American Journal of Pathology, Vol. 135, No. 6, December 1989

Diagnosis of Myelomonocytic and Macrophage Neoplasms in Routinely Processed Tissue Biopsies with Monoclonal Antibody KP1

Roger A. Warnke, Karen A. F. Pulford, Gorm Pallesen, Elisabeth Ralfkiaer, David C. Brown, Kevin C. Gatter, and David Y. Mason

From the Nuffield Department of Pathology, John Radcliffe Hospital, Oxford, England, the Institute of Pathology, Kommunehospitalet, University of Aarhus, and the Histopathology Department, Rigshospitalet, University of Copenhagen, Denmark

A new monoclonal antibody, KP1, against the CD68 antigen, which labels macrophages and other members of the mononuclear phagocyte lineage in routinely processed tissue sections, has been used to stain a range of lymphoid, histiocytic, and myelomonocytic proliferations. All 20 neoplasms of myeloid, myelomonocytic, and presumed macrophage derivation reacted with antibody KP1. None of the 22 cases of T cell neoplasia had positive reactions. Although 14 of 41 B lineage lymphomas and leukaemias were stained by antibody KP1, staining was usually confined to small dots of reactivity, in contrast to the strong and extensive cytoplasmic staining seen in the neoplasms of myeloid and macrophage/monocyte origin. Furthermore, positive B cell neoplasms were almost all small cell proliferations, which are unlikely to be confused with myelomonocytic malignancies. It was concluded that antibody KP1 is a valuable addition to a panel of monoclonal antibodies for phenotyping lymphomas, particularly in routinely fixed tissues. It should assist the pathologist in the recognition of extramedullary presentation of leukaemia, aid in the diagnosis of suspected cases of true histiocytic neoplasia, and allow for quantitation of macrophages infiltrating lymphomas and other solid tumors. (Am J Pathol 1989, 135: 1089–1095)

In recent years many monoclonal antibodies capable of detecting leucocyte-associated antigens in routinely processed pathologic material have been reported. Some of these react with antigens associated with T lymphocytes, eg, antibodies MT1 and UCHL1,[1,2] whereas others detect B cell associated antigens in pathologic material. The most widely studied antibodies are LN1 and LN2, MB1 and MB2, L26 and 4KB5.[1,3–7]

The availability of antibodies that can stain white cell antigens in formalin-fixed tissue has been of great interest to diagnostic pathologists and there have been several reports on the use of these paraffin reactive reagents for the categorization of non-Hodgkin's lymphomas into T and B lineages.[1,8–10] Combinations of these antibodies can also be used to distinguish between benign and malignant lymphoproliferations in routinely processed tissue sections.[11]

A monoclonal antibody capable of detecting macrophages and other members of the mononuclear phagocyte lineage in paraffin-embedded tissue would be a valuable addition to a lymphoma phenotyping panel. It could be used to identify cases of true histiocytic lymphoma, and also extramedullary deposits of monocytic leukaemia.[12] A pan-macrophage antibody of this sort could also be used to detect macrophages infiltrating lymphomas. These cells are sometimes present in large numbers and may complicate the diagnosis and classification of the underlying neoplasm.[13,14]

We recently described a new monoclonal antibody, KP1, that fulfills the criteria for a pan-macrophage reagent when tested on a wide range of routinely processed paraffin-embedded tissues.[15] Biochemical analysis showed that this antibody recognizes a glycoprotein with a molecular weight of approximately 110,000, which is identical to the molecule detected by a number of previously described monoclonal antibodies, eg, Y1/82A, Ki-M6, and EBM11.[16–19] This molecule was recently designated as

This work was supported by a grant from the Leukaemia Research Fund, UK.

Accepted for publication August 3, 1989.

Kevin C. Gatter is a Wellcome Senior Research Fellow in Clinical Science.

Roger A. Warnke was on sabbatical leave from the Department of Pathology, Stanford University School of Medicine, Stanford, California.

Address reprint requests to Dr. David Y. Mason, Department of Haematology, John Radcliffe Hospital, Oxford, OX3 9DU, UK.

the CD68 antigen at the Fourth International Conference on Human Leucocyte Differentiation Antigens (Vienna 1989). However, antibody KP1 differs from these other CD68 reagents in its ability to stain routinely fixed paraffin-embedded tissue.

Here we describe an evaluation of the reactivity of antibody KP1 on a range of lymphoid neoplasms and on histiocytic and myelomonocytic proliferations, with the aim of establishing its value in lymphoma diagnosis.

Materials and Methods

Fixed Tissue Samples

Paraffin-embedded sections of formalin-fixed tissues from 101 hematolymphoid malignancies and related conditions were obtained from the authors' departments. All tissues had been fixed in formol saline with the exception of bone marrow trephines, which were fixed in formol saline containing 1% glacial acetic acid.

All cases had been phenotyped in cryostat sections using panels of monoclonal antibodies against CD antigens and other conventionally recognized leucocyte markers, as described in earlier publications.[20-22] Cases of acute leukaemia were also studied by enzyme cytochemical methods, including staining for myeloperoxidase, Sudan black B, and nonspecific esterase (with and without pretreatment with sodium fluoride).

Blood Smears

Unfixed blood smears from cases of chronic lymphocytic leukaemia of B cell origin were obtained from the Haematology Department of the John Radcliffe Hospital.

Monoclonal Antibody KP1

The antibody KP1 was raised against a lysosomal fraction of human lung macrophages, as described previously.[15] Testing on a range of tissues showed that it labels all types of macrophages (eg, Kupffer cells and alveolar macrophages) and also myeloid precursors in the bone marrow.[15] The antibody was applied to tissue sections as undiluted tissue culture supernatant.

Immunoenzymatic Labeling

Tissue sections were stained using the alkaline phosphatase:anti-alkaline phosphatase (APAAP) method, a three-stage immunoperoxidase technique, or both.[23,24] Trypsinization of sections increased the number of cells stained by KP1 and also enhanced the intensity of the labeling reaction[15]; this was performed in most cases by incubating dewaxed, hydrated sections for 20 to 30 minutes in 0.1% trypsin in 0.1% calcium chloride solution (pH 7.8).[25] When using the immunoperoxidase technique, endogenous peroxidase activity was blocked by incubation with methanol/H_2O_2. Blood smears were stained by the APAAP technique after fixation in acetone:methanol.[24]

Table 1. *Staining of Human Hematolymphoid Neoplasms for CD68 Antigen with Monoclonal Antibody KP1*

Neoplasm	Number of cases positive/number of cases tested†
B lineage proliferations*	
Small lymphocytic/CLL	4/9‡
Follicular, all types (centroblastic/centrocytic)	0/7
Diffuse small cleaved cell (centrocytic)	2/2
Diffuse mixed cell (centroblastic/centrocytic)	0/1
Diffuse large cell (centroblastic)	1/9‡§
Immunoblastic	0/2
Multiple myeloma	0/1
Hairy cell leukemia	7/9‡
T lineage proliferations	
Lymphoblastic	0/2
Mycosis fungoides	0/1
Peripheral T	0/19§
Lymphomatoid papulosis	1/7‡
Leukemias	
Acute lymphoblastic	0/1
Acute myeloblastic	6/6
Acute myelomonocytic	10/10
Chronic myelogenous	2/2
Miscellaneous haematolymphoid proliferations	
Systemic mastocytosis	1/1
True histiocytic lymphoma	1/1
Undefined CD30 (Ki-1/Ber-H2)-positive lymphoma	0/4
Hodgkin's disease	0/7

* B lymphomas are classified according to the Working Formulation, with the Kiel classification in parenthesis.

† Reactivity of neoplastic cells in paraffin section (see Materials and Methods)

‡ Dot-like cytoplasmic staining, fine granular staining, or both.

§ In two B large cell lymphomas and six T large cell lymphomas most of the neoplastic cells expressed CD30 (Ki-1/BerH2).

Results

Myeloid and Monocyte/Macrophage Malignancies

All 20 neoplasms of myeloid, myelomonocytic, and presumed macrophage derivation were labeled by antibody KP1 (Table 1 and Figures 1 and 2). The biopsy specimens

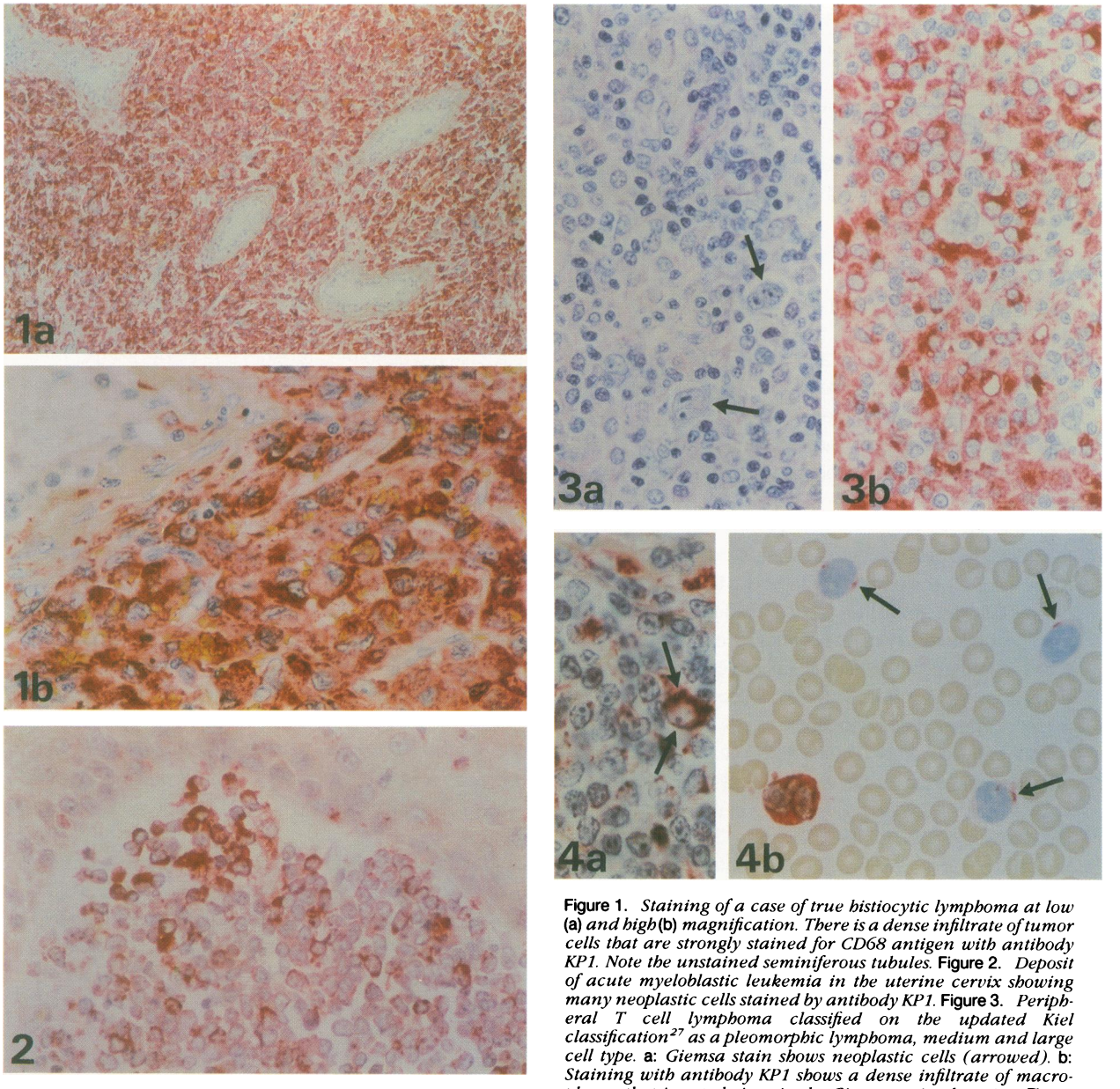

Figure 1. *Staining of a case of true histiocytic lymphoma at low* (a) *and high* (b) *magnification. There is a dense infiltrate of tumor cells that are strongly stained for CD68 antigen with antibody KP1. Note the unstained seminiferous tubules.* **Figure 2.** *Deposit of acute myeloblastic leukemia in the uterine cervix showing many neoplastic cells stained by antibody KP1.* **Figure 3.** *Peripheral T cell lymphoma classified on the updated Kiel classification[27] as a pleomorphic lymphoma, medium and large cell type.* **a:** *Giemsa stain shows neoplastic cells (arrowed).* **b:** *Staining with antibody KP1 shows a dense infiltrate of macrophages that is not obvious in the Giemsa stained section.* **Figure 4.** *Chronic lymphocytic leukemia stained with antibody KP1.* **a:** *Staining of a paraffin-embedded biopsy showing a strongly stained macrophage (arrowed) and also small lymphocytes with fine dot-like deposits of antigen.* **b:** *A blood smear from another case of B cell chronic lymphocytic leukemia shows a strongly stained neutrophil and three lymphocytes containing small intracytoplasmic KP1-positive inclusions (arrowed).*

from ten of the 20 cases were obtained from extramedullary sites including lymph nodes (four), skin (four), cervix (one), and testis (one). All cases showed intense, granular cytoplasmic staining of most of the neoplastic cells with the exception of two cases of acute myeloblastic leukemia in which a subpopulation of neoplastic cells showed faint and/or focal granular cytoplasmic staining in addition to a subpopulation with intense granular staining.

Two cases of myelomonocytic leukemia had initially been diagnosed as T cell lymphomas. One patient presented with a skin tumor and the other underwent lymph node biopsy that showed infiltrates in a T-zone distribution. Although the expression of CD2 and CD4 in both cases was consistent with a T cell lymphoma, the subsequent peripheral blood and bone marrow findings, as well as the clinical evolution, were characteristic of myelomonocytic leukemia.

The one case of true histiocytic lymphoma studied was a testicular mass in a 59-year-old man (Figure 1). Typing in cryostat sections showed previously that the large neoplastic cells expressed α-naphthyl acetate esterase activity and the monocyte/macrophage-associated mark-

ers CD4, CD11c, and CD35. They reacted with the anti-macrophage antibody EBM11, but lacked pan-T and pan-B antigens.[26]

In a case of systemic mastocytosis, KP1 labeled not only the nodular aggregates of oval and spindled cells in the bone marrow infiltrate, but also numerous early myeloid precursors, suggesting an associated myeloproliferative disorder.

Lymphoid and Related Disorders

Antibody KP1 gave strong and uniform staining of non-neoplastic macrophages present in cases of lymphoma and it was noted that the density of macrophage infiltration varied widely, from rare scattered cells in some cases, to numerous macrophages in other biopsies (Figure 3).

In fourteen of 40 B lineage lymphomas and leukemias, the neoplastic cells themselves were stained by antibody KP1 (Table 1). The pattern of staining in these cases was clearly different from the intense granular cytoplasmic staining observed in myelomonocytic cells, because it took the form of localized dots, usually only one per cell, of varying size (Figure 4a). These dots could also be seen in blood smears made from some cases of B cell chronic lymphocytic leukemia (Figure 4b). The only exception was a single case of diffuse large cell lymphoma that showed diffuse fine granular staining in the cytoplasm of the neoplastic cells. Of the 14 cases, 13 were small cell proliferations that one is unlikely to confuse morphologically with myelomonocytic malignancies, ie, four cases of chronic lymphocytic leukemia (CLL), two cases of diffuse small cleaved (centrocytic) lymphoma, and seven cases of hairy cell leukemia.

None of the 22 T lineage lymphomas was stained by antibody KP1. Of the seven examples of lymphomatoid papulosis, one showed weak-to-moderate staining of a number of atypical lymphoid-appearing cells in a pattern similar to that seen in the B large cell lymphoma. None of the cases of Hodgkin's disease or the 12 cases of anaplastic large cell lymphoma expressing the CD30 (Ki-1/Ber-H2) antigen was labeled by antibody KP1.

Discussion

In this study, the monoclonal anti-CD68 antibody KP1 stained all myeloid and monocyte/macrophage neoplasms in routinely processed paraffin-embedded tissue sections. This broad pattern of reactivity on myelomonocytic malignancies correlates well with the pattern observed in normal tissues, in which KP1 labeled granulocyte precursors, monocytes, and most macrophages.[15]

It is of practical interest that KP1 reacted with all myelomonocytic neoplasms that presented in extramedullary sites where they might be confused with lymphoma. Indeed, two cases of myelomonocytic leukemia were misinterpreted as T cell lymphomas. In this context it is relevant that the T cell associated markers, CD2 and CD4, may both be expressed by neoplastic cells of monocytoid origin[28]; it is possible that some myelomonocytic neoplasms, especially those that are localized (as was the case with one of the two cases referred to above) in the interfollicular areas of lymphoid tissue, may be miscategorized after phenotyping as T cell lymphomas. Although we have not performed an extensive study of leukemias, our study having focused on a limited number particularly in extramedullary sites, antibody KP1 appears to be a potentially valuable reagent in the histologic diagnosis of myeloid leukemic tissue infiltrates. It is superior in terms of the number of cases of acute myeloid leukemia labeled for CD15 (Leu-M1), as shown in a study to be reported elsewhere. It is not clear whether it labels more cases than chloroacetate esterase, which is itself known to be a better marker than CD15,[29,30] and a study aimed at clarifying this issue would be valuable. However, antibody KP1 offers an advantage over chloroacetate esterase, similar to that offered by lysozyme, in that it can label myeloid cells in acid decalcified tissues as well as in those fixed in B-5 fixative.[31] Antibody KP1 may be preferable to lysozyme as a marker of myeloid cells because it labels a wider spectrum of cells of monocyte/macrophage derivation.

Our initial experience in staining a variety of normal and reactive lymphoid lesions with antibody KP1 indicated that its reactivity is confined to cells of monocyte/macrophage and myeloid origin, and we have never observed normal lymphoid cells in paraffin sections reactive with the antibody. The positive reactions of a significant number of lymphomas (14 of 56) by antibody KP1, particularly those of low grade B cell type, were therefore an unexpected observation.[15] In general, however, the overall pattern of staining was different from that seen in myelomonocytic malignancies, comprising focal dot-like cytoplasmic reactivity. Recently, we used KP1 to stain cytospin preparations of fine needle aspirates and cell suspensions from lymphoid tissue and noted small dots of KP1 reactivity in some small lymphocytes (unpublished observation, 1989). Because we have not observed such reactivity in small lymphocytes in paraffin sections of normal and reactive lymphoid disorders, we suspect that fixation and processing render this small amount of antigen undetectable in paraffin sections. Presumably the amount of KP1 reactivity that occurs in the dot-like accumulations of reaction product in small B cell neoplasms is greater than that occurring in normal lymphocytes. Furthermore, we noted that the dot-like staining in hairy cell leukemia

cells in paraffin sections was often not seen when the trypsinization time was shortened to 20 minutes or less, suggesting that this reactivity represents antigen in small amounts close to the detection threshold.

The different pattern of reactivity that we observed in myelomonocytic *versus* lymphoid cells is reminiscent of the different patterns of reactivity previously observed with enzyme histochemical reactions for nonspecific esterases. For example, alpha naphthol acetate esterase (ANAE) was initially used as a marker for monocytes and macrophages[32] until several investigators showed that, if reaction conditions were suitably modified, it was possible to demonstrate dot-like staining in both normal and neoplastic T lymphocytes.[33–36] Similar dot-like reactivity for ANAE was also described in a subset of B cells[37] and low and high grade B cell neoplasms.[38,39] Furthermore, we recently observed weak KP1 staining of some epithelial neoplasms in cytospins from fine needle aspirates, a finding paralleled by the discovery of other researchers that touch preparations from a variety of epithelial neoplasms may show nonspecific esterase activity.[40] The target antigen for this KP1 antibody (CD68) is apparently not itself ANAE because sequence data for the CD68 molecule shows no homology to known esterases (D. Simmons, unpublished observation, 1988), but it is possible that the antigen is another lysosome-associated constituent, as suggested by recent studies of peripheral blood cells with CD68 antibodies.[41]

Malignant neoplasms presumed to derive from tissue histiocytes/macrophages and their precursors have become extremely controversial. Recent studies employing panels of monoclonal antibodies or probes to antigen receptor genes suggested that many cases diagnosed as examples of malignant histiocytosis or true histiocytic lymphoma represent unusual B or T cell lymphomas.[42–47] Antibody KP1 should be of value in resolving such diagnostic uncertainties. It may also be of value in the study of histiocytosis X, because it has been shown elsewhere that KP1 may react with the abnormal cell population in this condition.[48] The antibody may also have a place in the differential diagnosis of histiocytic *versus* nonhemopoietic neoplasms (eg, carcinoma and melanoma). The antibody has been extensively used in the authors' laboratories on a variety of nonhemopoietic neoplasms, and we have yet to encounter reactivity with such tumors in paraffin-embedded sections. However, in view of the evidence that weak staining of epithelial neoplasms in cytologic preparations can occur (see above), antibody KP1 should be used in conjunction with other markers (eg, S-100 and cytokeratin) when staining possible nonhemopoietic neoplasms.

Cases of lymphoid neoplasms masquerading as histiocytic tumors include many cases that express activation antigens such as CD30 (Ki-1/Ber-H2) and that are often large cell neoplasms of T lymphoid origin. Of note, in view of the possibility of confusing these neoplasms with histiocytic tumors, is the finding that none of the CD30-expressing lymphomas in our study was stained by antibody KP1. The striking number of macrophages that may occur in lymphomas may well account for some of the cases of T and B lymphomas that have been erroneously interpreted as histiocytic malignancies.[14] Antibody KP1 appears to be a reliable marker for identifying the large number of macrophages present in such lymphomas, and may, when used in combination with paraffin-reactive B and T lineage-associated markers, aid in the diagnosis and the precise classification of other macrophage-rich lymphomas. Antibody KP1 should also prove useful for assessing the number and distribution of macrophages in other malignancies, and thereby should help in determining their biological significance.

References

1. Poppema S, Hollema H, Visser L, Vos H: Monoclonal antibodies (MT1, MT2, MB1, MB2, MB3) reactive with leucocyte subsets in paraffin-embedded tissue sections. Am J Pathol 1987, 127:418–429
2. Norton AJ, Ramsay AD, Smith SH, Beverley PCL, Isaacson PG: Monoclonal antibody (UCHL1) that recognises normal and neoplastic T cells in routinely fixed tissues. J Clin Pathol 1986, 39:399–405
3. 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 tumours. J Immunol 1984, 133:1028–1036
4. Ishii Y, Takami T, Yausa 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, 58:183–192
5. Cartun RW, Coles FB, Pastuszak WT: Utilization of monoclonal antibody L26 in the identification and confirmation of B-cell lymphomas: A sensitive and specific marker applicable to formalin and B-5 fixed, paraffin-embedded tissues. Am J Pathol 1987, 129:415–421
6. Norton AJ, Isaacson PG: Monoclonal antibody L26: An antibody that is reactive with normal and neoplastic B lymphocytes in routinely fixed and paraffin wax embedded tissues. J Clin Pathol 1987, 40:1405–1412
7. Pulford KAF, Falini, B, Heryet A, Gatter KC, Mason DY: 4 KBS: 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 1987, p 828
8. Dobson CM, Myskow MW, Krajewski AS, Carpenter FH, Horne CHW: Immunohistochemical staining of non-Hodgkin's lymphoma in paraffin sections using the MBI and MTI monoclonal antibodies. J Pathol 1987, 153:203–212
9. Norton AJ, Isaacson PG: Detailed phenotypic analysis of B-cell lymphoma using a panel of antibodies reactive in rou-

tinely fixed wax-embedded tissue. Am J Pathol 1987, 128: 225–240
10. Strickler JG, Weiss LM, Bindl J, McDaid R, Buck D, Warnke R: Monoclonal antibodies reactive in routinely processed tissue sections of malignant lymphoma, with emphasis on T-cell lymphomas. Hum Pathol 1987, 18:808–814
11. Ngan BY, Picker LJ, Medeiros LJ, Warnke RA: Immunophenotypic diagnosis of non-Hodgkin's lymphoma in paraffin embedded sections: Coexpression of L60 (Leu22) and L26 antigens correlates with malignant histology. Am J Clin Pathol 1989, 91:579–583
12. Peterson L, Dehner LP, Brunning RD: Extramedullary masses as presenting features of acute monoblastic leukaemia. Am J Clin Pathol 1981, 75:140–148
13. Braylan RC, Long JC, Jaffe ES, Greco FA, Orr SL, Berard CW: Malignant lymphoma obscured by concomitant extensive epithelioid granulomas: Report of three cases with similar clinicopathologic features. Cancer 1977, 39:1146–1155
14. Jaffe ES, Costa J, Fauci AS, Cossman J, Tsokos M: Malignant lymphoma and erythrophagocytosis simulating malignant histiocytosis. Am J Med 1983, 75:741–749
15. Pulford KAF, Rigney EM, Jones M, Stross WP, Micklem KJ, Gatter K, Mason DY: KP1: a new monoclonal antibody detecting a monocyte/macrophage associated antigen in routinely processed tissue sections. J Clin Pathol 1989, 42: 414–421
16. Davey FR, Cordell JL, Erber WN, Pulford KAF, Gatter KC, Mason DY: Monoclonal antibody (Y1/82A) with specificity towards peripheral blood monocytes and tissue macrophages. J Clin Pathol 1988, 41:753–758
17. Parwaresch MR, Radzun HJ, Kriepe H, Hansmann M-L, Barth J: Monocyte/macrophage-reactive monoclonal antibody Ki-M6 recognizes an intracytoplasmic antigen. Am J Pathol 1986, 125:141–151
18. Kelly PMA, Bliss E, Morton JA, Burns J, McGee JO'D: Monoclonal antibody EBM/11: High cellular specificity for human macrophages. J Clin Pathol 1988, 41:510–515
19. Micklem K, Rigney E, Cordell J, Simmons D, Stross P, Turley H, Seed B, Mason DY: A human macrophage-associated antigen detected by five different monoclonal antibodies. Br J Haematol 1989, 73:6–11
20. Pallesen G: Immunopnenotypic markers for characterizing malignant lymphoma, malignant histiocytosis and tumors derived from accessory cells. Cancer Rev 1988, 8:1–65
21. Ralfkiaer E, Al Saati T, Bosq J, Delsol G, Gatter KC, Mason DY: Immunocytochemical characterization of cutaneous lymphomas other than mycosis fungoides. J Clin Pathol 1986, 39:553–563
22. Davey FR, Gatter KC, Ralfkiaer E, Pulford KAF, Krissansen GW, Mason DY: Immunophenotyping of non-Hodgkin's lymphomas using a panel of antibodies on paraffin-embedded tissues. Am J Pathol 1987, 129:54–63
23. Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz Z, MacDonald S, Pulford KAF, Stein H, Mason DY: Immunoenzymatic labelling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). J Histochem Cytochem 1984, 32:219–229
24. Mason DY, Erber WN, Falini B, Stein H, Gatter KC. Immunoenzymatic labelling of haematological samples with monoclonal antibodies. Methods in Haematology: Monoclonal Antibodies. Edited by PCL Beverley. Edinburgh Churchill Livingstone 1986, pp 145–181
25. 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
26. Kelly PMA, McGovern M, Gatter KC, Theaker JM, McGee, JO'D: EBM/11 reactivity in malignant histiocytosis. J Clin Pathol 1988, 41:1305–1309
27. Stansfeld AG, Diebold J, Noel H, Kapanci Y, Rilke F, Kelenyi G, Sundstrom C, Lennert K, Van Unnik JAM, Mioduszewska O, Wright DH: Updated Kiel classification for lymphomas. Lancet 1988, i:292–293
28. Mirro J, Antoun GR, Zipf TF, Melvin S, Stass S. The E rosette-associated antigen of T cells can be identified on blasts from patients with acute myeloblastic leukemia. Blood 1985, 65: 363–367
29. Ralfkiaer E, Pulford KAF, Lauritzen AF, Avnstrøm S, Guldhammer B, Mason DY: Diagnosis of acute myeloid leukaemia with the use of monoclonal anti-neutrophil elastase (NP57) reactive with routinely processed biopsy samples. Histopathology 1989, 14:637–643
30. Kurec AS, Cruz VE, Barrett D, Mason DY, Davey FR: Immunophenotyping of acute leukemias using paraffin embedded tissue sections. Am J Clin Pathol (In press)
31. Neiman RS, Barcas M, Berard C, Bonner H, Mann R, Rydell RE, Bennett JM: Granulocytic sarcoma: A clinicopathologic study of 61 biopsied cases. Cancer 1981, 48:1426–1437
32. Li CY, Lam KW, Yam LT: Esterases in human leucocytes. J Histochem Cytochem 1973, 21:1–12
33. Kulenkamff J, Janossy G, Greaves MF: Acid esterase in human lymphoid cells and leukemic blasts: a marker for T lymphocytes. Br J Haematol 1977, 36:231–240
34. Knowles DM II, Hoffman T, Ferrarini M, Kunkel HG: The demonstration of acid alpha-naphthyl acetate esterase activity in human lymphocytes: usefulness as a T-cell marker. Cell Immunol 1978, 35:112–123
35. Knowles DM II, Halper JP, Machin GA, Sherman W: Acid alpha-naphthyl acetate esterase activity in human neoplastic lymphoid cells. Am J Pathol 1979, 96:257–278
36. Ferrarini M, Cadoni A, Franzi AT, Ghigliotti C, Leprini A, Zicca A, Grossi CE: Ultrastructure and cytochemistry of human peripheral blood lymphocytes: Similarities between the cells of the third population and TG lymphocytes. Eur J Immunol 1980, 10:562–570
37. Ranki A, Totterman TH, Hayry P: Identification of resting T and B lymphocytes by acid alpha-naphthyl acetate esterase staining combined with rosette formation with Staphylococcus aureus strain Cowan I. Scand J Immunol 1976, 5:1129–1138
38. Tolksdorf G, Stein H: Acid alpha-naththyl acetate esterase in hairy cell leukaemia cells and other cells of the hematopoietic system. Blut 1979, 39:165–176
39. Cossman J, Jaffe ES, Fisher RI: Immunologic phenotypes of diffuse, aggressive, non-Hodgkin's lymphomas. Cancer 1984, 54:1310–1317

40. Tubbs RR, Savage RA, Crabtree RH, Sebek BA, Valenzuela R, Benjamin SP: Expression of monocytic-histiocytic cytochemical markers in epithelial neoplasia. Am J Clin Pathol 1979, 72:789–794
41. Pulford KAF, Sipos A, Mason DY. Cell distribution and heterogeneity of the CD68 macrophage-associated antigen. (Manuscript in preparation)
42. 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
43. Turner RR, Wood GE, Beckstead JH, Colby TV, Horning SJ, Warnke RA: Histiocytic malignancies: Morphologic, immunologic and enzymatic heterogeneity. Am J Surg Pathol 1984, 8:485–500
44. Weiss LM, Trela MJ, Cleary ML, Turner RR, Warnke RA, Sklar J: Frequent immunoglobulin and T-cell receptor gene rearrangements in "histiocytic" neoplasms. Am J Pathol 1985, 121:369–373
45. O'Connor NTJ, Stein H, Gatter KC, Wainscoat JS, Crick J, Al Saati T, Falini B, Delsol G, Mason DY: Genotypic analysis of large cell lymphomas which express the Ki-1 antigen. Histopathol 1987, 11:733–740
46. Delsol G, Al Saati T, Gatter KC, Gerdes J, Schwarting R, Caveriviere P, Rigal-Huguet F, Robert A, Stein H, Mason DY: Coexpression of epithelial membrane antigen (EMA), Ki-1 and interleukin-2 receptor by anaplastic large cell lymphomas: Diagnostic value in so-called malignant histiocytosis. Am J Pathol 1988, 130:59–70
47. Weiss LM, Picker LJ, Copenhaver CM, Warnke RA, Sklar J: Large cell hematolymphoid neoplasms of uncertain lineage. Hum Pathol 1988, 19:967–973
48. Ruco LP, Pulford KAF, Mason DY, Ceccamea A, Uccini S, Pileri S, Baglioni P, Baroni CD: Expression of macrophage-associated antigens in tissues involved by Langerhans' cell histiocytosis (Histiocytosis X). Am J Clin Pathol (In press)