

‖ Rare cells with focal weak immunoreactivity.

In three nodes involved by true histiocytic lymphoma and one involved by mastocytosis, infiltrating cells were nonreactive for Leu-M1 except for focal, weak staining in a few neoplastic cells of the histiocytic lymphomas.

Table 3 compares the staining pattern for Leu-M1 observed in Hodgkin's disease with that noted for B and T-cell neoplasms and true histiocytic lymphoma. Some of the neoplasms, particularly the immunoblastic sarcomas, contained cells morphologically resembling R-S cells. In contrast with cases of Hodgkin's disease in which Leu-M1 distinctly characterized the R-S cells and variants in 69 of 73 cases, only 4 T-cell and 1 B-cell lymphomas expressed this differentiation antigen. However, in those T- or B-cell proliferations, the staining pattern was clearly different from that observed in Hodgkin's disease.

Nonneoplastic Lymphoid Tissues

In order to assess further the specificity of staining for Leu-M1 antigen and to determine whether a nonneoplastic counterpart could be identified for the Leu-M1–positive cells in Hodgkin's disease, we evaluated a variety of lymphoid tissues. Specimens (38 total) included lymph nodes with follicular and interfollicular lymphoid hyperplasia of undetermined etiology (15 specimens), toxoplasmic lymphadenitis (6 specimens), dermatopathic lymphadenitis (3 specimens), sarcoidosis (4 specimens), an abnormal proliferation resembling immunoblastic lymphadenopathy (1 specimen),

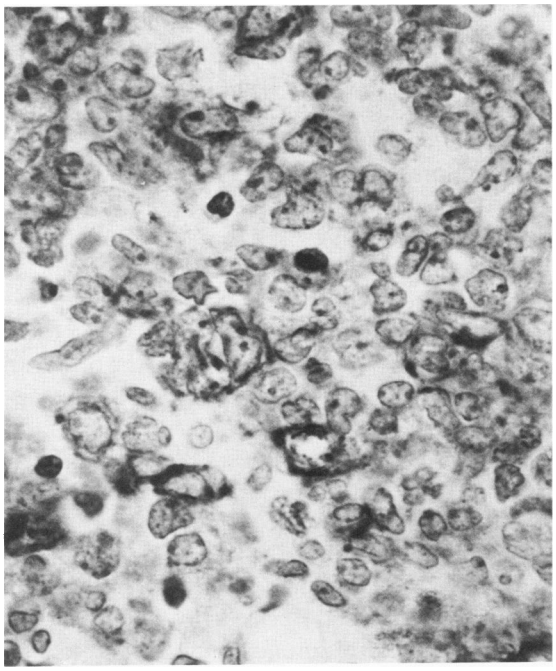

Figure 4—Immunoblastic sarcoma, B-cell type, B5 fixation. Neoplastic B cells, including a binucleated form (*near center*), exhibit immunoreactivity for Leu-M1, characterized mainly by membrane staining. Staining intensity was weak to moderate for most cells and is best defined on the larger lymphoid forms in the illustration. (Immunoperoxidase technique, hematoxylin counterstain, ×750)

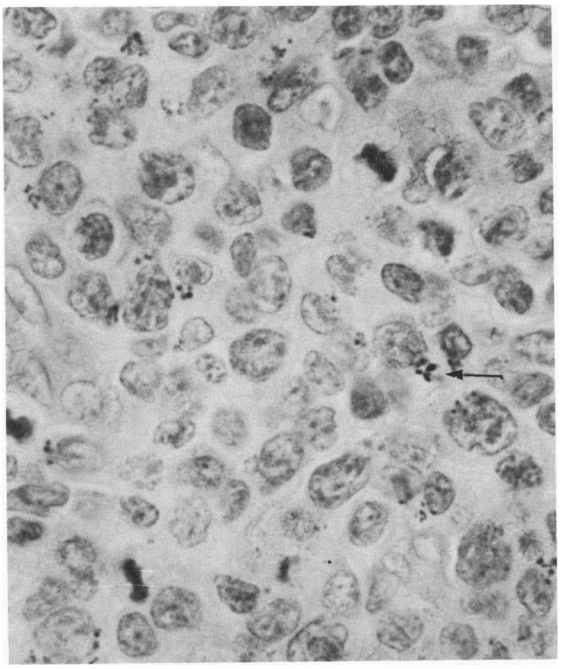

Figure 5—Immunoblastic sarcoma, T-cell type, B5 fixation. Many neoplastic cells reveal immunoreactivity for Leu-M1, characterized by finely granular cytoplasmic staining. The *arrow* designates one of the immunoreactive cells. (Immunoperoxidase technique, hematoxylin counterstain, ×850)

and a node with hyperplastic changes from a homosexual male with persistent adenopathy (1 specimen), hyperplastic tonsils (4 specimens), normal spleen (2 specimens), and infant thymus (1 specimen). Tissues were fixed in B5 solution (18), formalin (14), Bouin's (1), or multiple fixatives (5), including B5, formalin, Zenker's, and Bouin's solution. Myeloid cells were strongly immunoreactive for Leu-M1 in all specimens. In cases in which multiple fixatives were evaluated, immunoreactivity in formalin-fixed tissue appeared slightly diminished as compared with that observed for other types of fixatives.

Lymphoid cells in follicles and in interfollicular areas, including large transformed cells, were negative for Leu-M1 except for rare small lymphoid cells in interfollicular areas, which revealed a few cytoplasmic granules. Histiocytes were variable. Interdigitating reticulum cells were nonreactive. In follicular centers, only occasional tingible-body macrophages revealed focal weak cytoplasmic staining. The staining pattern in epithelioid histiocytes constituting sarcoid granulomas or those present in toxoplasmic lymphadenitis varied from nonreactive to finely granular or focally vesicular cytoplasmic staining. The staining pattern of histiocytes constituting the sinusoidal network of nodes was of particular interest. In nodes with nonspecific hyperplasia, occasional cells revealed finely granular or diffuse cytoplasmic staining and/or weak membrane staining. Rare cells of the sinusoidal network, however, exhibited a more intense membrane and cytoplasmic staining pattern, focally with a globular paranuclear staining pattern, reminiscent of that observed in R-S cells and variants in Hodgkin's disease. The number of nonneoplastic sinusoidal histiocytes demonstrating the latter staining pattern was particularly increased in toxoplasmic lymphadenitis (Figure 6). These cells were also conspicuous in the node from the homosexual male with persis-

Table 3—Comparison of Immunoreactivity for Leu-M1 in Hodgkin's Disease and Malignancies of B-Cell, T-Cell, and Histiocytic Types

Diagnosis	Number of cases evaluated	Number of cases with neoplastic cells immunoreactive for Leu-M1
Hodgkin's disease	73	69*
B-cell malignancies	37	1†
T-cell malignancies	20	4†
Histiocytic lymphoma‡	3	0

* R-S cells (L&H variants and diagnostic cells) were nonimmunoreactive in all 4 cases of nodular lymphocyte predominance type evaluated.
† Staining pattern distinctly different from that observed for R-S cells.
‡ Neoplastic cells exhibited markers of true histiocytes.

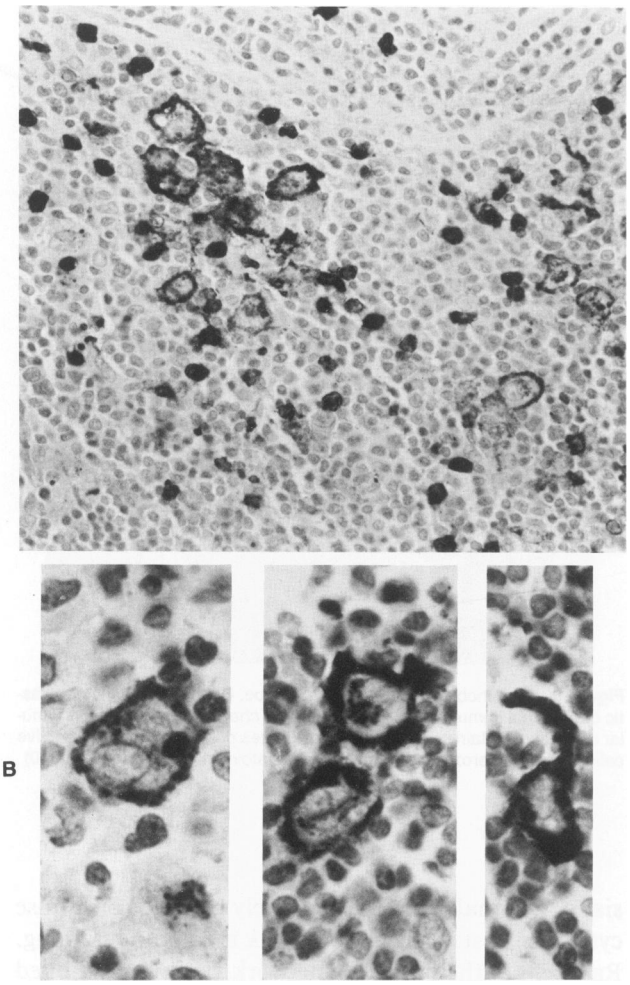

Figure 6—Toxoplasmic lymphadenitis, formalin fixation. **A**—Immunoreactivity for Leu-M1 is observed for histiocytes of the sinusoidal network (larger cells) and for myeloid cells (smaller immunoreactive cells). Lymphoid cells are nonreactive. **B**—The staining pattern in some sinusoidal histiocytes resembles that observed for R-S cells, with membrane staining and cytoplasmic granular positivity with occasional paranuclear globules. (Immunoperoxidase technique, hematoxylin counterstain, **A**, ×360; **B**—right and center, ×920, left, ×940)

tent adenopathy and were identified in smaller numbers in nodes with sarcoidosis or dermatopathic lymphadenitis. Monocytoid (perisinusoidal) cells, prominent in toxoplasmic lymphadenitis, were Leu-M1–negative.

In the node involved by an immunoblastic lymphadenopathy-like proliferation, lymphoid cells were not reactive for Leu-M1. In nonneoplastic spleens, immunoreactivity for Leu-M1 was restricted to myeloid cells and rare mononuclear cells of indeterminate type in red pulp. Thymic lymphocytes were also negative, though staining was observed for Hassall's corpuscles.

Discussion

Despite extensive morphologic, ultrastructural, histochemical, and immunologic studies,[1-12,23,24] the origin of the R-S cell remains controversial. Most studies have proposed either a lymphoid or a histiocytic derivation for R-S cells, and some have suggested a heterogeneous origin.[6,7,23] Other investigations, utilizing monoclonal antibodies TÜ9 and 3C4, have demonstrated immunoreactivity of R-S cells for granulocyte-related antigens.[11,14] Recently, Hsu and Jaffe[25] also found that R-S cells in 20 of 22 cases of Hodgkin's disease could be detected with the use of anti-Leu-M1, a reagent which identifies a differentiation antigen on human myelomonocytic cells.[15] Our study, using Leu-M1 monoclonal antibodies, further extends the observation of granulocyte-related antigens on R-S cells, raising the possibility that R-S cells may derive from granulocytic/monocytic cells. However, this reagent is also capable of detecting mitogen-activated T cells, particularly those of helper/inducer cell phenotype, some T-cell lines (eg, HSB-2 and MOLT-4), and a human histiocytic cell line (U-937) but is not reactive with B cells, B-cell lines, or activated B cells.[15] In addition, although initial studies with this monoclonal antibody failed to demonstrate immunoreactivity with epithelial cells,[15] in our study and in that of Hsu and Jaffe,[25] epithelial staining was observed. Monoclonal antibody 3C4, which defines antigens specific for cells in late stages of granulopoiesis and also detects R-S cells, similarly reacts with ductal epithelium of many organs.[26] Leu-M1 monoclonal antibody apparently detects a differentiation antigen on R-S cells which is shared by granulocytic cells and other cell types, eg, epithelial cells, and does not specifically define a granulocytic lineage for R-S cells. Further evidence against a granulocytic origin for R-S cells is the consistent lack of other markers for these cells, eg, peroxidase or chloroacetate esterase activity.[11]

Leu-M1 monoclonal antibody represents a highly effective reagent for the detection of R-S cells, especially since the antigen defined by this reagent is well preserved in fixed, paraffin-embedded tissues. In all of our cases (69 total) of Hodgkin's disease of nodular sclerosis, mixed cellularity, and lymphocyte depletion types (Table 1), diagnostic R-S cells, as well as lacunar, mononuclear, and multinucleated/multilobated variants, revealed strong immunoreactivity for Leu-M1 (Figures 1–3), most with a characteristic staining pattern (membranous, cytoplasmic, and/or paranuclear). Of our group of 60 non-Hodgkin's lymphomas (Table 2), only a single B-cell lymphoma and four T-cell malignancies were immunoreactive for Leu-M1. The staining pattern in these cases, however, distinctly differed from that of R-S cells, with a more delicate membrane pattern noted in the B-cell case and finely granular cytoplasmic positivity observed for T-cell lesions. Reactivity in some T-cell neoplasms is perhaps not surprising, since anti-Leu-M1 is known to detect certain

T-cell lines and mitogen-activated T cells.[15] None of the T- or B-cell malignancies (27 total) evaluated by Hsu and Jaffe[25] revealed staining for Leu-M1. On the basis of these results, even in T- and B-cell neoplasms, which may contain cells morphologically resembling R-S cells, particularly immunoblastic sarcomas, immunoreactivity patterns for Leu-M1 potentially provide a diagnostic discriminant. In some cases, the distinction between Hodgkin's disease and non-Hodgkin's lymphoma may be very difficult, especially if B- and T-cell marker studies are unavailable. Exemplifying this difficulty in a review of 287 cases initially diagnosed as Hodgkin's disease, the Southwest Oncology Group found that 13% were misdiagnosed.[27] Therapy and prognosis are obviously affected by these errors.

In our study and in that previously reported,[25] R-S cells and L&H variants of lymphocyte predominance Hodgkin's disease were consistently nonreactive for Leu-M1. Using the granulocyte-related monoclonal antibodies TÜ9, TÜ5, TÜ6, Stein et al[14] observed staining of R-S cells and variants in all cases of nodular sclerosis, nearly all cases of mixed cellularity, and most cases of lymphocyte depletion type Hodgkin's disease. However, only 8 of 21 cases of lymphocyte predominance Hodgkin's disease were reactive. Other studies have suggested that this form of Hodgkin's disease, in contrast to other types, originates in B-cell, rather than T-cell, zones.[24] Evidence has been presented for a B-cell derivation for the neoplastic cells in lymphocyte predominance Hodgkin's disease and a histiocytic origin in the other histologic types.[7] The unique excellent prognosis in nodular lymphocyte predominance type of Hodgkin's disease, even without therapy, has been well documented.[6,28] The disparities between lymphocyte predominance Hodgkin's disease and the other histologic types raise the possibility of a different pathogenesis for this disorder.

In our studies of nonneoplastic lymph nodes, occasional histiocytes of the sinusoidal network exhibited a staining pattern for Leu-M1 similar to that observed for R-S cells (Figure 6B). These cytologically benign cells were noted in small numbers in nodes with nonspecific reactive changes but were much more conspicuous in toxoplasmic lymphadenitis and in the node from the homosexual male with persistent adenopathy. The basis for the altered immunoreactivity of some of these cells is obscure. The similarity of their staining pattern to that of R-S cells is insufficient evidence to define these cells as their nonneoplastic counterpart. However, this observation does raise the possibility that some R-S cells or variants in Hodgkin's disease of nodular sclerosis, mixed cellularity, or lymphocyte depletion type may represent transformed neoplastic sinusoidal histiocytes. Using monoclonal antibody Ki-1, which selectively reacts with R-S cells and variants[10,11] and has also been observed on auto and allo-activated T-helper cells,[29] Stein et al[10,11] detected a distinct, as yet unidentified cell population in normal lymphoid tissue. In their study, the number of Ki-1–positive cells was also conspicuously increased in toxoplasmic lymphadenitis.[11] However, the cells defined by their antibody apparently exhibited a distribution different from our Leu-M1–positive cells, occurring around germinal centers and between follicles. In contrast to our studies, which demonstrated variable but definite immunoreactivity for Leu-M1 in some histiocytes of both neoplastic and nonneoplastic tissues, Hsu and Jaffe[25] did not observe staining in histiocytes. The basis for this discrepancy may be related to the different immunohistochemical techniques employed in these studies, our method apparently yielding greater sensitivity. In our laboratories, the three-step procedure described in this study, ie, monoclonal antibody followed sequentially by peroxidase-conjugated rabbit anti-mouse immunoglobulin serum and peroxidase-conjugated swine anti-rabbit immunoglobulin serum, is preferable to other methods, eg, avidin–biotin (ABC) techniques, peroxidase–antiperoxidase (PAP) techniques, or a two-step procedure with peroxidase-conjugated rabbit anti-mouse immunoglobulin serum. Our technique provides stronger enzyme labeling than the other methods (unpublished observations), readily characterized by a peroxidase reaction, and results in minimal to absent background staining.

In summary, this study demonstrates the presence of a granulocyte-related antigen, Leu-M1, for R-S cells and variants of nodular sclerosis, mixed cellularity and lymphocyte depletion types of Hodgkin's disease. L&H variants of lymphocyte predominance type (nodular) consistently lacked immunoreactivity for Leu-M1. In nonneoplastic tissues, only occasional sinusoidal histiocytes of lymph nodes exhibited the staining pattern observed for R-S cells. The absence of immunoreactivity in nearly all our cases of non-Hodgkin's lymphoma and the relatively characteristic staining pattern observed for R-S cells and variants in Hodgkin's disease may serve as helpful diagnostic discriminants in distinguishing between these neoplasms.

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