American Journal of Pathology, Vol. 129, No. 1, October 1987

Immunophenotyping of Non-Hodgkin's Lymphomas Using a Panel of Antibodies on Paraffin-Embedded Tissues

FREDERICK R. DAVEY, KEVIN C. GATTER, ELISABETH RALFKIAER, KAREN A. F. PULFORD, G. W. KRISSANSEN and DAVID Y. MASON

From SUNY Health Science Center at Syracuse, Syracuse, New York; Nuffield Department of Pathology, John Radcliffe Hospital, Oxford, England; ICRF Laboratories, Lincoln's Inn Fields, London, England; and the Histopathology Department, Finseninstitutet, Copenhagen, Denmark

The use of monoclonal and polyclonal antibodies for the immunophenotyping of non-Hodgkin's lymphomas in paraffin-embedded tissue has been limited by the fact that most antigens on lymphoid cells are denatured by histologic fixation, dehydration, and embedment. In this article the authors have analyzed a small panel of antibodies which represent exceptions to this rule, in that they identify denaturation-resistant determinants on leukocyte antigens in paraffin-embedded tissue. Monoclonal antibodies L27 and 4KB5 label preferentially B cells, monoclonal antibody UCHL1 stains predominantly T cells, and monoclonal antibody MAC 387 reacts with granulocytes and some macrophages. A polyclonal antiserum raised against purified CD3 (T3) antigen, a T-cell-specific molecule, was also employed. This antibody panel was used to immunophenotype routinely processed tissue biopsy specimens from 61 non-Hodgkin's lymphomas (all of which had been previously phenotyped in cryostat sections). The lineage of the neoplastic cells was correctly identified in 32 of 34 (94%) cases of B-cell lymphoma, in 19 of 19 (100%) cases of T-cell neoplasm, and in 2 of 4 (50%) cases of histiocytic malignancy. It is concluded that this combination of antibodies is helpful in immunophenotyping non-Hodgkin's lymphomas when only paraffin-embedded tissue sections are available, although additional reagents of higher specificity are required to improve the identification of lymphomas. (Am J Pathol 1987, 129:54–63)

CONSIDERABLE advances have been made in classifying non-Hodgkin's lymphomas according to neoplastic cell lineage.[1–5] This has been facilitated recently by immunohistologic staining of cryostat sections.[6–8] However, the application of this technique to routinely processed, paraffin-embedded tissue sections has been hindered by the inability of most monoclonal and polyclonal antibodies against human leukocyte antigens to give satisfactory immunostaining on this type of material.[9]

One exception to this rule is represented by monoclonal antibodies that react with leukocyte common antigen in formalin-fixed and paraffin-embedded sections. Such reagents have been of great assistance in the distinction of lymphoma from other poorly differentiated tumors.[10] Similarly, monoclonal antibodies against the CD15 antigen (X hapten) have achieved widespread use in the recognition of Reed–Sternberg cells and the diagnosis of Hodgkin's disease.[11,12]

In contrast, monoclonal antibodies recognizing antigens associated with B cells, T cells, and macrophages in routinely processed pathologic material have been more difficult to identify, and only recently have a few reagents of this sort been reported.[13–17] In the present study we assessed the usefulness of a panel of such monoclonal antibodies in the immunophenotyping non-Hodgkin's lymphomas in routinely processed tissue sections. A polyclonal antiserum specific for the CD3 (T3) molecule, previously shown to be of value in identifying routinely processed T-cell neoplasms, was also assessed.[18]

Accepted for publication May 15, 1987.

Address reprint requests to F. R. Davey, MD, Department of Pathology, SUNY Health Science Center at Syracuse, 750 East Adams Street, Syracuse, NY 13210.

Materials and Methods

Tissue Samples

Paraffin-embedded sections of formalin-fixed tissues from 61 cases of non-Hodgkin's lymphomas were obtained, together with small numbers of samples from tonsils showing reactive lymphoid hyperplasia and 3 cases of tissues containing invasive carcinomas (adenocarcinoma of the breast, lung, and colon). The diagnoses of non-Hodgkin's lymphomas and categorization according to the Kiel classification were made on hematoxylin and eosin-stained sections.[19]

Fresh tissue samples had been obtained from all cases at the time of surgical resection, snap-frozen immediately in liquid nitrogen, and stored at −70 C. The remainder of the tissue was fixed and processed according to standard methods in a routine busy histopathology laboratory. As a result, the fixation times varied somewhat from specimen to specimen and day to day. To determine the usefulness of other fixatives, we placed portions of tonsil in one of the following solutions: Bouin's solution, B5, neutral-buffered formalin, acidic formalin, unbuffered formalin, Carnoy's solution, and Zenker's solution. Following adequate fixation, tissues were processed according to standard methods and stained with monoclonal antibodies.

Monoclonal Antibodies

The antibodies used on paraffin sections in this study are briefly described in Table 1. The monoclonal antibodies used to immunophenotype cases of non-Hodgkin's lymphoma in cryostat tissue sections have been previously described: antibodies against IgM, IgD, kappa, lambda, CD20, and CD22 for identifying B cells; monoclonal antibodies to CD2, CD3, CD4, and CD8, polyclonal antibody to terminal deoxynucleotidyl transferase (TdT) for labeling T cells; and monoclonal antibodies against the Y1/82A, EBM11, and KB90 determinants for macrophages.[8,23,24]

Immunohistologic Phenotyping

Paraffin sections (at 3–4 μ) and cryostat sections (at 6 μ) were stained with either an immunoperoxidase or the alkaline phosphatase anti-alkaline phosphatase (APAAP) method.[18,25]

To determine whether treatment of paraffin embedded tissue sections with dilute concentrations of trypsin would enhance the reactivity of monoclonal antibodies, routinely processed tissue sections of human tonsil were incubated for 10, 20, 30, and 40 minutes with 0.1% trypsin in 0.1% $CaCl_2$ solution (pH 7.8) prior to immunostaining.[26]

Results

Reactivity of Antibodies With Non-Neoplastic Tissues

Monoclonal antibodies L27 and 4KB5 stained B-cell areas of lymph node, spleen, ileum, colon, tonsil, and thymus (from a patient with myasthenia gravis). Germinal center cells stained strongly with monoclonal antibody L27 and moderately to weakly with monoclonal antibody 4KB5. In contrast, lymphocytes of the mantle zone reacted strongly with monoclonal antibody 4KB5 and moderately with monoclonal antibody L27. Monoclonal antibodies L27 and 4KB5 stained occasional lymphocytes in the dermis, interstitium of the lung, and sinusoids and

Table 1—Antibodies Used for Immunophenotyping Non-Hodgkin's Lymphoma in Paraffin-Embedded Tissues

Antibody	Type of antibody	Molecular weight of antibody	Cluster designation	Reactivity with normal cells	Reference
L27	Monoclonal	35 kD	CD20	Pan-peripheral B cells	16,20
4KB5	Monoclonal	170 kD	CD45	Germinal center and mantle zone B cells, subset T cells and monocytes	15
UCHL1	Monoclonal	180 kD	CD45R	T cells, subset of B cells, macrophages, mature granulocytes	13,14, 21
CD3	Polyclonal	25–29 kD	CD3	T lymphocytes, squamous epithelium (weakly)	22
MAC 387	Monoclonal	No Data	—	Granulocytes, subset of macrophages	17

periportal areas of the liver. However, plasma cells did not stain with either monoclonal antibody L27 or 4KB5.

Monoclonal antibody UCHL1 stained intensely T-cell areas of the lymph node, spleen, ileum, colon, thymus, and tonsil and also rare lymphocytes in the dermis, interstitium of the lung, and the periportal areas of the liver. In addition, monoclonal antibody UCHL1 reacted weakly with epithelial cells and other mononuclear cells of tonsil. B-cell areas were generally unreactive with monoclonal antibody UCHL1.

The anti-CD3 (T3) serum, as described in another publication from this laboratory,[18] stained T lymphocytes in thymic and peripheral lymphoid tissue and was unreactive with other cell types with the exception of weak staining of squamous epithelium.

Monoclonal antibody MAC 387 reacted with granulocytes and a subpopulation of macrophages located in the lymph node, spleen, ileum, colon, thymus, skin, and lung. It did not react with dendritic reticulum cells, tingible body or other germinal center macrophages, Langerhans cells of the skin, or Kupffer cells in the liver. In addition, monoclonal antibody MAC 387 reacted with stratified squamous epithelium and with occasional epithelial cells in skin appendages. Staining in the skin was strongest in areas overlying tumors or inflammatory lesions and was weak or negative on normal skin.

Reactivity of Antibodies with Non-Hodgkin's Lymphomas

The phenotypes (as determined by immunostaining in cryostat sections) and the histopathologic diagnosis of the 61 cases of non-Hodgkin's lymphomas investigated in this study are presented on Table 2, together with the staining patterns obtained on paraffin sections. Most of the known histologic variants of non-Hodgkin's lymphomas were represented in this study.

The neoplastic cells in paraffin-embedded sections from 32 of 34 (94%) B-cell neoplasms were stained by L27 and/or 4KB5. Monoclonal antibody L27 was positive in 29 cases, and monoclonal antibody 4KB5 positive in 30 cases. However, monoclonal antibody L27 reacted with two cases of centroblastic lymphoma not marked by monoclonal antibody 4KB5,

Table 2—Immunohistologic Labeling of 61 Cases of Non-Hodgkin's Lymphomas Stained in Paraffin Sections

Diagnosis	No. of cases	B-cell markers		T-cell markers		Macrophage marker
		L27	4KB5	UCHL1	CD3	Mac387
B-cell lymphomas (34 cases)						
Centroblastic	8	+	+	−	−	−
	2	+	−	−	−	−
Immunoblastic	1	−	−	+	−	−
Lymphoblastic	1	+	+	−	−	−
	2	−	+	−	−	−
Centroblastic-centrocytic	11	+	+	−	−	−
Centrocytic	4	+	+	−	−	−
Chronic lymphocytic leukemia	1	+	+	−	−	−
	1	−	+	−	−	−
Immunocytoma (lymphoplasmacytic)	1	+	+	−	−	−
Plasmacytoma (multiple myeloma)	1	−	−	−	−	−
Hairy-cell leukemia	1	+	+	−	−	−
T-cell lymphomas (19 cases)						
Pleomorphic T cell	7	−	−	+	+	−
	3	−	−	−	+	−
Malignant histiocytosis of intestine (MHI)	1	−	−	+	−	−
Cutaneous T cell	1	−	−	+	+	−
Lymphoblastic	3	−	−	+	+	−
	2	−	+	−	+	−
	2	−	−	−	+	−
Macrophage/monocyte malignancies (4 cases)						
Malignant histiocytes	1	−	−	−	−	+
	1	−	−	+	−	+
	2	−	−	−	−	−
Unclassified lymphomas (4 cases)						
Pleomorphic	1	−	−	−	−	−
Pleomorphic	1	−	−	+	+	−
Immunoblastic	1	−	−	−	−	−
Cutaneous	1	−	−	−	−	−

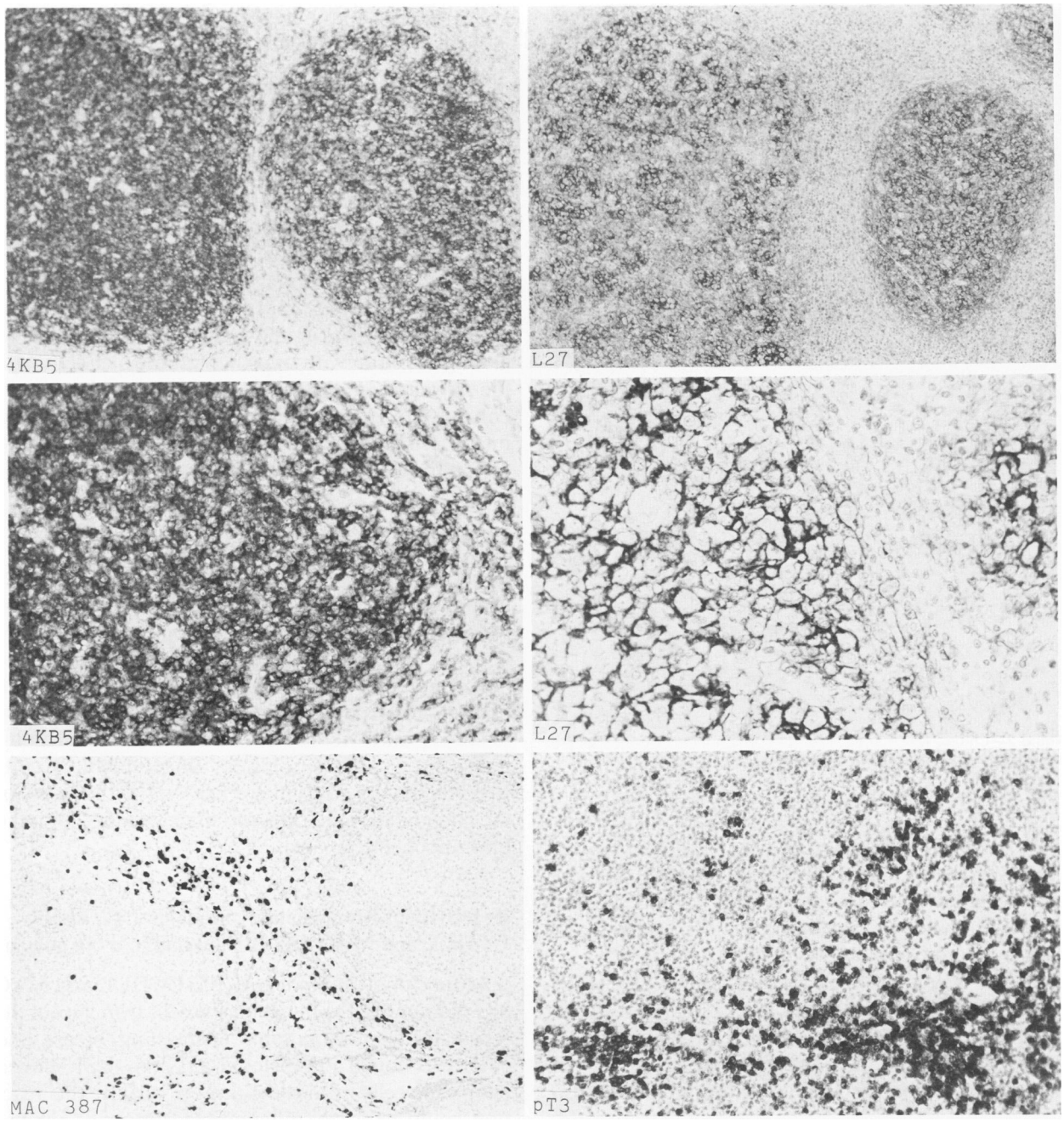

Figure 1—Centroblastic/centrocytic lymphoma in which the neoplastic germinal centers are positively stained by the anti-B cell monoclonal antibodies 4KB5 and L27. Surrounding macrophages and T cells are stained by monoclonal antibody MAC 387 and polyclonal anti-CD3 (pT3) serum, respectively, but the lymphoma cells are negative.

whereas monoclonal antibody 4KB5 reacted with 2 cases of lymphoblastic lymphoma and with 1 case of chronic lymphocytic leukemia which were unstained with monoclonal antibody L27. In the cases positive for both antibodies, monoclonal antibody L27 gave a more intense staining of follicular center cell lymphomas than monoclonal antibody 4KB5; whereas monoclonal antibody 4KB5 reacted more strongly with chronic lymphocytic leukemia, lymphoblastic lymphoma, and lymphoplasmacytic lymphoma.

In 1 case of B-cell lymphoma, none of the monoclonal antibodies reacted with the neoplastic cells. In 1 other case, clearly of B-cell type when stained on cryostat section, only monoclonal antibody UCHL1 reacted with the malignant cells.

Monoclonal antibody UCHL1 stained neoplastic

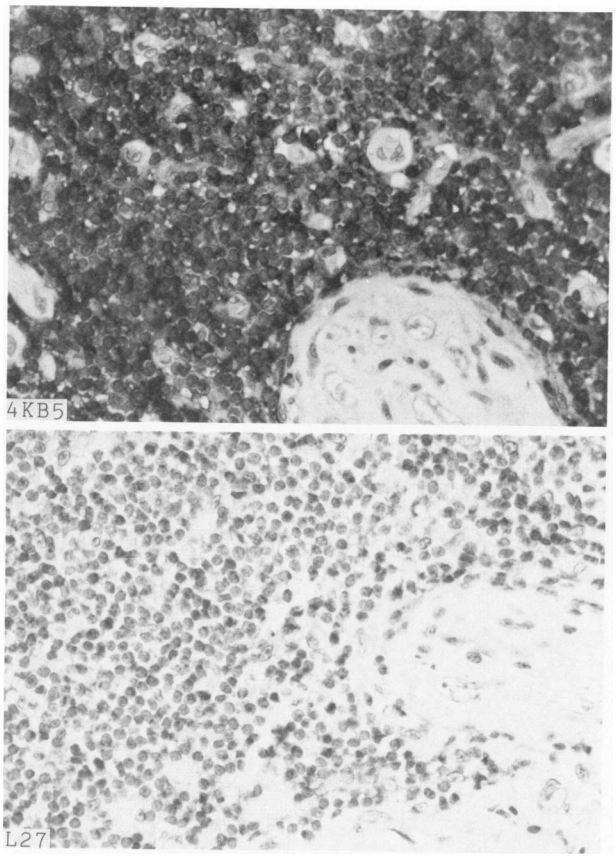

Figure 2—Chronic lymphocytic leukemia in which the malignant cells are labeled by monoclonal antibody 4KB5 not by L27.

cells in 12 of 19 cases (63%) of T-cell lymphoma. However, among the 7 UCHL1-negative cases, 5 (26%) were unreactive with any of the monoclonal antibodies; and the remaining 2 cases were stained only by monoclonal antibody 4KB5.

The polyclonal antiserum specific for CD3 (T3) antigen reacted with 18 out of 19 (95%) cases of T-cell lymphoma and was unreactive with all other types of lymphoma. The single unreactive case was that of malignant histiocytosis of the intestine which also lacked the T3 antigen on frozen tissue examination, but possessed the UCHL1 determinants.

In 2 of the 4 (50%) histiocytic lesions, monoclonal antibody MAC 387 stained a subpopulation of malignant cells and in 1 of these cases, monoclonal antibody UCHL1 also stained the neoplastic histiocytes.

In 4 cases of non-Hodgkin's lymphoma, the lineage of the neoplastic cells could not be determined on frozen tissue sections despite the use of an extensive panel of monoclonal antibodies. When the panel of four monoclonal antibodies was applied to the fixed sections from these cases, monoclonal antibody UCHL1 and polyclonal antibody CD3 reacted in 1 case; whereas the remaining 3 cases were negative with all four antibodies.

Trypsinization of Paraffin-Embedded Tissues

The intensity of the staining reaction with monoclonal antibodies L27 and 4KB5 was diminished when formalin-fixed paraffin sections were previously exposed to trypsin. In contrast, the staining reaction with monoclonal antibody UCHL1 was enhanced, but B-cell areas also showed reactivity. Staining of granulocytes, macrophages, and epithelium with MAC 387 was greatly enhanced in intensity following digestion with trypsin. Staining of T cells with the polyclonal anti-CD3 (T3) serum was also greatly enhanced by exposure to trypsin. In general, the optimal effects of trypsin were observed within the first 10–20 minutes of incubation.

Effects of Different Fixatives

The effects of different fixatives on the reactivity of monoclonal antibodies L27, 4KB5, UCHL1, and MAC 387 were determined. Although slight variations occurred among the monoclonal antibodies, the best results were generally observed with formalin, neutral buffered formalin, and acidic formalin. Intermediate results were observed with B5, Bouin's solution, and Carnoy's solution; unacceptable results were obtained with Zenker's fixative. With the use of the latter fixative, all cells and tissues stained with the immunochemical label.

Reactivity of Monoclonal Antibodies L27, 4KB5, UCHL1, and MAC 387 in Nonlymphoid Neoplasms

Monoclonal antibodies 4KB5, UCHL1, and MAC 387 did not react with neoplastic cells from any of the 3 cases of adenocarcinoma of the lung, breast, and colon. Although monoclonal antibody L27 stained neoplastic cells from these tumors weakly, this reaction could be easily differentiated from the intense staining observed in adjacent lymphocytes and in neoplastic lymphocytes from cases of non-Hodgkin's lymphoma.

Discussion

In this study, a panel of antibodies, four monoclonal, one polyclonal, against leukocyte-associated antigens was examined for their reactivity on routinely processed tissue sections. Two antibodies reacted predominantly with B cells, two with T cells, and the fifth with granulocytes and macrophages. The reactivity of

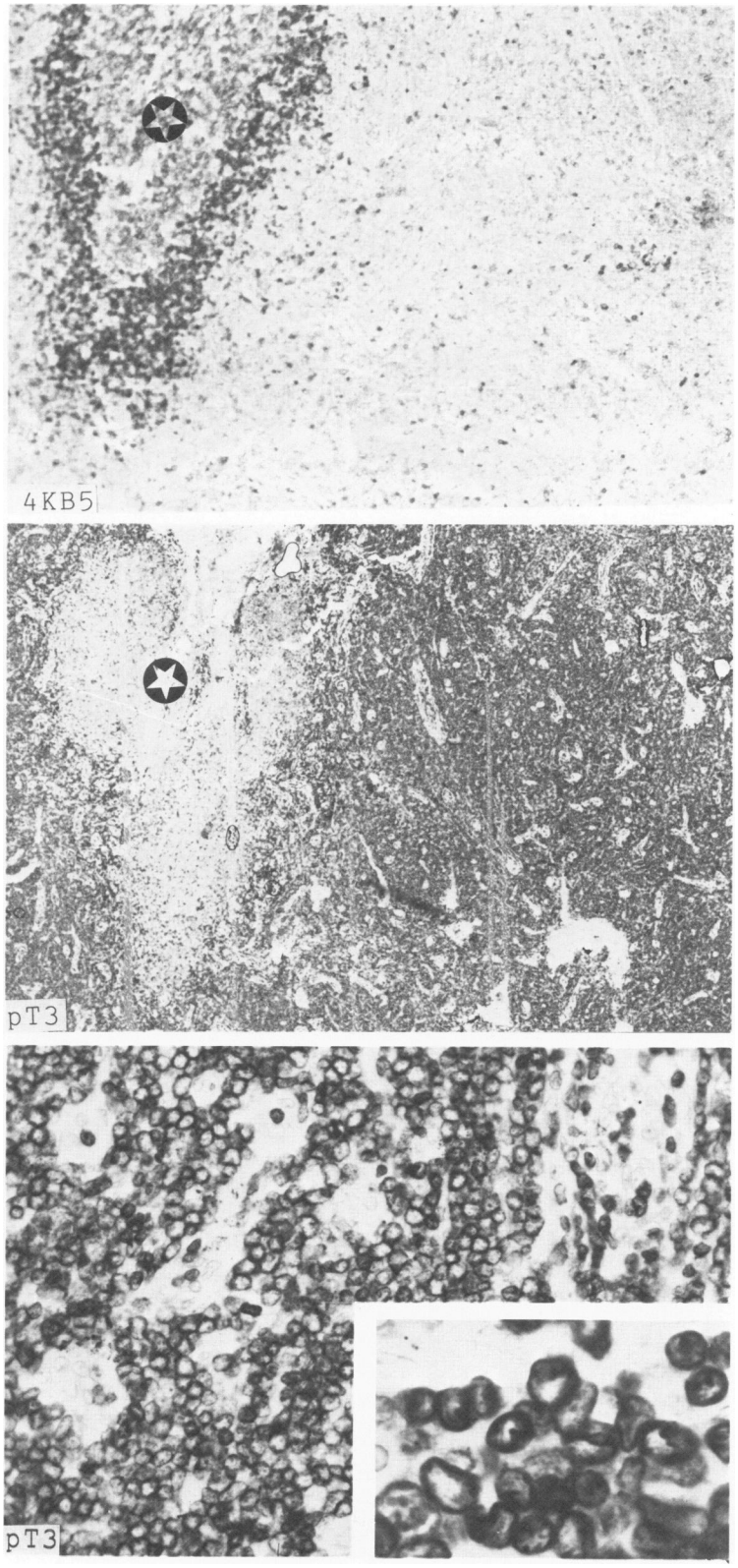

Figure 3—T-lymphoblastic lymphoma showing staining of the neoplastic cells by the polyclonal anti-CD3 (pT3) serum. High-power magnification of the neoplastic cells (**inset** at bottom) shows the strong cytoplasmic expression of the CD3 antigen. The tumor cells are negative with monoclonal antibody 4KB5, which outlines a reactive follicle (*) unstained by polyclonal anti-CD3 (pT3).

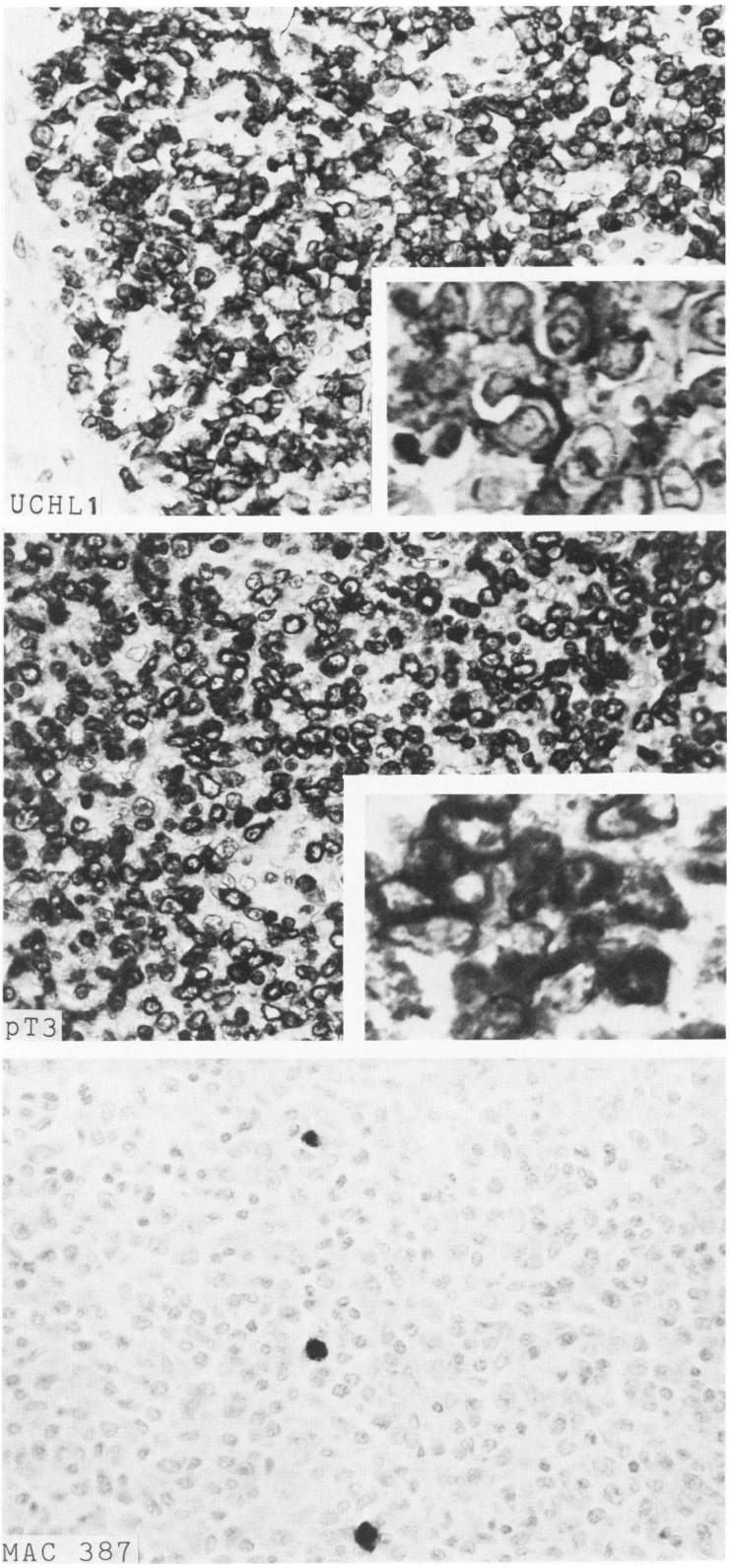

Figure 4—Pleomorphic T-cell lymphoma stained by monoclonal antibody UCHL1 and polyclonal anti-CD3 serum (pT3). High-power **insets** show the predominant membrane staining with anti-UCHL1 and the cytoplasmic staining with anti-CD3. The tumor cells were unstained by the monoclonal anti-B-cell antibody 4KB5 (not illustrated).

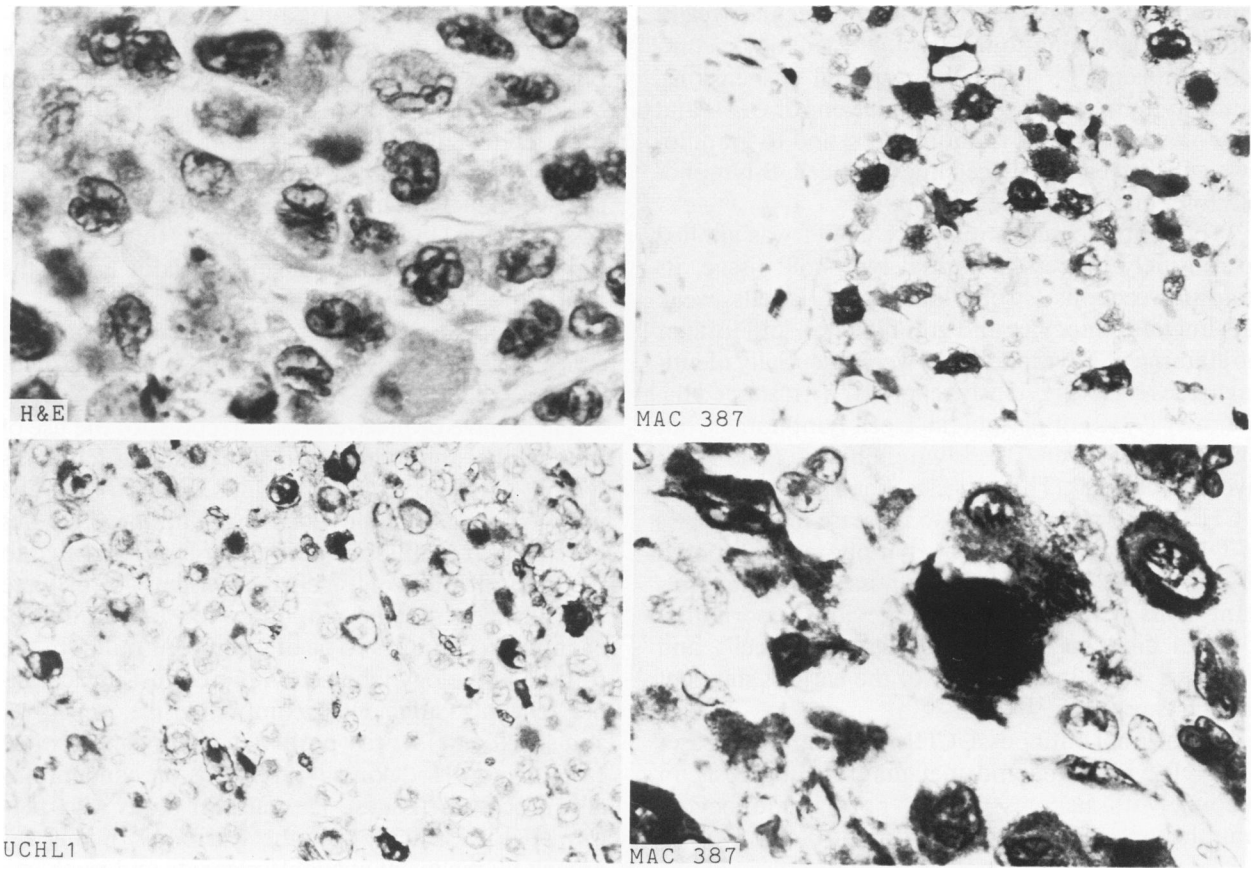

Figure 5—A case of malignant histiocytosis involving the testis in which the large pelomorphic cells seen in the hematoxylin and eosin stain are labeled by the monoclonal antibody MAC 387. The higher power view below shows that the labeled cells are clearly neoplastic. In this case some of the malignant cells are also positive with monoclonal antibody UCHL1.

the antibodies was assessed first on nonneoplastic tissue and then on a wide range of neoplastic lymphoid samples.

Previous studies have shown that monoclonal antibody L27 reacts with the CD20 B-cell-associated antigen,[20] a molecule found on most B cells in the peripheral blood and peripheral lymphoid tissues, but only on a limited range of B cells at later stages of maturation.[16,20] In the current study, monoclonal antibody L27 stained germinal-center B cells more intensely than mantle-zone lymphocytes and (among non-Hodgkin's lymphomas) reacted with neoplastic cells from 85% of the cases of B-cell lymphoma. In contrast, none of the cases of T-cell lymphoma or malignant histiocytosis were reactive. It is of interest that antibody L27 tended to react more intensely with neoplastic cells from follicular center cell lymphomas than with those from lymphoblastic lymphomas, immunoplasmacytic lymphomas, and multiple myeloma.

Monoclonal antibody 4KB5 recognizes a high-molecular-weight form of the leukocyte common antigen family (CD45R) which tends to be preferentially expressed by B cells.[15,20,27] In fresh tissues or cell suspensions, monoclonal antibody 4KB5 reacts with peripheral blood B cells as well as a subpopulation of T cells and monocytes. However, in paraffin-embedded tissue sections, the antibody stains predominantly lymphocytes of B-cell areas, particularly mantle-zone lymphocytes.

In the current study, monoclonal antibody 4KB5 stained the majority (88%) of B-cell lymphomas but also reacted with 2 cases of T-cell lymphoblastic lymphomas (out of 19 tested), which suggested that in paraffin-embedded sections it recognizes early T cells (thymocytes).

When both monoclonal antibodies L27 and 4KB5 were used, almost all (94%) B-cell lymphomas were identified. The exceptions (immunoblastic lymphoma and multiple myeloma) are not unexpected, because the CD20 and CD45R antigens, in common with other B-cell-associated antigens,[15,16,27] are not present on normal plasma cells.

Monoclonal antibody UCHL1 appears (in com-

mon with 4KB5) to recognize a low-molecular-weight molecule belonging to the family of leukocyte common antigens[13,14,21] and in fresh cell suspensions, binds to thymocytes, to subpopulations of CD4- and CD8-positive T cells, to monocytes, and to granulocytes. It also reacts with cell lines of T-cell, B-lymphoblastoid, and plasma-cell origin.[13]

When monoclonal antibody UCHL1 was applied to routinely processed normal lymphoid tissue, its staining reactions (strong labeling of T cells, weak labeling of monocytes and granulocytes, and little or no staining of B lymphocytes) were essentially identical to the results previously reported.[13,14] It stained the majority (9/12) of peripheral T-cell lymphomas, but only three of seven T-cell lymphoblastic (TdT-positive) lymphomas. This suggests that expression of the UCHL1 determinant tends to increase in later stages of T-cell maturation, and that lymphomas composed of progenitor T cells may not possess this determinant. This is in keeping with previous reports that UCHL1 binds to memory T cells, thymic cells, and mature T cells, but not cells of the earliest stages of thymic expression.[13,14]

Monoclonal antibody UCHL1 stained single cases of B-cell lymphoma and malignant histiocytosis, in keeping with its presence on normal monocytes, granulocytes, and a subpopulation of B cells.[13,14]

The reactions of the polyclonal antiserum against the CD3 (T3) antigen are of particular interest because this molecule, unlike other molecules studied in paraffin-embedded lymphoma tissues in this and other reports, is known to be restricted to a single white cell lineage. In keeping with this property, the reactions of this antibody were highly selective for T-cell neoplasms. Because this polyclonal antibody was prepared by immunizing rats with a purified preparation of the T3 molecule, the strategy of raising monoclonal antibodies against purified preparations of well-characterized T-cell, B-cell, and macrophage-associated antigens is likely to prove increasingly fruitful in the future.

In 1 case, monoclonal antibody UCHL1 and polyclonal antibody CD3 reacted with neoplastic cells, the lineage of which could not be identified after immunophenotyping on frozen tissue sections. Because no other antibodies reacted with this case, the neoplastic cells are probably derived from T cells.

Monoclonal antibody MAC 387 binds to a cytoplasmic determinant present in cells of the monocyte/macrophage series.[17] Although a specific cluster designation has not been assigned to this determinant, it was classified in the Third Workshop in a group together with other antibodies recognizing monocytes, macrophages, and granulocytes.[28] In paraffin-embedded tissues, the antibody stained predominantly granulocytes and a subpopulation of macrophages. Although it stained the squamous epithelium of skin and tonsil biopsies, it did not react with malignant epithelial cells in adenocarcinoma biopsies. Monoclonal antibody MAC 387 stained 2 of the 4 cases of malignant histiocytosis and none of the other cases of non-Hodgkin's lymphoma. However, a definitive judgement of its diagnostic value (given the rarity and difficulties in diagnosing malignancies of true histiocytic origin) requires the analysis of a large series of fully phenotyped putative histiocytic neoplasms.

There have been several other reports of the use of monoclonal antibodies in the study of non-Hodgkin's lymphomas in paraffin-embedded tissues.[13,29-33] The monoclonal antibodies employed in these studies have proved useful, but were either largely limited to the identification of B-cell lymphomas[30,31] or were not lineage-restricted. In order for immunohistochemical techniques to be of significant utility in the routine pathology laboratory, there must be accessible reagents sufficiently comprehensive, sensitive, and specific to identify positively the lineage of most cases of non-Hodgkin's lymphomas on conventionally processed tissues. The current study was performed for evaluation of the feasibility of this approach by means of available reagents and methods.

This panel of antibodies has proven to be particularly helpful in the immunophenotyping of non-Hodgkin's lymphomas in routinely processed tissues. In most cases, these antibodies accurately reflected the immunophenotype provided by frozen tissue examination. Because of the superior morphology of paraffin-embedded tissues, the ease of performing immunoenzymatic studies and the precision of interpreting results, we believe that this panel of antibodies could be very useful to the pathologist in the immunologic analysis of non-Hodgkin's lymphomas. However, there is a continued need for additional monoclonal antibodies which are lineage-specific and will stain paraffin-embedded tissues, particularly for the identification of neoplastic T cells and histiocytes. In the former type of neoplasm, a monoclonal antibody duplicating the specificity of the polyclonal anti-CD3 serum would be of considerable value.

References

1. Lennert K, Mohri N, Stein H, Kaiserling E: The histopathology of malignant lymphoma. Br J Haematol 1975, 31(Suppl):193–203
2. Lukes RJ, Parker J, Taylor CR, Tindle BH, Cramer AD, Lincoln TL: Immunologic approach to non-Hodgkin's lymphomas and related leukemias: Analysis

of results of multiparameter studies of 425 cases. Seminars Hematol 1978, 15:322–351
3. Stein H, Lennert K, Feller AC, Mason DY: Immunohistological analysis of human lymphoma: Correlation of histological and immunological categories. Adv Cancer Res 1984, 42:67–147
4. Borowitz MJ, Croker BP, Metzgar RS: Comparison of histological and immunologic heterogeneity of non-Hodgkin's lymphomas. Am J Pathol 1981, 105:97–106
5. Knowles DM II, Halper JP: Human T-cell malignancies: Correlative clinical, histopathologic, immunologic and cytochemical analysis of 23 cases. Am J Pathol 1982, 106:187–203
6. Borowitz MJ, Bousvaros A, Brynes RK, Cousar JB, Crissman JD, Whitcomb CC, Kerns BJ, Byrne GE: Monoclonal antibody phenotyping of B-cell non-Hodgkin's lymphoma. Am J Pathol 1985, 121:514–521
7. Shimizu M, Miura M, Kitahara M, Katayama I: Non-Hodgkin's lymphomas: Analysis of 109 Japanese cases with the use of LSGJ classification. Am J Pathol 1982, 106:30–39
8. Stein H, Mason DY: Immunological analysis of tissue sections in diagnosis of lymphoma. Recent Advances in Haematology. Edited AV Hoffbrand. London, Churchill Livingstone, 1985, 4:127–169
9. Gatter KC, Mason DY: The use of monoclonal antibodies in the histopathologic diagnosis of human malignancies, Poorly Differentiated Neoplasms and Tumors of Unknown Origin. Edited by MF Fer, FA Greco, RK Oldham. Orlando, Grune & Stratton 1986, p 399
10. Warnke RA, Gatter KC, Falini B, Hildreth P, Woolston RE, Pulford K, Cordell JL, Cohen B, DeWolf-Peeters C, Mason DY: Diagnosis of human lymphoma with monoclonal antileukocyte antibody. N Engl J Med 1983, 309:1275–1281
11. Stein H, Gerdes J, Schwab U, Lemke H, Mason DY, Ziegler A, et al: Identification of Hodgkin and Sternberg-Reed cell as a unique cell type derived from a newly-detected small-cell population. Int J Cancer 1982, 30:445–459
12. Hsu S, Jaffe E: Leu M1 and peanut agglutinin stain the neoplastic cells of Hodgkin's disease. Am J Clin Pathol 1984, 82:29–32
13. Norton AJ, Ramsay AD, Smith SH, Beverley PCL, Isaacson PG: Monoclonal antibody (UCHL1) that recognizes normal and neoplastic T cells in routinely fixed tissues. J Clin Pathol 1986, 39:399–405
14. Smith SH, Brown MH, Rowe D, Callard RE, Beverley PCL: Functional subsets of human helper-inducer cells defined by a new monoclonal antibody, UCHL1. Immunol 1986, 58:63–70
15. Pulford KAF, Falini B, Heryet A, Gatter KC, Mason DY: A new monoclonal anti-B cell antibody for routine diagnosis of lymphoid tissue biopsies, Leucocyte Typing III. Edited by A McMichael et al. Oxford University Press (In press)
16. Ishii Y, Takami T, Yuasa H, Takei T, Kikuchi K: Two distinct antigen systems in human B lymphocytes: Identification of cell surface and intracellular antigens using monoclonal antibodies. Clin Exp Immunol 1984, 56:183–192
17. Flavell DJ, Jones DB, Wright DH: Identification of tissue histiocytes on paraffin sections by a new monoclonal antibody. J Histochem Cytochem (In press)
18. Mason DY, Krissansen GW, Davey FR, Crumpton MJ, Gatter KC: Antisera against denaturation-resistant epitopes on the T3 (CD3) T cell antigen can detect reactive and neoplastic T cells in paraffin embedded biopsies. (Manuscript submitted)
19. Gerard-Marchant R, Hamlin I, Lennert K, Rilke F, Stansfeld AG, van Unnik JAM: Classification of non-Hodgkin's lymphoma. Lancet 1974, 2:406–408
20. Ling NR, MacLennan ICM, Mason DY: Analysis of the B cell and plasma cell panel.[15]
21. Cobbold S, Hale G, Waldmann H: Summary of studies performed on the non-lineage, LFA-1 family and leucocyte common antigen panels of antibodies.[15]
22. Krissansen GW, Verbi W, Totty NF, Crumpton MJ: Purification and identification by amino acid sequence analysis of the subunits of the CD3 antigen of human tonsil T-lymphocytes. (Manuscript submitted)
23. Franklin WA, Mason DY, Pulford K, Falini B, Bliss E, Gatter KC, Stein H, Clarke LC, McGee, JO'D: Immunohistological analysis of human mononuclear phagocytes and dendritic cells by using monoclonal antibodies. Lab Invest 1986, 54:322–335
24. Stass SA, Schumacher HR, Keneklis TP, Bollum FJ: Terminal deoxynucleotidyl transferase immunofluorescence of bone marrow smears. Am J Clin Pathol 1979, 72:898–903
25. Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz Z, MacDonald S, Pulford KAF, Stein H, Mason DY: Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). J Histochem Cytochem 1984, 32:219–229
26. Mepham BL, Frater W, Mitchell BS: The use of proteolytic enzymes to improve immunoglobulin staining by the PAP technique. Histochem J 1979, 11:345–357
27. Dalchau R, Fabre JW: Identification with a monoclonal antibody of predominantly B lymphocyte-specific determinants of human leucocyte common antigen: Evidence for structural and possible functional diversity of the human leukocyte common molecule. J Exp Med 1981, 153:753–765
28. Hogg N, Horton MA: Joint report of the myeloid section of the Third International Workshop on Human Leucocyte Differentiation Antigens.[15]
29. West KP, Warford A, Fray I, Allen M, Campbell AC, Lauder I: The demonstration of B-cell, T-cell and myeloid antigens in paraffin sections. J Pathol 1986, 150:89–101
30. Epstein AL, Marder RJ, Winter JN, Fox RI: Two new monoclonal antibodies (LN-1, LN-2) reactive in B5 formalin-fixed, paraffin embedded tissues with follicular center and mantle zone human B lymphocytes and derived tumors. J Immunol 1984, 133:1028–1036
31. Marder RJ, Variakojis D, Silve J, Epstein AL: Immunohistochemical analysis of human lymphomas with monoclonal antibodies to B cell and Ia antigens reactive in paraffin sections. Lab Invest 1985, 52:497–504
32. Poppema S, Hollema H, Visser L, Vos H: Monoclonal antibodies (MT1, MT2, MB1, MB2, MB3) reactive with lymphocyte subsets in paraffin embedded tissue sections. Am J Pathol 1987, 127:418–429
33. Epenetos AA, Bobrow LG, Adams TE, Collins CM, Isaacson PG, Bodmer WF: A monoclonal antibody that detects HLA-D region antigen in routinely fixed, wax embedded sections of normal and neoplastic lymphoid tissues. J Clin Pathol 1985, 38:12–17