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Monoclonal Antibodies (MT1, MT2, MB1, MB2, MB3) Reactive With Leukocyte Subsets in Paraffin-Embedded Tissue Sections

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The absence of reactivity on routinely prepared tissue sections has hampered the use of monoclonal antileukocyte antibodies in diagnostic histopathology. Here we describe five new antibodies reactive with leukocyte subsets in formaldehyde-fixed, paraffin-embedded tissue sections. Antibody MT1 is reactive with mature and immature T cells and not with mature B cells. MT2 is reactive with mature T cells and B cells, but not with immature T cells, activated T cells, and germinal center B cells. Antibody MB1 is reactive with all B cells, with about 50% of mature T cells, and not with immature T cells. MB2 is reactive with all B cells and not with T cells. However, MB2 also stains endothelial cells and several types of epithelial cells. MB3 is reactive with B cells and histiocytes, but not with T cells. The antibodies were tested on a series of lymphomas that were also immunophenotyped with a panel of well-established reagents on frozen tissue sections. The results indicate that the MB and MT antibodies are useful tools in the study of reactive and neoplastic disorders of the lymphoid system. (Am J Pathol 1987, 127:418–429)

MONOCLONAL ANTIBODIES, reactive with lymphocyte differentiation antigens, can be used for the identification of cells in frozen tissue sections.[1] Few antibodies have been found to be reactive on paraffin-embedded, formaldehyde-fixed tissue sections. These are the anti-pan-leukocyte antibodies PD7/26 and 2B11, both reactive with 200-kd glycoproteins on the surface of T and B lymphocytes,[2] antibodies reactive with human HLA-DR antigens,[3,4] antibodies LN1 and LN2, which are reactive with B-cell subsets,[5] and antibodies Leu-M1 and Clonab Tü-9, reactive with lacto-N-fucopentaose antigens, present in granulocytes and Reed–Sternberg cells.[6,7] Immunohistologic staining on paraffin tissue sections has some obvious advantages over frozen tissue section staining. Morphologic details will be better preserved, and staining can be performed on material also used for routine histologic examination.

In general, two different approaches can be chosen —first, the development of fixation, dehydration, and paraffin-embedding procedures permitting the use of the present generation of monoclonal antibodies.[8,9] So far, these procedures have led to inadequate morphology and absence of reactivity with some of the more important antibodies like T4 and T8. A second approach will be the development of a new generation of monoclonal antibodies reactive on routinely prepared paraffin-embedded tissue sections. To obtain optimal immunohistologic staining it is necessary to employ standard fixation procedures. Sublimate-containing fixatives, like the B5 fixative, have been described to give excellent morphologic and immunohistologic results.[5]

Here we report five new antibodies reactive with lymphocyte subsets in formaldehyde-fixed, paraffin-embedded tissue sections and describe their staining patterns in B5-fixed lymph nodes, thymuses, and spleens.

Materials and Methods

Production of Monoclonal Antibodies

BALB/c mice were immunized intraperitoneally at least twice at 2-week intervals with: A) cell suspen-

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sions of a lymph node involved by Hodgkin's disease; B) cell suspensions of a lymph node involved by B-chronic lymphatic leukemia; C) cells of Hodgkin's cell line DEV,[10] respectively. No adjuvants were used; the cells were suspended in saline. Four days after intravenous boosting, spleen cell suspensions were fused with X63 mouse myeloma cells according to standard procedures.[11] Supernatants of the resulting hybrid clones were tested with an indirect immunoperoxidase method on frozen tissue sections of human lymph nodes.[12] A total of 50 clones with restricted anti-lymphocyte specificities was selected for further study. These were further tested on paraffin tissue sections, resulting in 5 hybrids with strong reactivity. These were recloned twice in soft agar, and large quantities of supernatants were produced. Acites was produced by intraperitoneal inoculation of hybridoma cells in pristane-primed BALB/c mice. The antibodies were denominated MT1 (from immunization A), MT2 and MB3 (from immunization B), and MB1 and MB2 (from immunization C).

Testing of Immunoglobulin Subclasses

The immunoglobulin subclass of the antibodies was tested with subclass-specific peroxidase-conjugated rabbit anti-mouse antibodies (Serotec, Blackthorn, U.K.) by an indirect immunoperoxidase method.

Immunohistology

Frozen tissue sections of lymph nodes were prepared as previously described.[1] Paraffin tissue sections were prepared as follows. To determine optimal fixation conditions, tissue blocks were fixed at room temperature for 3 or 24 hours in the following fixatives:

I. Formalin: 10 ml 37% formaldehyde dilution, 90 ml tap water, pH 6.5, as used for routine fixation.
II. Ten percent phosphate-buffered formalin: 10 ml 37% formaldehyde dilution, 90 ml phosphate buffer (0.4 g $NaH_2PO_4 \cdot H_2O$ and 0.65 g Na_2HPO_4 in 90 ml aqua dest) pH 7.2.
III. Bouin's fixative: 75 ml 1.2% picric acid, aqueous solution, 25 ml neutral 37% formaldehyde solution, 5 ml glacial acetic acid, pH 1.5.
IV. B5 fixative: 90 ml aqua dest, 6 g mercuric chloride, 2.074 sodium acetate ($CH_3COONa \cdot 3H_2O$), 10 ml 37% formaldehyde solution, pH 5.7.
V. Zenker's fixative: 95 ml aqua dest, 2.5 g K_2CrO_4, 5 g $HgCl_2$, 5 ml glacial acetic acid.

Subsequently the blocks were transferred to 70% ethanol and dehydrated and embedded in paraffin according to standard procedures. Tissue sections were cut at 2 μ thickness and deparaffinized, rehydrated, and desublimated (if necessary) according to standard procedures. In addition, formalin-fixed tissue blocks from our routine files and from referring institutions were obtained. Tissue sections were pretreated for 30 minutes with 0.3% methanol H_2O_2 to block endogenous peroxidase activity. First-step incubations were with 100 μl diluted supernatant containing monoclonal antibody (MT1, MT2, MB1, MB2, MB3, and for comparison LN1, LN2, LN3, Clonab Tü-9 [Biotest, Dreieich, West Germany] and Leu-M1 [B&D], for 30 minutes. As a control, supernatant reactive with hemocyanin was used. After a 5-minute wash the sections were incubated with 25 μl peroxidase-conjugated rabbit anti-mouse Ig antibodies, diluted 1:20 (Dakopatts, Copenhagen, Denmark), for 15 minutes. In some instances, when on routinely prepared tissue sections only weak staining was obtained, an extra third step with peroxidase-conjugated swine anti-rabbit IgG antibodies, diluted 1:20 (Dakopatts) was applied, for 15 minutes. As an alternative, the avidin–biotin complex method (ABC method) was used. These reagents were also obtained from Dakopatts and applied according to the manufacturer's instructions. After another 5-minute wash the sections were stained with 3.3′-diaminobenzidine tetrahydrochloride and H_2O_2. Nuclear counterstaining was performed with Mayers' hemalum.

Immunoblotting

Cells of a suspension of a hyperplastic lymph node were lysed with 1% NP-40 in 0.01 N triethanolamine, pH 7.8, 0.15 M NaCl, 1 mM phenylmethylsulfonyl fluoride, and 0.02 mg trypsin inhibitor per milliliter. The lysates were centrifuged at 13,000*g* for 15 minutes for removal of nuclei. The supernatant was used for sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing conditions, according to the method of Laemmli.[13] Acrylamide concentration of the separation gels was 12.5, or 10%. Appropriate molecular weight markers were used. The electrophoretic transfer of proteins to nitrocellulose was performed according to the procedures of Towbin et al[14] at 45 V for 16 hours. After protein transfer, the unspecific binding sites of the nitrocellulose were blocked by a solution of 5% bovine serum albumin (BSA) in 0.01 M tris-HCl containing 0.15 moles NaCl/l, pH 7.4 (BSA/TBS), for 30 minutes at 40 C. The nitrocellulose was then transferred to a solution of BSA/TBS containing 10% normal rabbit serum (BSA/TBS/

NRS) and MAb IgG (MT1, MT2, MB1, MB2, MB3, LN1, and LN2) at a concentration of 5.0 mg/l. The incubation was performed overnight at room temperature. After the nitrocellulose was washed with phosphate buffered saline, it was incubated with peroxidase-conjugated anti-mouse IgG produced in rabbits (Dakopatts, Copenhagen, Denmark) diluted 1:500 in BSA/TBS/NRS (1 hour at room temperature). After washing with 0.05 M Tris-HCl, pH 7.4, the bound peroxidase was incubated with 0.05 M Tris-HCl, pH 7.4, containing 1.26 mmol 3.3′-diaminobenzidine tetrahydrochloride and 0.005% H_2O_2 per liter. The staining reaction was stopped by rinsing the nitrocellulose with distilled water.

Enzyme Digestion

To characterize the biochemical nature of the antigens recognized by antibodies MT1, MT2, MB1, MB2, and MB3, frozen acetone-fixed tissue sections were pretreated with neuraminidase (Sigma, Type X, 0.05 U/ml in saline, pH 7.2) for 30 minutes. Subsequently the sections were stained with the antibodies as described above.

Results

Generation of the Antibodies

A series of fifty monoclonal anti-lymphocyte antibodies produced in our laboratory were tested for staining reactivity on B5-fixed paraffin-embedded tissue sections. Five different clones with strong positive reactions were selected for further study. The immunoglobulin subclasses of these antibodies as determined with the subclass-specific antibodies were IgG1 for MT1, MT2, MB1 and MB2, and IgG2B for MB3.

Influence of Fixatives

The results obtained with different fixation procedures were as follows (Table 1). Positive staining was obtained on routine paraffin sections after 3 or 24 hours' fixation in formalin, or in buffered formaldehyde, although after longer fixation periods, ie, more than 24 hours, the staining intensity decreased. A third-step peroxidase-conjugated swine anti-rabbit Ig antibody on top of the second step peroxidase-conjugated rabbit anti-mouse Ig antibody or application of the ABC method could enhance weak staining. Trypsin and pronase pretreatment of sections did not improve the staining results. Bouin's fixative and Zenker's fixative only gave weak staining after 3 hours' fixation. Optimal results were obtained on B-5-fixed, paraffin-embedded tissue sections. Fixation periods of 3–24 hours in B5 fixative resulted in excellent morphology and optimal immunohistochemical results. Therefore, the reaction patterns of the antibodies were defined on B5-fixed, paraffin-embedded tissue sections of reactive lymph nodes, thymuses, and spleens, on tissues of a series of lymphomas, and on a panel of tissues obtained at autopsy.

Immunohistologic Staining of Normal Tissues

The staining results are summarized in Table 2, and illustrated in Figures 1 and 2. Antibody MT1 was reactive with a membrane antigen on all T lymphocytes and thymocytes and not on mature B lymphocytes. In addition, it was reactive with monocytes and macrophages, including Langerhans cells of the epidermis and Kupffer cells of the liver, with myeloid cells and with erythrocyte precursors. Antibody MT2 was reactive with a membrane antigen on mature T lymphocytes and on medullary thymocytes, but not

Table 1–Influence of Fixation on the Immunohistochemical Demonstration of Leukocyte Antigens

	Formalin	Buffered formaldehyde	Bouin's fixative	B5 fixative	Zenker's fixative
MT1					
3 hours	+	+	±	++	±
24 hours	+	+	−	++	−
MT2					
3 hours	+	+	+	++	+
24 hours	+	+	−	++	−
MB1					
3 hours	+	+	+	++	±
24 hours	++	+	−	++	−
MB2					
3 hours	+	+	+	++	+
24 hours	+	+	++	++	−
MB3					
3 hours	+	+	++	++	+
24 hours	+	+	++	++	−

−, no staining; ±, weak staining; +, good staining; ++, excellent staining.

Table 2—Reactivity of MT1, MT2, MB1, MB2, and MB3 With Lymphoid and Hematopoietic Cells

Cell type	MT1	MT2	MB1	MB2	MB3
B lymphocytes					
Germinal center cells	−	−	+	+	+
Mantle zone cells	−	+	+	+	+
Marginal zone cells	−	+	+	+	+
Plasma cells	−	−	−	−	−
T lymphocytes					
Cortical thymocytes	+	−	−	−	−
Medullary thymocytes	+	+	+/−	−	−
Paracortical lymphocytes	+	+	+/−	−	−
Periarteriolar lymphocytes in spleen	+	+	+/−	−	−
Histiocytes					
Macrophages	+	−	+/−	−	+
Langerhans cells	+	−	−	−	+
Interdigitating cells (T-cell areas)	+	−	−	−	+
Dendritic reticulum cells (B-cell follicles)	−	−	−	−	−
Kupffer cells	+	−	−	−	+
Hematopoietic cells					
Monocytes	+	−	+/−	−	+
Myeloid cells	+	−	−	−	+
Erythroid precursors	+	−	−	−	−
Erythrocytes	−	−	−	−	−
Megakaryocytes	+	−	−	−	−
Thrombocytes	−	−	−	−	−

+, reactive with most cells; −, not reactive; +/−, reactive with 20–50% of cells.

on cortical thymocytes. In addition, it was reactive with mantle zone lymphocytes of follicles in lymph nodes and spleen and also with marginal zone lymphocytes in spleens. However, it was unreactive with germinal center B cells.

Antibody MB1 was reactive with all B cells, excluding mature plasma cells, and with about 50% of mature T lymphocytes. No reactivity was found with cortical thymocytes. Antibody MB2 was reactive with a cytoplasmic antigen in all B lymphocytes, excluding mature plasma cells. No reactivity was found with T lymphocytes and thymocytes in paraffin tissue sections, although in frozen tissue sections a very weak T-cell staining could be observed. In addition, MB2 was weakly reactive with endothelial cells and with several types of epithelial cells (Table 3). MT1, MT2, and MB1 showed no reactivity with nonlymphoid cell types. Antibody MB3 was reactive with a cytoplasmic antigen in B lymphocytes and in monocytes, reticulum cells, macrophages, and reticuloepithelial cells of the thymus in a distribution pattern similar to HLA Class II antigens but lacking the membranous staining of regular anti HLA-DR reagents. MB3 was not reactive with T lymphocytes.

For comparison, we stained sections with monoclonal antibodies LN1, LN2, and LN3 (Biotest, Dreieich, West Germany). The staining pattern of LN1 was restricted to germinal center cells in lymph nodes and some large blasts in the interfollicular areas (Figure 2B). The staining pattern of LN2 was identical to that of antibody MB3, and antibody LN3 gave a classical membranous HLA Class II staining pattern.

Immunobiochemical Characterization of Antigens

The molecular weights of the antigens recognized by these antibodies in the immunoblot procedure were as follows. MT1 reacted with three bands with apparent molecular weights of 190, 110, and 100 kd. MT2 reacted with two bands of 200 and 190 kd, MB1 with three bands of 200, 110, and 100 kd, MB2 with one band of 28 kd, and MB3 with a strong band of 31 kd. We also tested LN1 and LN2. LN1 reacted with 8 bands between 45 and 85 kd, and LN2 reacted with a strong band, identical to that of MB3, at 31 kd (Figure 3).

The enzymatic digestion studies on frozen sections demonstrated that neuraminidase pretreatment of sections subsequently stained with MT2 led to disappearance of the T-lymphocyte staining and persistance of the small B-lymphocyte staining (Figure 4). The staining with MT1 also clearly diminished after neuraminidase pretreatment. This indicates that these antigens are at least partly carbohydrate and also that the expression of MT2 antigen on T cells and B cells is different. We also confirmed the neuraminidase sensitivity of the antigen recognized by LN1, because the germinal center staining by this antibody was completely absent after neuraminidase treatment.

Immunohistologic Staining of Malignant Lymphomas

To evaluate the usefulness of these antibodies in lymphoma diagnosis we tested them on a series of lymphomas that were also immunophenotyped with established monoclonal reagents on frozen tissue sections. The results are summarized in Table 4. A mixture of MT1 and MB1 stained all malignant lymphomas, including true histocytic tumors, except for most plasmacytomas. MT1 was reactive with T-cell lymphomas (Figure 5A) and histiocytic tumors, but also with some B-cell lymphomas. Strong reactions were found with lymphoblastic B-cell lymphomas, whereas small lymphocytic lymphomas and centrocytic (small cleaved, diffuse) lymphomas stained weakly (Figure 6B). Centroblastic/centrocytic lymphomas (cleaved cell/non-cleaved cell), centroblastic lymphomas (large non-cleaved, diffuse), B-immunoblastic lymphomas, and plasmacytomas were all

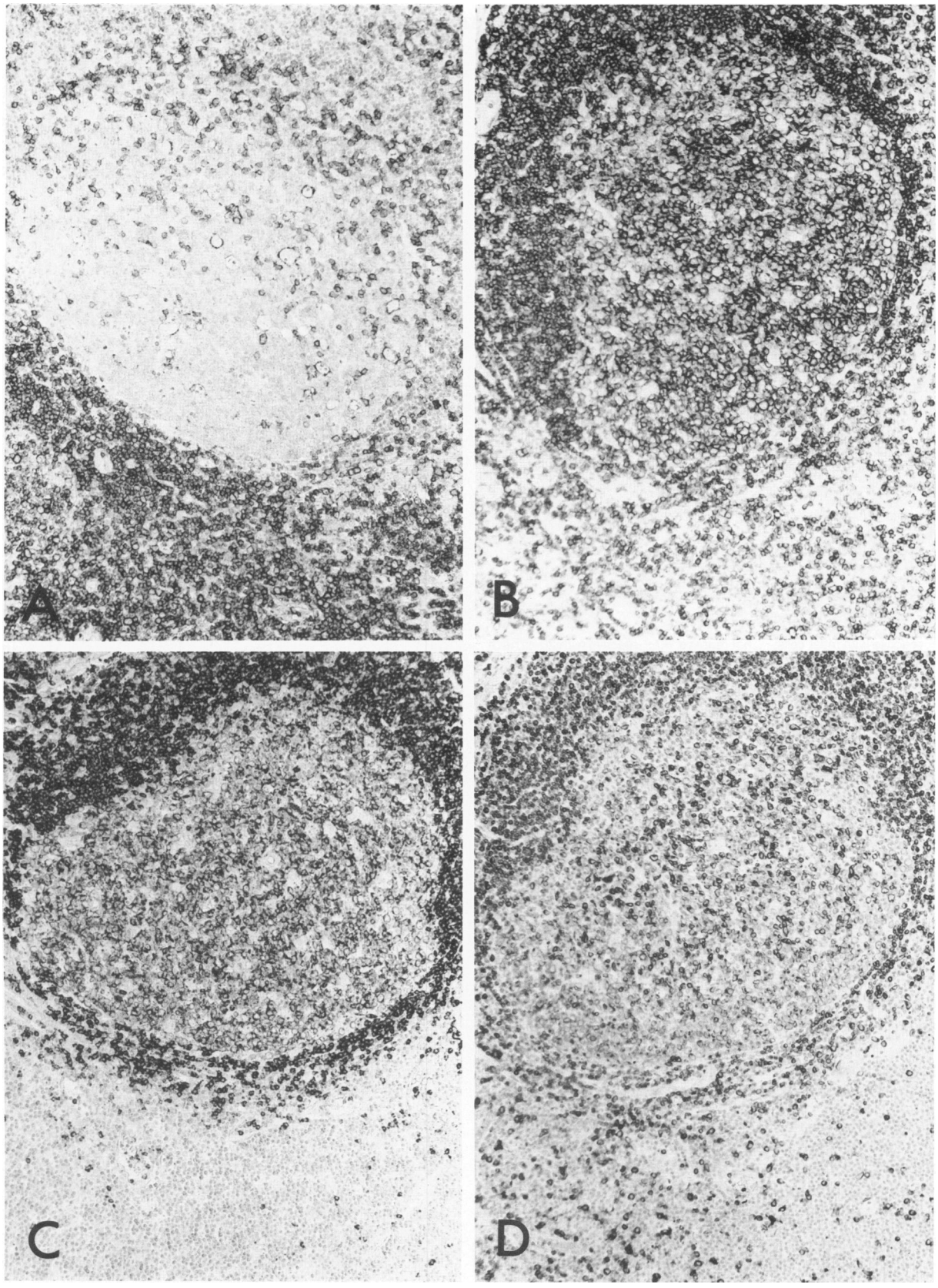

Figure 1—B5-fixed paraffin-embedded tissue sections of hyperplastic lymph node stained with MT1 (**A**), MB1 (**B**), MB2 (**C**) and MB3 (**D**). The upper half of the pictures consists of a secondary follicle, whereas the lower half is a T-cell area. **A**—Positive reactions of T lymphocytes in the T-cell area and also in the germinal center on the border with the mantle zone. In addition, staining of several starry-sky macrophages can be noted. **B**—Positive reactions of all B lymphocytes in the mantle zone as well as in the germinal center. In addition, about 30% of the lymphocytes in the T-cell areas stain. **C**—Strong cytoplasmic staining of all B lymphocytes in the mantle zone and germinal center. Only few scattered positive cells are found in the T-cell area. **D**—Cytoplasmic staining of all B lymphocytes and of interdigitating reticulum cells in the T-cell area. (Two-step immunoperoxidase staining, ×140)

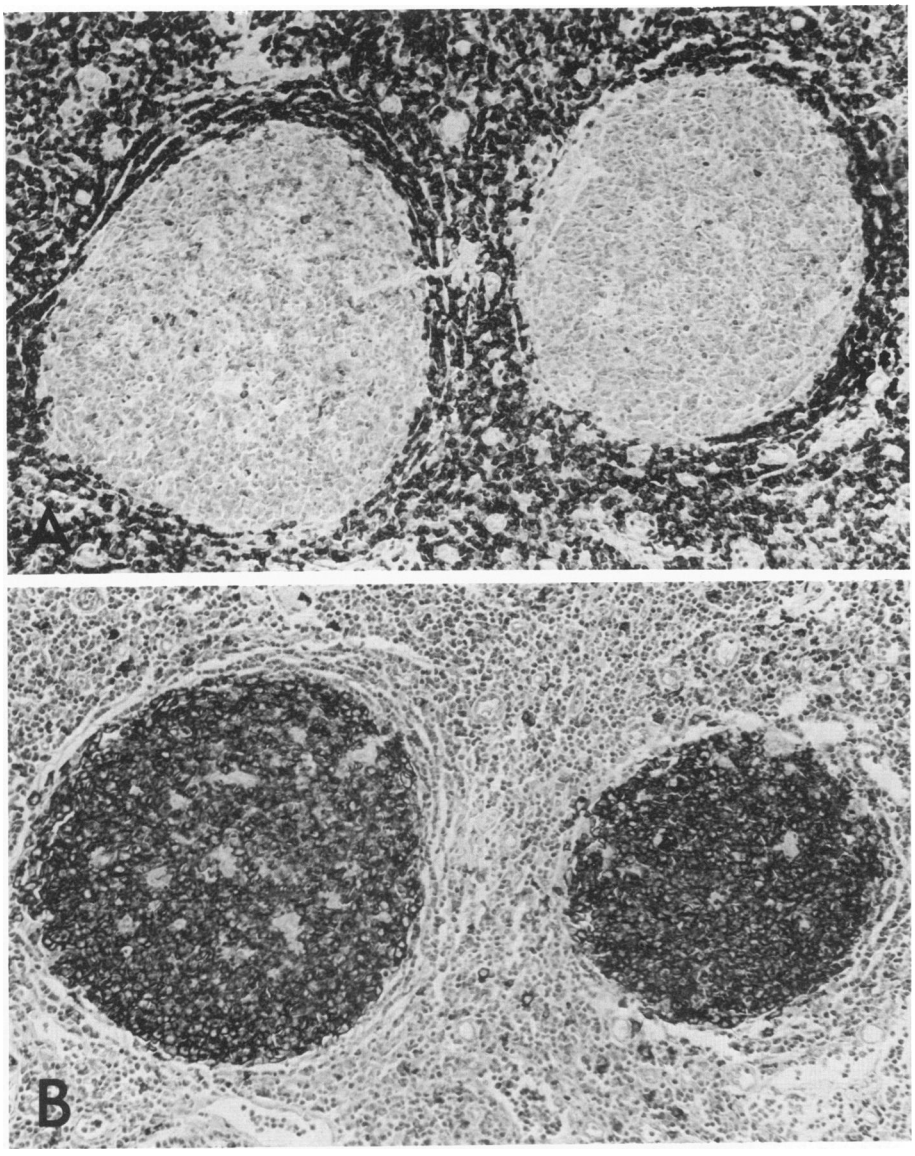

Figure 2—B5-fixed paraffin-embedded tissue sections of hyperplastic lymph node stained with MT2 (**A**) and LN1 (**B**). MT2 strongly stains mantle-zone B cells as well as small lymphocytes of the T-cell area. Germinal-center B cells are not stained (**A**). LN1 stains germinal-center B cells, but not mantle-zone B lymphocytes and T cells. Note the presence of scattered large immunoblasts in the interfollicular region. (Two-step immunoperoxidase, ×140)

negative for MT1 (Figures 5D and 6D). MT2 was not reactive with lymphoblastic B-cell lymphomas, but positive staining was found on all other B-cell lymphomas, except for plasmacytomas. T-cell lymphomas were not reactive with MT2, despite the clear staining of normal mature T cells with this reagent (Figure 5B).

MB1 was reactive with all B-cell lymphomas, except for most plasmacytomas (Figure 6A). It was not reactive with lymphoblastic T-cell lymphomas, but did react with several peripheral T-cell lymphomas, in accordance with the finding that up to 50% of normal mature T cells are reactive with MB1. MB2 was reactive with all B-cell lymphomas, except for plasmacytomas (Figures 5C and 6C). The antigen localization appeared to be cytoplasmic in all cases. T-cell lymphomas were not reactive with MB2. MB3 was reactive with the B-cell lymphomas, except for plasmacytomas, and did not react with lymphoblastic T-cell lymphomas, whereas several peripheral T-cell lymphomas were positive. In addition, all histiocytic tumors were clearly reactive with MB3. The antigen localization appeared to be cytoplasmic in all cases.

In Hodgkin's disease, a majority of MT1-positive T lymphocytes could be distinguished in most cases (Figure 7A). These T lymphocytes were reactive with neither MT2 or MB1 (Figure 7B). Reed–Sternberg cells were generally not reactive with MT1, MT2, MB1, or MB2, but clearly stained with MB3, Leu-M1, and Clonab Tü-9 (Figure 7C). However, L&H type Reed–Sternberg cells in the cases of lymphocyte predominance type of Hodgkin's disease showed clear

Table 3—Reactivity of MB2 and LN1 With Nonlymphoid Tissues

	MB2		LN1	
Epidermis	+	(excluding squamous cell layer)	+	(squamous cell layer)
Breast	+	(epithelium)	+	(epithelium)
Lung	±	(epithelium)	−	
Kidney	+	(distal tubules)	+	(distal tubules)
Prostate	+	(glands)	+	(glands)
Endometrium	+	(glands)	−	
Ovary	+	(stroma cells)	−	
Adrenal	+	(cortex)	−	
Thyroid	−		−	
Pancreas	±	(epithelium)	−	
Liver	−	(hepatocytes)	+	(hepatocytes)
	+	(bile ducts)	+	(bile ducts)
Stomach	+	(epithelium)	−	
Colon	+	(epithelium)	−	
Heart	−		−	
Smooth muscle	+/−		+/−	
Skeletal muscle	−		−	
Cartilage	−		−	
Peripheral nerve	−		−	
Brain	−		−	
Testis	+	(spermatocytes)	−	
Bladder	+	(epithelium)	−	
Fallopian tube	+	(epithelium)	−	
Red blood cells	−		+	

+, clearly positive; ±, weakly positive; +/−, positive in some tissues, negative in others; −, negative.

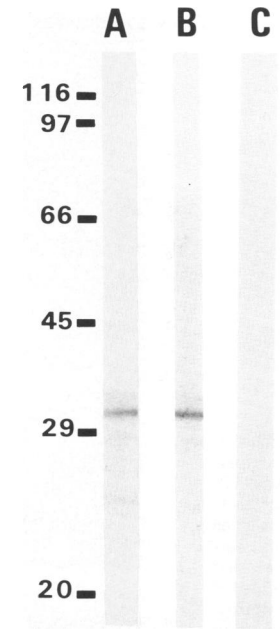

Figure 3—Immunoblot of lymph node suspension cells stained with monoclonal antibody MB3 (lane A), LN2 (lane B), and control supernatant (lane C). Identical bands can be noted at 31 kd in lanes A and B. Some additional weak, but specific bands can be identified in both lanes.

cytoplasmic staining with MB2 and weak or absent staining with Leu-M1 and Clonab Tü-9.

Discussion

The majority of monoclonal anti-leukocyte antibodies are reactive with cells in suspensions and on frozen tissue sections, but not on formaldehyde-fixed, paraffin-embedded tissue sections. This has been an obstacle to the use of these reagents in routine diagnostic procedures, because these are dependent on the morphologic evaluation of well-fixed, paraffin-embedded tissue sections.

Here we describe five new monoclonal antibodies reactive with leukocyte subsets in paraffin-embedded tissue sections. Optimal results were obtained on B5-fixed tissues, although good staining was also found after other fixations, in particular after formalin or

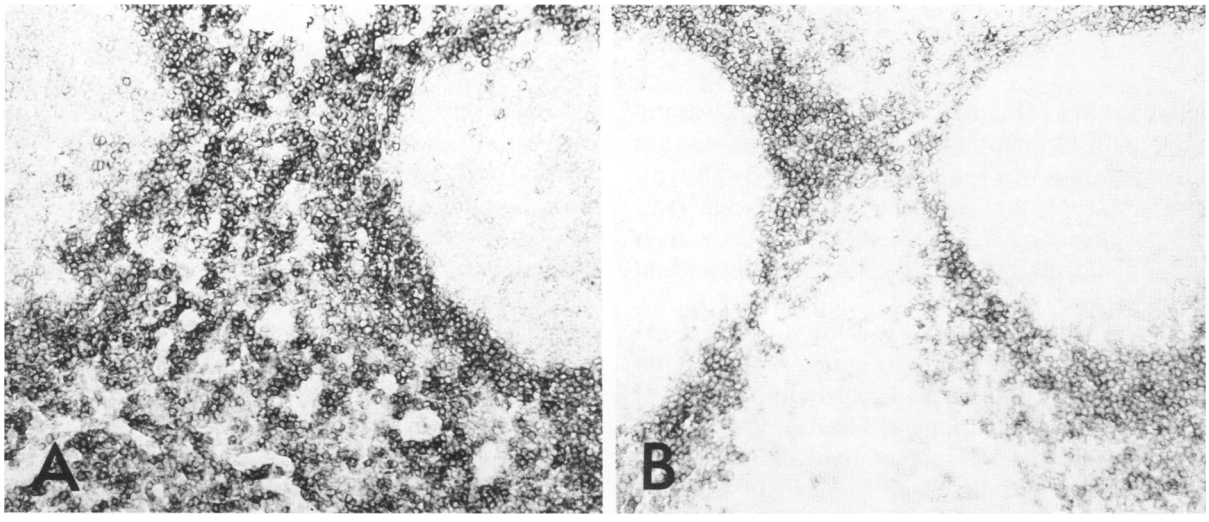

Figure 4—Immunoperoxidase staining on frozen tissue sections of hyperplastic lymph node, employing MT2, without (**A**) and with (**B**) neuraminidase pretreatment (Sigma, Type X, 0.05 U/ml, 37 C, 30 minutes). Note that T-cell staining is absent after neuraminidase pretreatment (**B**). (Two-step immunoperoxidase, ×140)

Table 4—Reactivity of Anti-Leukocyte Antibodies MT1, MT2, MB1, MB2, and MB3 With Non-Hodgkin's Malignant Lymphomas

Type	Number	MT1+MB1	MT1	MT2	MB1	MB2	MB3
B-cell lymphomas*							
CALLA lymphoma†	2	2	2	0	2	2	2
Pre-B-cell lymphoma‡	2	2	2	0	2	2	2
B lymphoblastic (Burkitt type)	4	4	4	0	4	4	4
Centrocytic (cleaved cell, diffuse, small or large)	12	12	12††	12	12	12	12
Small lymphocytic	7	7	7††	7	7	7	7
Centroblastic/centrocytic (cleaved cell/non-cleaved cell, follicular)	10	10	0	10	10	10	10
Centroblastic (large non-cleaved, diffuse)	6	6	0	6	6	6	6
Immunoblastic	7	7	0	5	7	7	7
Plasmacytoma	5	1	0	0	1	0	0
T-cell lymphomas§							
T-lymphoblastic	5	5	5	0	0	0	0
Sézary's syndrome	2	2	2	0	0	0	0
T-CLL	1	1	1	0	0	0	0
Mycosis fungoides	2	2	2	0	0	0	2
Polymorphic T-cell	5	5	5	0	2	0	4
T-immunoblastic	5	5	5	0	3	0	3
Histiocytic tumors							
Histiocytosis-X‖	5	5	5	0	0	0	5
Histiocytic sarcoma¶	2	2	2	0	0	0	2
Malignant histiocytosis**	1	1	1	0	1	0	1

*Reactive with B4 antibody, except for plasmocytomas.
†Reactive with anti CALLA, OKI1, B4; non-reactive with B1, OKT11, OKT6, and anti-Ig.
‡Reactive with anti CALLA, OKI1, B4, anti μ; non reactive with B1, OKT11, OKT6, and anti-light chains.
§Reactive with OKT11 antibody.
‖Reactive with OKT6 and LKI1 antibodies.
¶Reactive with OKM1, Leu-M5, and OKI1 antibodies.
**Reactive with OKT6, Leu-M5, OKM1, and OKI1 antibodies.
††Weakly reactive with lymphoma cells.

buffered formaldehyde fixation as generally applied in routine pathology. The staining results on reactive lymphoid tissues demonstrate that these reagents can distinguish between T cells, B cells, and macrophages and also between different maturation stages of B cells and T cells. The study of a series of well-defined lymphomas shows that a combination of MT1 and MB1 can identify all hematopoietic neoplasms except plasmacytomas.

Antibodies MT1, MT2, and MB1 are reactive with different maturation stages of B cells and T cells. Because all three are reactive with antigens with molecular weights near 200 kd, they are probably directed against members of the so-called leukocyte common antigen family. These antigens are very prominent on leukocytes and not on other types of cells, but their function is presently unknown. Because MT1 and MT2 antigen are sensitive to neuraminidase treatment, they are probably sialo-antigens. MT2 is reactive with two different bands at 200 and 190 kd in Western blot procedures. Because the 200-kd band is shared with MB1, predominantly reactive with B cells, and the 190-kd band with MT1, predominantly reactive with T cells, we presume that the two MT2 bands reflect different antigens as present on B cells (200 kd) and T cells (190 kd). This idea is supported by the differential sensitivity of MT2 antigen on B cells and T cells for neuraminidase treatment. Our findings are in agreement with previous studies on leukocyte common antigens in rat and mouse, indicating antigens with different molecular weights on T and B cells.[15,16] The bands at 100 and 110 kd shared between MT1 and MB1 also appear to be members of the common leukocyte antigen family, because antigens with similar molecular weights were identified in studies on human and rat leukocytes.[17]

It is likely that sialo-antigens modify easily during lymphocyte maturation and differentiation. The absence of MT2 reactivity with T-cell lymphomas, despite its reactivity with normal mature T cells, is also reflected in the disappearance of MT2 antigen from phytohemagglutinin-stimulated T lymphocytes (data not shown). MT2 also identifies differences between reactive germinal center cells, which are MT2-negative, and lymphomas of germinal center cells that are MT2-positive. MT1 is not reactive with normal mature B cells, but is positive on lymphoblastic lymphomas derived from immature B cells and also, though more weakly, on small B-lymphocytic lymphomas and centrocytic lymphomas (ML, small cleaved cell, diffuse). In this respect MT1 resembles antibodies of CD5 (like OKT1 and Leu-1) that are reactive with a 67-kd glycoprotein present on mature T cells and also on small B-lymphocytic lymphomas

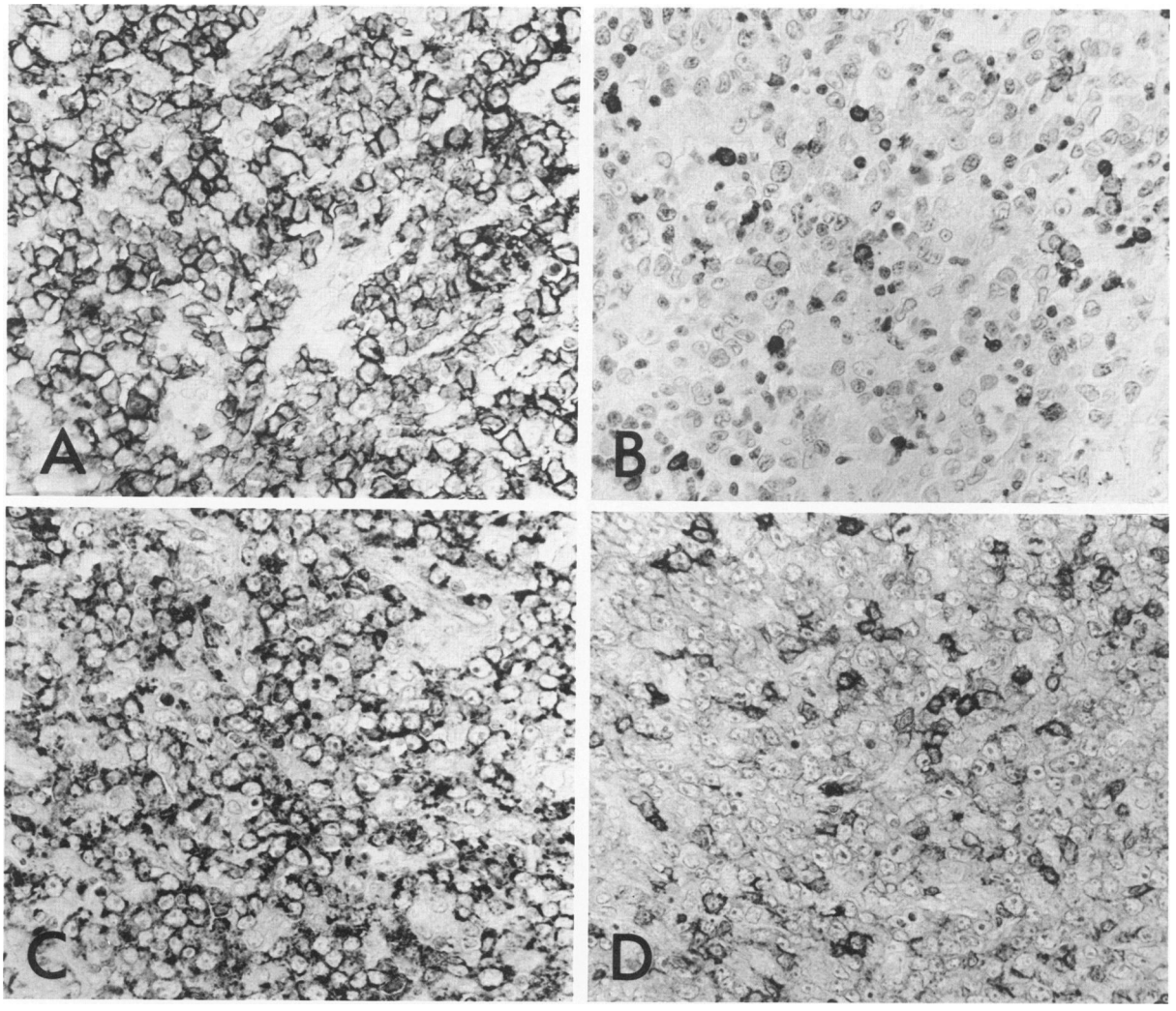

Figure 5—B5-fixed paraffin-embedded tissue sections of T-immunoblastic lymphoma (**A** and **B**) and B-immunoblastic lymphoma (**C** and **D**). MT1 strongly stains the T immunoblasts (**A**), whereas MT2 stains only scattered small lymphocytes (**B**). MB2 strongly stains the B immunoblasts (**C**), whereas scattered reactive T lymphocytes are identified by MT1 (**D**). (Two-step immunoperoxidase, ×350)

and centrocytic lymphomas.[17,18] The general reaction pattern of MT1 is quite different from CD5 antibodies, and the molecular weight of the antigen identified is not the same. The reactivity of MT1 with centrocytic (ML small cleaved, diffuse) and not with centroblastic/centrocytic lymphomas (ML cleaved cell/non-cleaved cell) confirms previous studies indicating that the centrocytes of these two entities are different cells.[18]

MB1 is reactive with all B cells and with a variable number of T cells ranging from 20% to 50% between different individuals. The positive cells include T4- as well as T8-positive lymphocytes (data not shown). Surprisingly, the T cells in Hodgkin's disease tissue, which are mainly T4-positive cells,[19] are MB1- as well as MT2-negative.

MB2 is reactive with a cytoplasmic antigen with a molecular weight of 28 kd in all B cells and B cell lymphomas. We compared this antibody with LN1, which is predominantly reactive with membrane and cytoplasmic antigens in germinal center B cells.[5] The nature of these antigens has not been identified. In Western blots we found that LN1 reacts with eight bands between 45 and 85 kd. Both antibodies are reactive with antigens outside the lymphoid system, but these show clearly different distributions patterns.

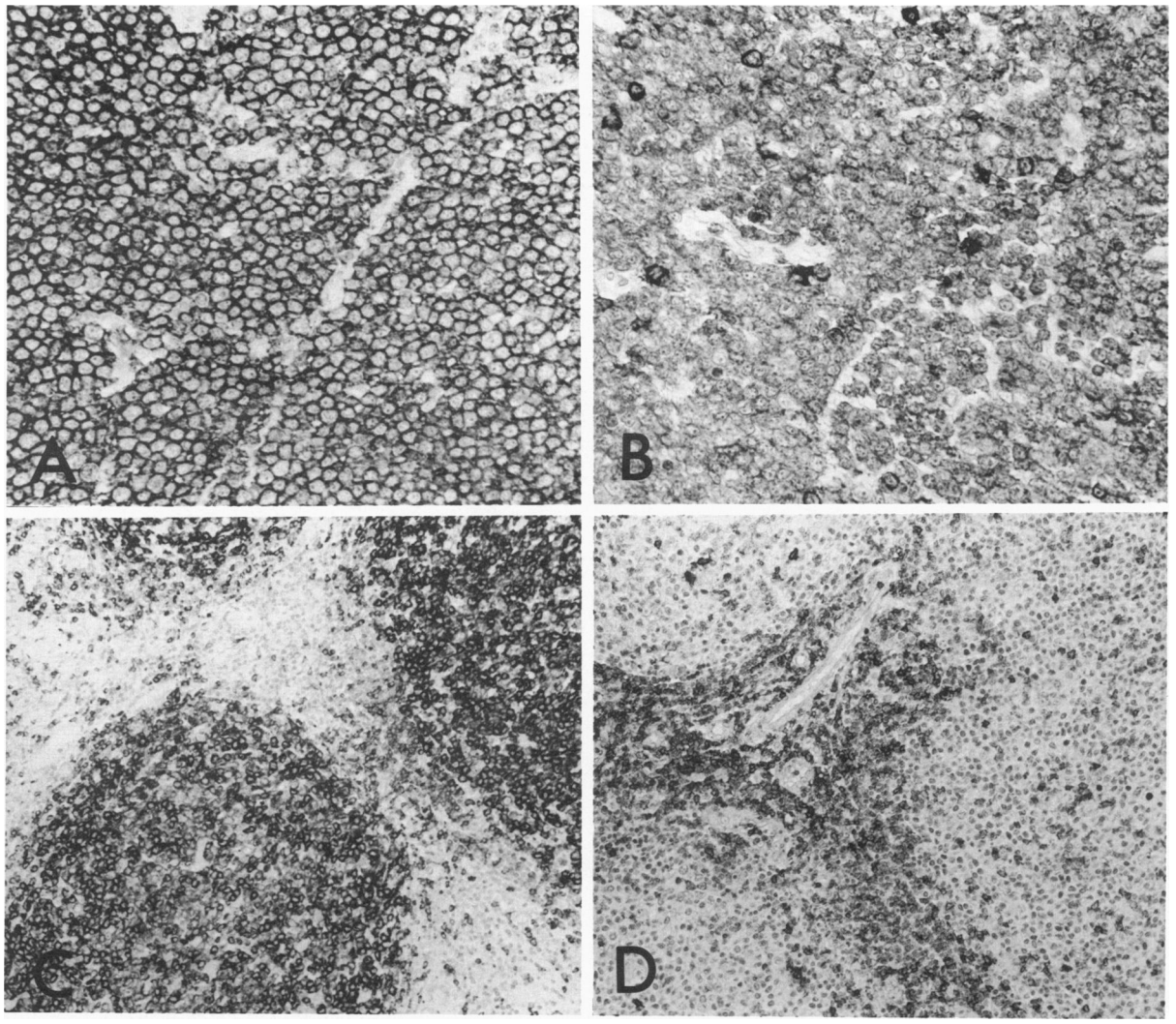

Figure 6A and **B**—B5-fixed paraffin-embedded tissue sections of centrocytic lymphoma (ML small cleaved, diffuse) and of centroblastic/centrocytic lymphoma (ML cleaved cell/non-cleaved cell, diffuse). MB1 stains all cells of centrocytic lymphoma (**A**), whereas MT1 also weakly stains the lymphoma cells (**B**). Scattered strongly, positive cells probably represent reactive T lymphocytes. **C**—Strong MB2 staining of the cells of a follicular centroblastic centrocytic lymphoma. **D**—Presence of interfollicular T cells and absence of MT1 staining of the lymphoma cells. (Two-step immunoperoxidase, **A** and **B**, ×350; **C** and **D**, ×140)

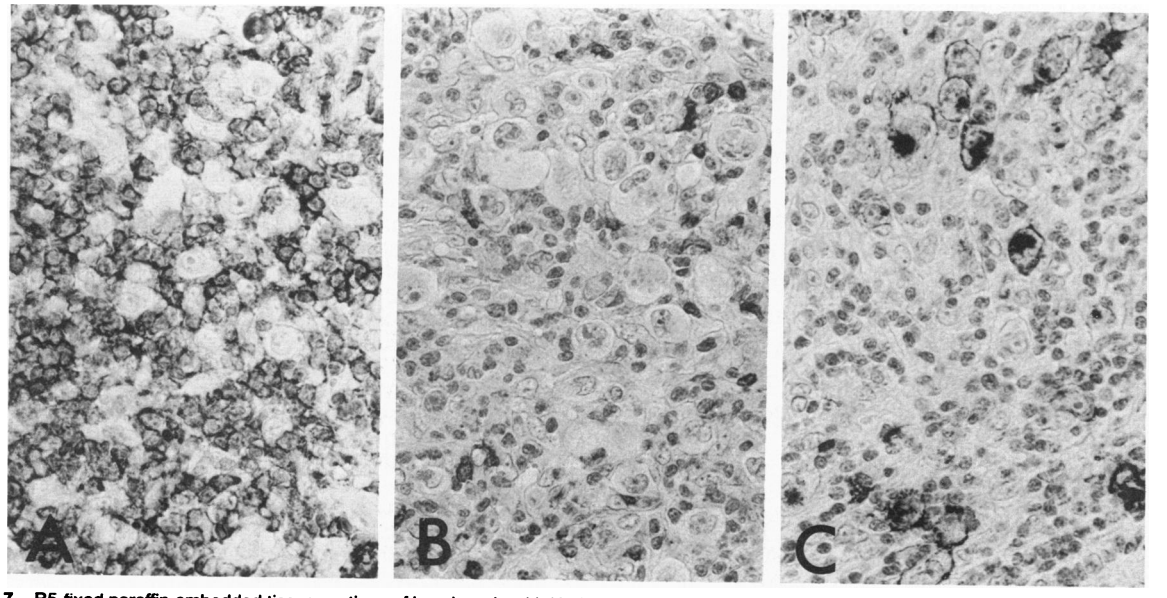

Figure 7—B5-fixed paraffin-embedded tissue sections of lymph node with Hodgkin's disease, nodular sclerosis subtype, stained with MT1 (**A**), MT2 (**B**), and with Leu-M1 (**C**). MT1 stains most of the T lymphocytes (**A**), whereas MT2 stains only a very few cells (**B**). Reed–Sternberg cells are identified by Leu-M1 (**C**). (Two-step immunoperoxidase, ×350)

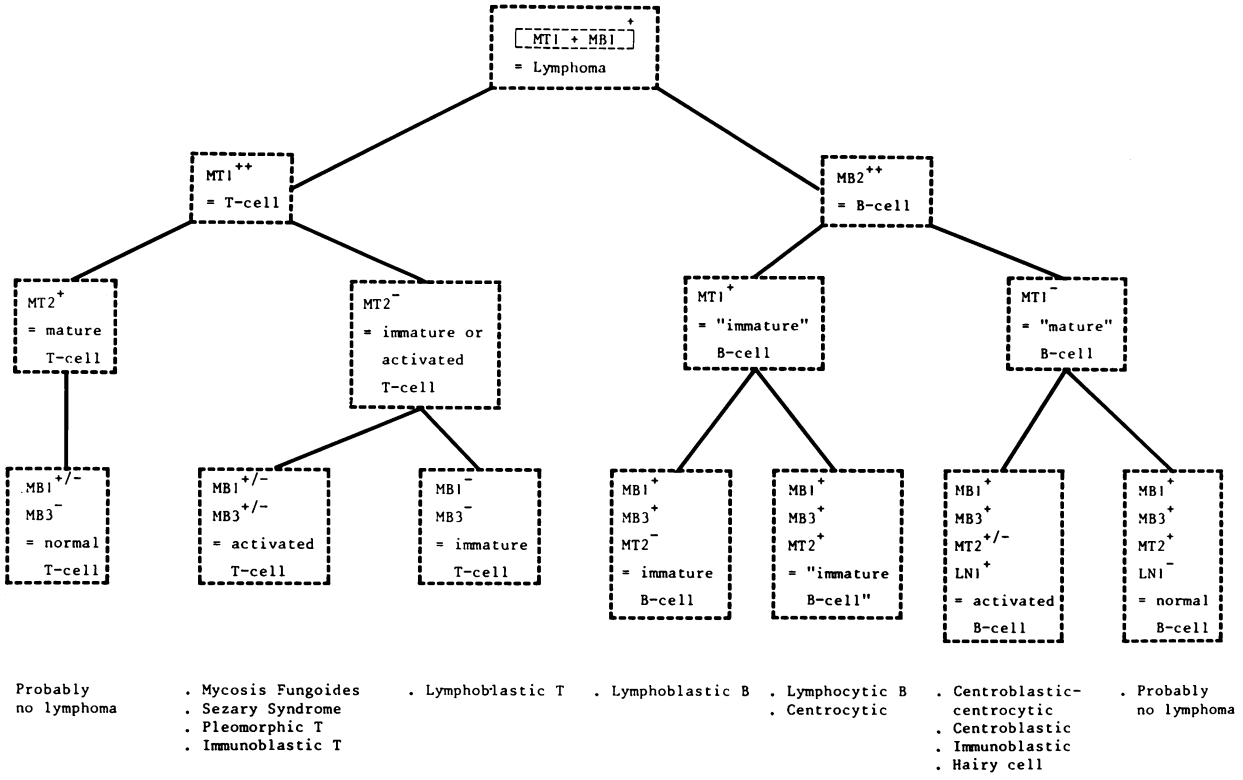

Figure 8—Flow diagram illustrating the possible use of the paraffin-reactive anti-leukocyte monoclonals in the immunophenotyping of lymphomas.

In Hodgkin's disease, MB2 was reactive with L&H type Reed–Sternberg cells of the lymphocyte predominance subtype of Hodgkin's disease. This is in agreement with other studies indicating a B-cell origin of these cells.[20,21]

Antibody MB3 has a staining pattern that is identical to that of LN2[5]; both antibodies identify cytoplasmic antigens in the same cells also reactive with anti-HLA Class II antibodies, and both are reactive with a 31-kd antigen. Therefore, one may speculate that these antibodies recognize an antigen associated with HLA Class II expression, probably the so-called invariant chain, described to be restricted to the cytoplasm of HLA Class II-positive cells.[22]

In conclusion, the antibodies described in this study are reactive with different leukocyte antigens preserved in paraffin tissue sections. They promise to be of value in the study and differential diagnosis of disorders of the lymphoid system. A possible flow diagram for the use of these reagents in the immunophenotyping of lymphomas is given in Figure 8.

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