Role of paraffin-section immunohistochemistry in the diagnosis of malignant lymphoma

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ABSTRACT. Immunohistochemistry plays an integral role in the diagnosis of malignant lymphoma. The recent development of antibodies against fixative-resistant epitopes, the improvement of antigen retrieval techniques, and the introduction of highly sensitive detection systems have expanded the possibility of immunophenotyping of malignant lymphoma in routinely processed paraffin sections. This review provides precise information regarding antibodies useful in paraffin-section immunohistochemistry, as well as strategies for the immunohistochemical diagnosis of malignant lymphoma based on up-to-date classification.

Key words: immunohistochemistry — lymphoma — paraffin — antibody

Lymphoma diagnosis not only requires knowledge of classical histology but also of immunohistochemistry, flow cytometry, and molecular studies because up-to-date lymphoma classification is based on recent understanding of normal lymphoid cell differentiation.[1,2] Although the immunohistochemical evaluation of malignant lymphoma has been limited in the past due to a paucity of antibodies that work on paraffin sections, the recent development of antibodies against fixative-resistant epitopes, the improvement of antigen retrieval techniques,[3] and the introduction of highly sensitive detection systems[4] have expanded the possibility of immunophenotyping of malignant lymphoma in routinely processed paraffin-embedded sections. As a result, paraffin-section immunohistochemisty is becoming an integral part of the diagnostic process. The aim of this review is to describe a recent advance in paraffin-section immunohistochemistry with special attention paid to antibodies useful for the differential diagnosis of malignant lymphoma.

Antibodies useful for the diagnosis of malignant lymphoma

1. Non-lineage-specific antigens

1-1. Leukocyte Common Antigen (LCA)

LCA (CD45) is a major glycoprotein on cells of hemopoietic origin showing considerable heterogeneity in both structure and expression. Biochemical heterogeneity has been attributed to differences in the primary sequence and glycosylation. This antigen is composed of a family of five or more high molecular mass glycoproteins (180, 190, 205, and 220 kD) present on the surface of the majority of human leukocytes[5] but absent from mature erythrocytes, platelets, plasma cells, and non-hematopoietic cells.[6] Normal

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lymphocytes are positive for this antigen. Macrophages react to a variable degree with the antibody. Polymorphs are usually weakly labeled. LCA works on Carnoy's-, ethanol-, and B5-fixed paraffin-embedded sections but can also be used on formalin-fixed material without antigen retrieval. However, overfixation with formalin gives false negative results. If normal lymphocytes are not stained with anti-LCA monoclonal antibody (mAb) in a formalin-fixed paraffin-embedded section, one should consider the possibility of overfixation. LCA is quite useful for the differential diagnosis of small round cell tumor. Granulocytic sarcoma has frequently been positive for this antigen.[7] One should remember that some large-cell lymphomas will not be stained in paraffin-sections, even when lymphocytes in the same tissue are positive. Since LCA is not expressed on the majority of plasmacytomas and multiple myeloma, it cannot be used in distinguishing anaplastic myeloma from carcinoma. Reed-Sternberg (R-S) cells and their mononuclear variants are negative for this antigen.[8]

1-2. Terminal deoxynucleotidyl transferase (TdT)

TdT is a unique DNA polymerase enzyme that can add deoxynucleotides to the end of a DNA molecule without a template.[9] This enzyme is a marker for T cell precursors in the thymus as well as bone marrow B cell precursors. TdT has been detected by immunofluorescence or immunoperoxidase on imprints or frozen sections. A simple TdT immunostaining method for paraffin-sections has been recently reported.[10] The method involves the use of microwave pretreatment, polyclonal rabbit anti-TdT antiserum, and the Elite kit (Vector Laboratory) as a detection system. The pattern of staining is nuclear. TdT has been demonstrated in most cases of ALL regardless of their immunological phenotype, in lymphoblastic crises of CML, and consistently in lymphoblastic lymphoma (Fig 1). It is also present in the thymocytes of thymomas and in the blastic T cells of rare cases of acute myeloid lenkemia.[11,12]

1-3. Ki-1 antigen (CD30)

CD30 antigen is a single chain glycoprotein with a molecular weight (MW) of 120 kD. It has been postulated that it functions as a growth factor receptor.[13] It reacts with scattered large lymphoid cells (immunoblasts) at the rim of germinal centers and interfollicular areas in most lymphoid tissues. This antigen is also expressed in B cells, T cells activated *in vitro,* some plasma cells, immature erythroblasts, and megakaryocytes in paraffin-embedded sections. The staining may be membrane-and Golgi-associated (dot-like) or cytoplasmic. It is useful in confirming the diagnosis of anaplastic large cell lymphoma (ALCL) or Hodgkin's disease in conjunction with morphological features and other antigens.[14] Occasional large cells in other non-Hodgkin's lymphomas may be positive. Fixation strongly influences the immunostaining. In routine work, we do not absolutely rely on this antigen for the diagnosis of HD because it is not unusual for typical HD to lack detectable CD30 expression. Carcinomas, especially embryonal cell carcinoma may occasionally express CD30.[15] Immunostaining for cytokeratin and placental alkaline phosphatase (PLAP) solves the differential diagnostic problem between ALCL and embryonal carcinoma.

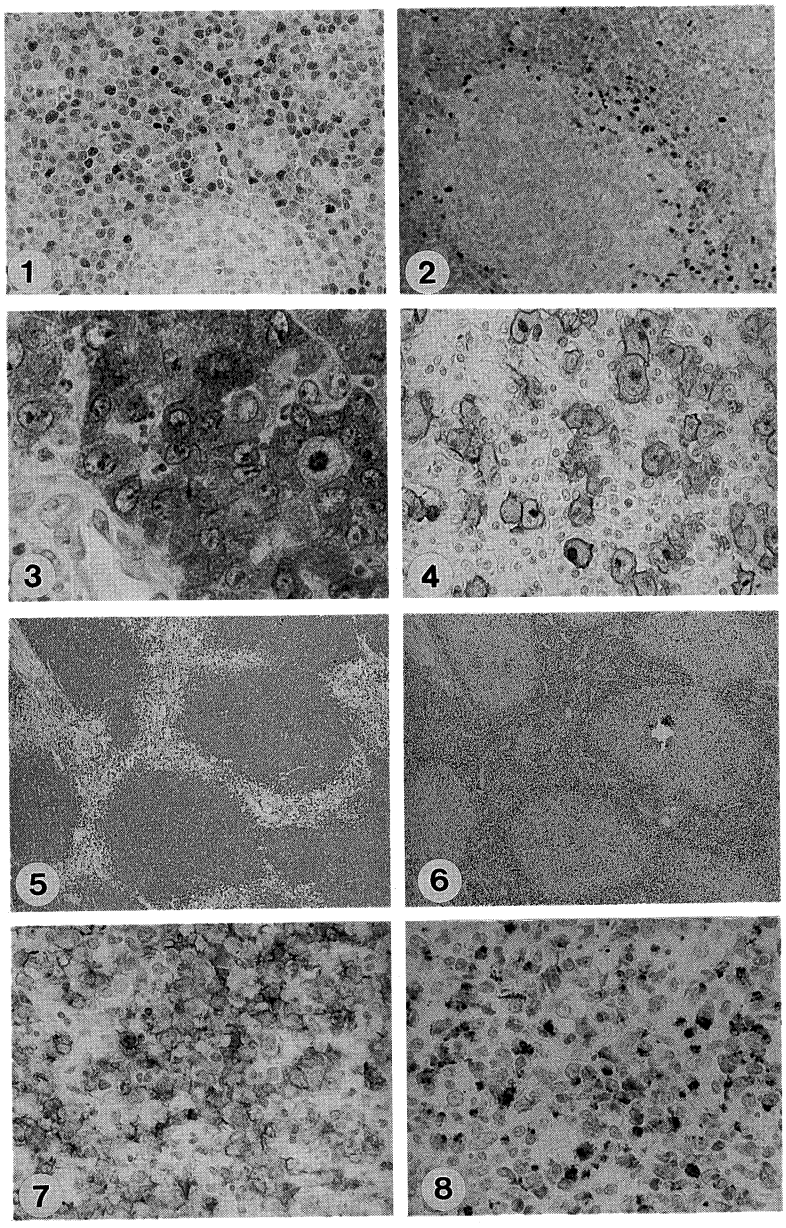

Fig 1. Nuclear expression of TdT in T-lymphoblastic lymphoma. No staining is seen in reactive germinal center cells. Original magnification, x 80.
Fig 2. Cyclin D1 expression in mantle cell lymphoma. No staining is seen in reactive germinal center cells. Original magnification, x 40.
Fig 3. Immunostaining of anaplastic large cell lymphoma with ALK1. Original magnification, x 132.
Fig 4. Leu-M1 expression of Hodgkin's disease. Original magnification, x 100.
Fig 5. CD20 expression in follicular lymphoma. Original magnification, x 25.
Fig 6. CD45RO expression in follicular cell lymphoma. Positive cells outline neoplastic follicles. Original magnification, x 25.
Fig 7. CD56 expression of nasal NK cell lymphoma. Original magnification, x 132.
Fig 8. TIA-1 expression is nasal NK cell lymphoma. Original magnification, x 132.

1-4. Ki-67 antigen

The utility of the proliferation index in predicting the outcome in lymphoma patients has been confirmed.[16] Ki-67 is a nuclear antigen associated with proliferating cells and expressed in all nucleated cells except Go cells.[17] It is detected in frozen and paraffin-sections; staining is nuclear. In our laboratory, microwave treatment in a pressure cooker is used to obtain a good immunostaining result with MIB-1, a monoclonal antibody reacting with Ki-67 antigen. The Ki-67 labeling index (the number of Ki-67 positive cells/the total number of cells) can help in an indivisual approach of the proliferation rate of each tumor and this may be an important parameter for prediciting the biological behavior of non-Hodgkin's lymphomas; i.e. high-grade NHLs display a higher proliferation rate than low grade ones. Actually, a statistically significant difference has been found between Ki-67 labeling of low-and high-grade lymphomas as classified by the Kiel classification[18] and the International Working Formulation,[19] although remarkable variations in MIB-1 expression have been found among individual cases of the same histological group.

1-5. Bcl-2

The Bcl-2 protein (MW: 25 kD) is an integral membrane protein localized within mitochondria and is encoded by a gene involved in (14; 18) chromosomal translocation.[20] This protein plays a role in preventing programmed cell death as an anti-apoptosis factor. The gene is normally inactive in germinal-center B cells, which have a high rate of cell death, but is active in most long-lived T and B cells and in most B cell lymphomas.[21] Therefore, the monoclonal antibody has been able to detect this protein in small B cells in the marginal zone, many cells within T cell area in lymph nodes and many thymocytes in medulla of thymus. In formalin-fixed paraffin-embedded sections, microwave pretreatment is mandatory to obtain a positive result. Detection of the Bcl-2 protein is very useful in distinguishing between reactive follicles and follicular lymphoma[22,23] but not in the diagnosis or subclassification of other B cell lymphomas and T cell lymphomas.

1-6. Cyclin D1

Cyclin D1 protein belongs to a family of proteins which function primarily in the cell cycle by regulating the activity of cyclin dependent kinase.[24] There are two representative monoclonal antibodies that work on paraffin-sections, 5D4[25] and DCS-6.[26] Under normal conditions, these antibodies react with some type of epithelial cells and stromal cells. Chromosomal translocation t (11; 14) (q13: q32), involving rearrangement of the bcl-1 locus, is closely associated with mantle cell lymphomas (MCL) and increased cyclin D1 expression. Thus, cyclin D1 immunostaining is diagnostic in MCL (Fig 2). However, it has been hard to obtain satisfactory results from cyclin D1 immunostaining on paraffin-sections. The following is our protocol for the immunostaining of DCS-6: (i) Microwave treatment in citrate buffer (pH 6.3) for 10 min in a pressure cooker; (ii) 60 min incubation in DCS-6 at room temperature; and (iii) incubation in Elite kit as the detection system. Recently, cyclin D1 has been shown to be expressed in multiple myelomas with the t (11; 14) (q13: q32).[27]

1-7. Chimeric protein $p80^{NPM/ALK}$

Some anaplastic large cell lymphomas (ALCLs) carry a specific chromosomal translocation, t (2 ; 5) (p23 ; q35).[28] The incidence of t (2 ; 5) (p23 ; q35) is higher in neoplasms from children than from adults. Antiserum against a hyperphosphorylated 80 kD protein tyrosine kinase (p80), a fusion protein of two different genes on chromosomes 2p23 and 5q35, in accordance with the sequence of the NPM/ALK gene, has been shown to be efficient for detecting lymphomas producing this chimeric protein.[29] Microwave treatment is mandatory to the immunostaining. A monoclonal antibody, ALK-1, reacts with the product of ALK but does not stain normal tissues.[30] Immunostaining of this antibody is clearer than that of the polyclonal antibody against p80 (Fig 3). The antigen has been found in approximately 40%～60% of the nodal ALCL. Skin ALCL were constantly negative. Clinicopathologic comparison of p80-positive and-negative ALCLs showed that p80-positive cases occurred in a far younger patient age group and that the patients had a far better 5-year survival rate. Lymphohistiocyiic ALCLs show positivity for ALK protein,[31] whereas HD-like ALCL is negative for $p80^{NPM/ALK}$. ALK1 immunostaining is particularly useful in detecting residual lymphoma cells and investigating bone marrow involvement of ALCL.[32]

2. B cell-associated antigen

2-1. Immunoglobulin (Ig)

Normally, populations of human B cells carry κ or λ light chains in approximately a 2 : 1 ratio. Since neoplastic populations arising from a single progenitor cell should contain only a single light chain, κ or λ, the presence of light-chain restriction is evidence of neoplasia. Cytoplasmic immunoglobulin immunostaining of formalin-fixed paraffin-embedded sections is particularly valuable in the diagnosis of plasmacytoma and monoclonal plasma cell differentiation in mucosa-associated lymphoid tissue (MALT) lymphoma.[33] Enzymatic digestion or high temperature antigen retrieval of formalin-fixed sections results in clearer staining.[34] There may be a high level of background staining because immunoglobulin is normally present in plasma. This is particularly true for Ig G, may be a problem with Ig A, κ and λ. Therefore, experience is required to interpret the immunoglobulin staining correctly.

2-2. CD20

CD20 is a membrane-embedded glycoprotein of approximately 33-37 kD[35] which contains four membrane-spanning domains and intracytoplasmic phosphorylation sites. CD20 expression is restricted to normal and neoplastic B cells. A representative monoclonal antibody, L26, stains bone marrow pre-B cells, germinal centers and mantle zone B cells but not plasma cells and epithelial cells. No cross reactivity with non-hematopoietic neoplasm has been shown with L26, whereas staining of smooth muscle cells is often seen. The pattem of staining is membranous. As with other paraffin-reactive antibodies, its reactivity varies with tissue fixation. Without antigen retrieval, no positive staining of L26 has been found in Bouin's-fixed samples, but immunostaining is recovered with microwave antigen retrieval.[36] CD20 antigen, detected by L26, is expressed in more than 90% of B cell lymphomas (Fig 5), 50% precursor

B-lymphoblastic lymphomas, 60~70% of ALL, 60~100% of Hodgkin's disease of nodular lymphocyte predominance (NLPHD), 6~75% of other forms of HD, 6~20% of multiple myelomas, 3% acute myeloid leukemias (AMLs), and 25~30% chronic myelogenous leukemias (CMLs) blastic transformation. In NLPHD, Read-Sternberg (RS) cells show membrane and paranuclear globular staining. Although the author have not had an experience, this antigen has been expressed in some cases of T cell lymphoma.[37] Immunomorphologic analysis using L26 is a more sensitive method for detecting residual hairy cells than morphology alone.[38]

2-3. CD79

The CD79 molecule with a molecular weight of 70 kD is the dimeric protein associated with Ig[39] and comprised of two polypeptide chains, CD79a and CD79b. CD79a is B cell specific, appearing before the pre-B cell stage and persisting to the plasma cell stage. Expression of CD79b starts later than that of CD79a and ceases earlier. The monoclonal antibody, JCB117, has reacted with CD79a. It stains the great majority (97%) of B cell neoplasms, covering the full range of B cell maturation, including multiple myeloma/plasmacytoma.[40] The antibody can label precursor B cell acute lymphoblastic leukemia/lymphoma samples, making it a rather reliable B cell marker detectable in paraffin-embedded specimens. All neoplasms of T cell or nonlymphoid origin have been negative. Thus, JCB117 may be of value for the identification of B cell neoplasms of all maturation stages. However, the staining intensity of CD79a in B cell lymphomas seems to be variable as compared with that of CD20.

2-4. CD10

The CD10 (CALLA) antigen is expressed in a wide variety of epithelial and nonepithelial tissues.[41] CD10 can be specifically and sensitively detected on paraffin sections using the monoclonal antibody 56C6 or NCL-CD 10-270 after epitope retrieval.[42] Strong staining has been seen in lymphoid germinal centers, renal tubules, glomeruli, syncytiotrophoblasts, hepatic parenchymal canaliculi, follicle center lymphomas, Burkitt lymphomas, and precursor B-lineage acute lymphoblastic leukemias (ALL). Therefore, these monoclonal antibodies are especially useful for the differential diagnosis of small-B cell lymphoma and subtyping of lymphoblastic leukemia.[43]

3. T cell-associated antigens

3-1. CD3

CD3 is expressed on both thymocytes and mature T cells.[44] The polyclonal antisera raised against the synthetic peptide sequence from the intracytoplasmic portion of CD3ε-chain, have resulted in strong labeling in routine paraffin embedded sections, with negligible background staining. This antibody works well in Bouin's-fixed paraffin-embedded sections but less well in formalin-or B5-fixed sections.[45] The CD3ε-chain is also expressed in NK cell lymphomas. When compared with other paraffin-reactive T cell antibodies (UCHL1, MT1, Leu-22, DF-T1 and MT2), the anti-CD3ε antibody is the most specific of the anti-T cell antibodies.[46]

3-2. T cell receptor β chain

The T cell receptor (TCR) complex is composed of the TCRα/β heterodimer and the associated CD3 complex.[47] TCR are expressed on both thymocytes and mature T cells. The monoclonal antibody βF1 reacts with a common framework determinant of the β subunit of the T cell receptor. Antibody βF1 is a specific and relatively sensitive marker of T cell phenotype in formalin-fixed paraffin sections of malignant lymphomas.[48] In pronase-treated formalin-fixed paraffin-sections, it is equivalent to surface CD3 in T cell specificity, but staining may be weak. βF1 immunostaining is particularly valuable in differentiating α/β T cell lymphomas from NK cell lymphomas and γ/δ T cell lymphomas which are not stained with this antibody.[49]

3-3. CD45RO

The monoclonal antibody UCHL-1 recognizes CD45RO antigen, lowest molecular mass isoform (180 kD) of CD45. This antigen is expressed by most thymocytes, a subpopulation of resting T cells of both $CD4^+$ and $CD8^+$ cells, and mature activated T cells (Fig 6). However, 25-50% of normal T cells (unstimulated or virgin T cells) lack the antigen. Presently, UCHL-1 is being used widely for detecting T cell lymphomas.[50] The advantage of this antibody is that it shows strong membranous immunostaining in formalin fixed sections without antigen retrieval.[51] In angioimmunoblastic T cell lymphoma, clear cells are clearly outlined with this antibody. However, UCHL-1 is not specific for T cells. This antibody also stains monocytes and macrophages. It rarely stains B cell lymphomas (centroblastic and immunoblastic lymphoma).[52] Other types of hematopoietic tumors, such as NK cell lymphoma and granulocytic sarcoma, are labeled with UCHL-1. The author have frequently experienced severe background staining with this antibody in Bouin-fixed bone marrow sections.

3-4. CD4

CD4 is a single chain transmembrane glycoprotein with a molecular weight of 59 kD.[53] This antigen is present on 80% of thymocytes and 45% of peripheral blood lymphocytes, monocytes, and macrophages. CD4 antigen can be detected in paraffin-sections using the monoclonal antibody 1F6 and microwave treatment in 1 mM EDTA solution (pH 8.0), but the staining is frequently faint and diffuse. Most cases of cutaneous T cell lymphoma, including mycosis fungoides.[54] HTLV-I associated adult T cell leukemia/lymphoma, and plasmacytoid T cell lymphoma, have been stained with this antibody.

3-5. CD8

CD8 is expressed mostly as an α/β heterodimer by most thymocytes and one third of peripheral T cells and splenic sinus lining cells. A proportion of γ/δ T cells and NK-cells express CD8 α/α homodimers.[55] Mason *et al* reported monoclonal antibodies, raised against a 13 amino acid peptide sequence from the cytoplasmic portion of the α chain of the human CD8 molecule.[56] This antigen can be detected in paraffin-sections without prior treatment of the sections if the tissue has been fixed in Bouin' s fixative. When

tissues have been fixed in formalin, microwave treatment is required. This regent is of value for identification of $CD8^+$ peripheral T cell lymphoma.

3-6. CD56

CD56 is a heterodimeric glycoprotein of 145, 185 kD which has been identified as a neural cell adhesion molecule (NCAM).[57] CD56 expression can be analyzed using a clone, 123C3 or 1B6, not only on frozen tissues but also on paraffin-sections with prior antigen retrieval.[58] This antibody reacts with autopsy sections.[59] Neoplastic cells show membrane staining. In normal or reactive lymphoid tissues from a variety of sites, only a few small lymphocytes (0.1%) have shown cell membrane staining with 123C3. This antibody has reacted with putative natural killer (NK) cell lymphomas (Fig 7), which shows a predilection for the upper aerodigestive tract, skin, skeletal muscle, and other extranodal sites and pursues an aggressive clinical course; intestinal T cell lymphomas; and γ/δ T cell lymphomas.[60] Cases of CD56-positive B cell lymphoma and multiple myeloma have also been recently reported.[61,62]

3-7. TIA-1

T cell intracellular antigen (TIA)-1 is a 15 kD cytotoxic granule-associated protein whose expression appears to be restricted to a subpopulation of peripheral blood lymphocytes possessing cytolytic potential.[63] Because TIA-1 binds poly(A) in vitro and induces apoptosis in permeabilized thymocytes, it may be involved in the induction of apoptosis in target cells during lymphocyte attack. This antigen is expressed in 55% of $CD8^+$ T lymphocytes, 6% of $CD4^+$ T lymphocytes, and 100% of NK cells, but not in B lymphocytes. Although TIA-1 recognizes a 15 kD protein in unstimulated T cells, activated T cells are induced to express several higher molecular weight proteins that are also recognized by TIA-1 monoclonal antibody. Neutrophils and monocytes/macrophages are also labeled with this antibody. This antibody is useful for detecting cytotoxic T cells and NK cells in allograft rejection and GVHD, and for making diagnosis of T/NK cell lymphoma with a cytotoxic phenotype[64] (Fig 8). Malignant lymphoma mimicking fulminant hepatitis frequently expresses TIA-1.[65]

3-8. Granzyme B

Granzyme B (a serine protease) is a major constituent of the cytotoxic granules of activated T cells and NK cells.[66] It activates casepase-3, which induces the apoptosis of target cells. Most extranodal T cell lymphomas appear to be derived from cytotoxic T cells, which express perforin, TIA-1 and granzyme B. Therefore, the role of granzyme B in tissue damage by cytotoxic T cell lymphomas is particularly intriguing issue.[65] However, these cytologic features have not been shown to be useful in predicting the clinical course of T cell lymphomas.[67,68] In contrast to anti-TIA-1 monoclonal antibody, anti-granzyme B monoclonal antibody does not react with myelomonocytic cells.

4. Myeloid cells-associated antigens

4-1. CD15

The structure recognized by the monoclonal antibody Leu-M1 is

Lacto-N-Fucose Pentosyl III (X-hapten).[69] This antigen is expressed on 90% of circulating human granulocytes (neutrophils and eosinophils) and 30-60% of circulating monocytes. It is also present on some epithelial cells, but reacts with few tissue macrophages. The immunostaining of Leu-M1 is cytoplasmic but some membrane labeling may also be noted, particularly in epithelial cells. Cytoplasmic dot-like staining has been reported in a case of granulocytic sarcoma.[70] This antibody stains not only myeloid leukemia but also Reed-Sternberg (R-S) cells (Fig 4) and their variants in all types of HD other than that of lymphocyte predominance.[71] Typical R-S cells show both peripheral membrane staining and paranuclear (Golgi) staining of Leu-M1. Interestingly, Leu-M1 has been reported to react with large cells infected with cytomegalovirus infection.[72] Combined immnunohistochemistry of CD15 and Epstein-Barr virus encoded RNA-1 (EBER-1) *in situ* hybridization has revealed CD15-positve R-S cells to be positive for EBER-1.[73]

4-2. CD68

The CD68 antigen is associated with lysosomes[74] and is recognized by the monoclonal antibodies KP1 and PG-M1. It is strongly expressed in cytoplasmic granules and weakly on the surface. KP1 labels cells of monocyte/macrophage lineage, myeloid precursors, peripheral blood granulocytes, plasmacytoid T cells, and a subpopulation of B cells in routinely processed tissue sections. Neoplasms of myelomonocytic cells, and some B cell lymphomas and leukemias (hairy cell leukemia) have been stained by antibody KP1, but staining has usually been confined to small dots of reactivity. KP1 is a valuable tool for recognition of extramedullary presentation of leukemia and true histiocytic neoplasia.[75] Some anaplastic large cell lymphomas, granular cell tumos, melanomas, renal cell carcinomas, and MFHs were also stained with KP1. PG-M1 was produced by immunizing BALB/c mice with fresh spleen cells from a patient with Gaucher's disease. PG-M1 reacts strongly with a fixative-resistant epitope of an intracytoplasmic molecule, selectively expressed by virtually all macrophages. In formalin-fixed samples, enzymatic predigestion or high temperature antigen retrieval is preferred. Unlike other CD68 antibodies, PG-M1 detects only the macrophage-restricted form of the CD68 antigen.[76] In daily diagnostic practice, PG-M1 seems to be particularly valuable for the diagnosis of myelomonocytic or monocytic leukemia and neoplasms of true histiocytic origin in routine paraffin-sections.[77]

4-3. Myeloperoxidase

Myeloperoxidase is a major constituent of primary granules of neutrophilic myeloid cells. Myeloid cells of both neutrophilic and eosinophilic types, at all stages of maturation and myeloblasts and immature myeloid cells of acute myelogenous leukemia, progranulocytic leukemia, monomyelocytic leukemia, erythroleukemia, myeloblastomas, and other hematopoietic disorders stain with rabbit polyclonal antibody to human myeloperoxidase. Myeloperoxidase has not been observed in the neoplastic cells of a wide variety of epithelial tumors and sarcomas.[78] This is a very useful marker for the identification of granulocytic sarcoma.[79]

Application of paraffin-section immunohistochemistry in the differential diagnosis of malignant lymphomas

1. Lymphoma versus non-lymphoid tumor

Paraffin-section immunohistochemistry is very useful in differentiating between malignant lymphomas and other undifferentiated malignant tumors. Apanel of antibodies should always be used in such cases, because unexpected cross-reactions leading to misdiagnosis are not uncommon. The pitfalls in the differential diagnosis may be summarized as follows: (i) CD45 is the most useful antigen for a confirmation of malignant lymphomas but some lymphomas lack reactivity for this antigen[80]; (ii) Some lymphomas express vimentin[81] and epithelial membrane antigen (EMA)[82]; (iii) Rare lymphomas may be positive for cytokeratin.[83]

2. Reactive hyperplasia versus malignant lymphoma

The first important step in studying tissues suspected of malignant lymphoma, is to differentiate the lesion from a reactive lesion. As described above, Bcl-2 immunostaining is particularly useful in differentiating follicular lymphoma from follicular hyperplasia. Monotypic immunoglobulin light chains (light chain restriction; κ : λ ratio exceeding 10 : 1 or 1 : 10) are the only reliable findings for confirming a diagnosis of low grade B cell lymphoma versus lymphoid hyperplasia when morphologic features are inconclusive or equivocal. However, polyclonal immunoglobulin immunostain does not exclude a diagnosis of lymphoma because reactive plasma cells are commonly found in the lamina propria even for lymphomas. MALT lymphoma cells may express CD43 (42% or 81%). Thus, expression of CD43 in a B cell population in an entire population should be regarded as atypical.[84] Demonstration of clonal T cell receptor gene rearrangements and an aberrant phenotype, as well as demonstration of many admixed $CD8^+$ T cells and B cells can be considered as useful criteria in the differentiation between pseudo-T cell lymphoma and cutaneous T cell lymphoma.[85] Sometimes one or more of the pan T cell markers are negative in cutaneous lymphoma. This is of diagnostic use in distinguishing pseudo-T cell lymphomas from malignant lymphomas.[86]

3. Hodgkin's disease (HD) versus non-Hodgkin's lymphoma (NHL)

Differentiation of HD from NHL is important because their clinical management differs. However, the morphologic and immunohistochemical features of HD and NHL occasionally overlap, making the differential diagnosis difficult. In general, the most helpful immunohistochemical findings in the differential diagnosis between usual HD and B cell lymphoma are the presence of $CD15^+CD30^+CD45^-$R-S cells and absence of B cell markers in HD. However, striking morphological similarities exist between T cell-rich B cell lymphoma and lymphocyte-predominant Hodgkin's disease (LPHD), making the distinction between them extremely difficult.[87] Occasional cases of HD may have some cytological atypia in the background lymphocytes, and some peripheral T cell lymphomas may have R-S-like cells, causing problems in the differential diagnosis. Anaplastic large cell lymphomas (ALCL) are divided to four subtypes: common, giant cell rich, lympho-histiocytic, and Hodgkin-related/Hodgkin's-like. There is a biological spectrum of disease

between HD and ALCL. Distinguishing HD from HD-like ALCL with immunophenotyping can be difficult.[88]

4. Classification of small B cell lymphomas.

The small B cell lymphomas include small lymphocytic lymphomas/B cell chronic lymphocytic leukemia (B-CLL), lymphplasmacytoid lymphoma, mantle cell lymphoma, marginal zone lymphoma and follicular lymphoma. These are distinct entities but can not always be diagnosed on the basis of routine histological study alone. A panel of monoclonal antibodies aids in the classification of small B cell lymphomas (Table 1).[1,89] Cyclin D1 is the best marker for mantle cell lymphoma. CD10 immunostaining separates follicular lymphoma from other small B cell lymphomas.

Table 1. Immunohistochemical classification of small B-cell lymphomas according to REAL classification.

Lymphoma Type	c-Ig	CD23	CD43	cyclin D1	CD20	CD10
B-CLL/SLL	−/+	+	+	−	+	−
Lymphoplasmacytoid	+	−	−/+	−	+	−
Mantle cell	−	−	+	+	+	−
Follicle center	−	−/+	−	−	+	+
Marginal zone	40%+	−/+	−/+	−	+	−

CLL, chronic lymphocytic lymphoma; SLL, small lymphocytic lymphoma,

CONCLUSIONS

As noted above, we are now able to select a panel of antibodies that work on paraffin-embedded sections. Although optimal condition of tissue fixation is different in each antibody, the antigen retrieval technique can be used to standardize paraffin-section immunohistochemisty. Figure 9 is a representative

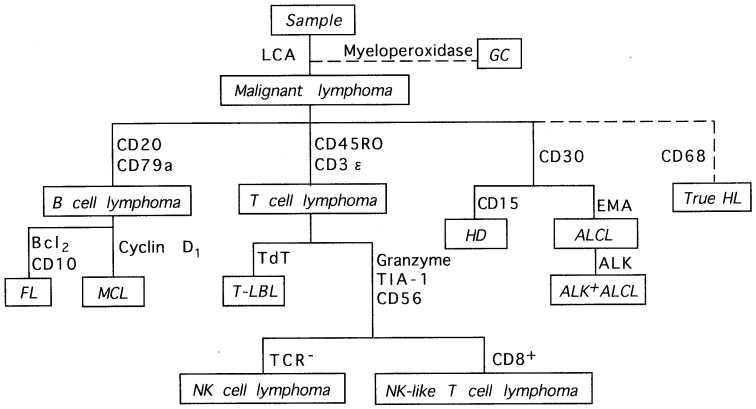

Fig 9. The schema of an immunohistochemical diagnostic method for malignant lymphomas. **Abbreviations**: ALCL, anaplastic large cell lymphoma; ALK, anaplastic lymphoma kinase; EMA, epithelial membrane antigen; GC, granulocytic sarcoma; HD, Hodgkin's disease; HL, histiocytic lymphoma; LBL, lymphoblastic lymphoma; LCA, leukocyte common antigen; MC, mantle cell lymphoma; TCR, T cell receptor; TdT, terminal deoxynucleotidyl transferase. GC and true HL belong to the myelomonocytic cell lineage.

schema for classification of malignant lymphomas in routine work and the following antibodies are recommended for this purpose: 123C3 (CD56), ALK1 ($p80^{NPM/ALK}$), Ber-H2 (CD30), DCS-6 (cyclin D1), L26 (CD20), Leu-M1 (CD15), UCHL-1 (CD45RO), and TIA-1 (TIA-1).

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REFERENCES

1) Harris NL, Jaffe ES, Stein H, Banks PM, Chan JK, Cleary ML, Delsol G, De Wolf-Peeters C, Falini B, Gatter KC: A revised European-American classification of lymphoid neoplasms: a proposal from the Intemational Lymphoma Study Group. Blood **84**: 1361-1392, 1994
2) Harris NL, Jaffe ES, Diebold J, Flandrin G, Müller-Hermelink HK, Vardiman J, Lister TA, Bloomfield CD: The World Health Organization classification of hematological malignancies report of the Clinical Advisory Committee Meeting, Airlie House, Virginia, November 1997. Mod Pathol **13**: 193-207, 2000
3) Shi SR, Cote RJ, Taylor CR: Antigen retrieval immunohistochemistry: past, present, and future, J Histochem Cytochem **45**: 327-343, 1997
4) Sabattini E, Bisgaard K, Ascani S, Poggi S, Piccioli M, Ceccarelli C, Pieri F, Fraternali-Orcioni G, Pileri SA: The En Vision++ system: a new immunohistochemical method for diagnostics and research. Critical comparison with the APAAP, ChemMate, CSA, LABC, and SABC techniques. J Clin Pathol **51**: 506-511, 1998
5) Thomas ML: The leukocyte common antigen family. Annu Rev Immunol **7**: 339-369, 1989
6) Kurtin PJ, Pinkus GS: Leukocyte common antigen-a diagnostic discriminant between hematopoietic and nonhematopoietic neoplasms in paraffin sections using monoclonal antibodies: correlation with immunologic studies and ultrastructural localization. Hum Pathol **16**: 353-365, 1985
7) Fellbaum C, Hansmann ML: Immunohistochemical differential diagnosis of granulocytic sarcomas and malignant lymphomas on formalin-fixed material. Virchows Arch A Pathol Anat Histopathol **416**: 351-355, 1990
8) Dorfman RF, Gatter KC, Pulford KA, Mason DY: An evaluation of the utility of anti-granulocyte and anti-leukocyte monoclonal antibodies in the diagnosis of Hodgkin's disease. Am J Pathol **123**: 508-519, 1986
9) Beutler E, Blume KG: Terminal deoxynucleotidyl transferase: biochemical properties, cellular distribution, and hematologic significance. Prog Hematol **11**: 47-63, 1979
10) Suzumiya J, Ohshima K, Kikuchi M, Takeshita M, Akamatsu M, Tashiro K: Terminal deoxynucleotidyl transferase staining of malignant lymphomas in paraffin sections: a useful method for the diagnosis of lymphoblastic lymphoma. J Pathol **182**: 86-91, 1997
11) Fukayama M, Maeda Y, Funata N, Koike M, Saito K, Sakai T, Ikeda T: Pulmonary and pleural thymoma. Diagnostic application of lymphocyte markers to the thymoma of unusual site. Am J Clin Pathol **89**: 617-621, 1988
12) Drexler HG, Sperling C, Ludwig WD: Terminal deoxynucleotidyl transferase (TdT) expression in acute myeloid leukemia. Leukemia **7**: 1142-1150, 1993
13) Gruss HJ, Dower SK: Tumor necrosis factor ligand superfamily: involvement in the pathology of malignant lymphomas. Blood **85**: 3378-3404, 1995
14) Chittal SM, Caveriviere P, Schwarting R, Gerdes J, Al Saati T, Rigal-Huguet F, Stein H, Delsol G: Monoclonal antibodies in the diagnosis of Hodgkin's disease. The search for a rational panel. Am J Surg Pathol **12**: 9-21, 1988
15) Latza U, Foss HD, Durkop H, Eitelbach F, Dieckmann KP, Loy V, Unger M, Pizzolo

G, Stein H : CD30 antigen in embryonal carcinoma and embryogenesis and release of the soluble molecule. Am J Pathol **146** : 463-471, 1995
16) Tusenius KJ, Bakker PJ, van Oers MH : Measurement of proliferation indices in non-Hodgkin's lymphoma-is it useful ? Leuk Lymphoma **7** : 181-187, 1992
17) Gerdes J : Ki-67 and other proliferation markers useful for immunohistological diagnostic and prognostic evaluations in human malignancies. Semin Cancer Biol **1** : 199-206, 1990
18) Kalogeraki A, Tzardi M, Panagiotides I, Koutsoubi K, Bolioti S, Rontogianni D, Stefanaki K, Zois E, Karidi E, Darivianaki K, Delides G, Kanavaros P : MIB1 (Ki-67) expression in non-Hodgkin's lymphomas. Anticancer Res **17** : 487-491, 1997
19) Weiss LM, Strickler JG, Medeiros LJ, Gerdes J, Stein H, Warnke RA : Proliferative rates of non-Hodgkin's lymphomas as assessed by Ki-67 antibody. Hum Pathol **18** : 1155-1159, 1987
20) Korsmeyer SJ. Bcl - 2 initiates a new category of oncogenes : regulators of cell death. Blood **80** : 879-886, 1992
21) Kondo E, Nakamura S, Onoue H, Matsuo Y, Yoshino T, Aoki H, Hayashi K, Takahashi K, Minowada J, Nomura S : Detection of bcl-2 protein and bcl-2 messenger RNA in normal and neoplastic lymphoid tissues by immunohistochemistry and in situ hybridization. Blood **80** : 2044-2051, 1992
22) Zutter M, Hockenbery D, Silverman GA, Korsmeyer SJ : Immunolocalization of the Bcl-2 protein within hematopoietic neoplasms. Blood **78** : 1062-1068, 1991
23) Utz GL, Swerdlow SH : Distinction of follicular hyperplasia from follicular lymphoma in B5-fixed tissues : comparison of MT2 and bcl-2 antibodies. Hum Pathol **24** : 1155-1158, 1993
24) Campo E, Raffeld M, Jaffe ES : Mantle-cell lymphoma. Semin Hematol **36** : 115 - 127, 1999
25) Yatabe Y, Nakamura S, Seto M, Kuroda H, Kagami Y, Suzuki R, Ogura M, Kojima M, Koshikawa T, Ueda R, Suchi T : Clinicopathologic study of PRAD1/cyclin Dl overexpressing lymphoma with special reference to mantle cell lymphoma. A distinct molecular pathologic entity. Am J Surg Pathol **20** : 1110-1122, 1996
26) de Boer CJ, Schuuring E, Dreef E, Peters G, Bartek J, Kluin PM, van Krieken JH : Cyclin D1 protein analysis in the diagnosis of mantle cell lymphoma. Blood **86** : 2715-2723, 1995
27) Vasef MA, Medeiros LJ, Yospur LS, Sun NC, McCourty A, Brynes RK : Cyclin D1 protein in multiple myeloma and plasmacytoma : an immunohistochemical study using fixed, paraffin-embedded tissue sections. Mod Pathol **10** : 927-932, 1997
28) Morris SW, Kirstein MN, Valentine MB, Dittmer KG, Shapiro DN, Saltman DL, Look AT : Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science **263** : 1281-1284, 1994
29) Shiota M, Nakamura S, Ichinohasama R, Abe M, Akagi T, Takeshita M, Mori N, Fujimoto J, Miyauchi J, Mikata A : Anaplastic large cell lymphomas expressing the novel chimeric protein p80NPM/ALK : a distinct clinicopathologic entity. Blood **86** : 1954 - 1960, 1995
30) Pittaluga S, Wiodarska I, Pulford K, Campo E, Morris SW, Van den Berghe H, De Wolf-Peeters C : The monoclonal antibody ALK 1 identifies a distinct morphological subtype of anaplastic large cell lymphoma associated with 2p23/ALK rearrangements. Am J Pathol **151** : 343-351, 1997
31) Pileri SA, Pulford K, Mori S, Mason DY, Sabattini E, Roncador G, Piccioli M, Ceccarelli C, Piccaluga PP, Santini D, Leone O, Stein H, Falini B : Frequent expression of the NPM-ALK chimeric fusion protein in anaplastic large-cell lymphoma, lympho-histiocytic type. Am J Pathol **150** : 1207-1211, 1997
32) Sadahira Y, Hata S, Sugihara T, Manabe T : Bone marrow involvement in NPM-ALK-positive lymphoma : report of two cases. Pathol Res Pract **195** : 657-661, 1999
33) Harris N : Diagnosis and classification of lymphomas. *In* Diagnostic Immunopathology, second edition, edited by Colvin RB, Bhan AK, and McCluskey RT, Raven Press, New York. 1995, p501-534
34) Ashton-Key M, Jessup E, Isaacson PG : Immunoglobulin light chain staining in paraffin-embedded tissue using a heat mediated epitope retrieval method. Histopathology **29** : 525-531, 1996
35) 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 **58** : 183-192, 1984
36) O'Briain DS, Murphy C : L26 (CD20) staining of Bouin fixed bone marrow biopsies. J

Clin Pathol **52**: 931-932, 1999
37) Quintanilla-Martinez L, Preffer F, Rubin D, Ferry JA, Harris NL: $CD20^+$ T cell lymphoma. Neoplastic transformation of a normal T cell subset. Am J Clin Pathol **102**: 483-489, 1994
38) Ellison DJ, Sharpe RW, Robbins BA, Spinosa JC, Leopard JD, Saven A, Piro LD: Immunomorphologic analysis of bone marrow biopsies after treatment with 2-chlorodeoxyadenosine for hairy cell leukemia. Blood **84**: 4310-4315, 1994
39) Engel P, Tedder TF: New CD from the B cell section of the Fifth International Workshop on Human Leukocyte Differentiation Antigens. Leuk Lymphoma 13 Suppl **1**: 61-64, 1994
40) Mason DY, Cordell JL, Brown MH, Borst J, Jones M, Pulford K, Jaffe E, Ralfkiaer E, Dallenbach F, Stein H: CD79a: a novel marker for B cell neoplasms in routinely processed tissue samples. Blood **86**: 1453-1459, 1995
41) Jones M, Mason DY: CD10: workshop panel report. *In* Leukocyte Typing VI White cell differentiation antigens, edited by Kishimoto T, Kikutani H, Vondem Borne AEG, Goyert SM, Mason DY, Miyasaka M, Moretta L, Okumura K, Shaw S, Springer TA, Sugamura K, Zola H. New York, Garland Publishing. 1998, pp 131-132
42) McIntosh GG, Lodge AJ, Watson P, Hall AG, Wood K, Anderson JJ, Angus B, Horne CH, Milton ID: NCL-CD10-270: a new monoclonal antibody recognizing CD10 in paraffin-embedded tissue. Am J Pathol **154**: 77-82, 1999
43) Kaufmann O, Flath B, Spath-Schwalbe E, Possinger K, Dietel M: Immunohistochemical detection of CD10 with monoclonal antibody 56C6 on paraffin sections. Am J Clin Pathol **111**: 117-122, 1999
44) Saito T, Yamazaki T: CD3: workshop panel report. *In* Leukocyte Typing VI White cell differentiation antigens, edited by Kishimoto T, Kikutani H, Vondem Borne AEG, Goyert SM, Mason DY, Miyasaka M, Moretta L, Okumura K, Shaw S, Springer TA, Sugamura K, Zola H. New York, Garland Publishing. 1998, pp 44-48
45) Mason DY, Cordell J, Brown M, Pallesen G, Ralfkiaer E, Rothbard J, Crumpton M, Gatter KC: Detection of T cells in paraffin wax embedded tissue using antibodies against a peptide sequence from the CD3 antigen. J Clin Pathol **42**: 1194-1200, 1989
46) Kurtin PJ, Roche PC: Immunoperoxidase staining of non-Hodgkin's lymphomas for T cell lineage associated antigens in paraffin sections. Comparison of the performance characteristics of four commercially available antibody preparations. Am J Surg Pathol **17**: 898-904, 1993
47) Chadburn A, Knowles DM: Paraffin-resistant antigens detectable by antibodies L26 andpolyclonal CD3 predict the B- or T cell lineage of 95% of diffuse aggressive non-Hodgkin's lymphomas. Am J Clin Pathol **102**: 284-291, 1994
48) Clevers H, Alarcon B, Wileman T, Terhorst C: The T cell receptor / CD3 complex: a dynamic protein ensemble. Annu Rev Immunol **6**: 629-662, 1988
49) Said JW, Shintaku IP, Parekh K, Pinkus GS: Specific phenotyping of T cell proliferations in formalin-fixed paraffin-embedded tissues. Use of antibodies to the T cell receptor βF1. Am J Clin Pathol **93**: 382-386, 1990
50) Macon WR, Salhany KE: T cell subset analysis of peripheral T cell lymphomas by paraffin section immunohistology and correlation of CD4 / CD8 results with flow cytometry. Am J Clin Pathol **109**: 610-617, 1998
51) Chott A, Augustin I, Wrba F, Hanak H, Ohlinger W, Radaszkiewicz T: Peripheral T cell lymphomas: a clinicopathologic study of 75 cases. Hum Pathol **21**: 1117-1125, 1990
52) Clark JR, Williams ME, Swerdlow SH: Detection of B-and T cells in paraffin-embedded tissue sections. Diagnostic utility of commercially obtained 4KB5 and UCHL-1. Am J Clin Pathol **93**: 58-69, 1990
53) Piatier-Tonneau D: CD4: workshop panel report. *In* Leukocyte Typing VI White cell differentiation antigens, edited by Kishimoto T, Kikutani H, Vondem Borne AEG, Goyert SM, Mason DY, Miyasaka M, Moretta L, Okumura K, Shaw S, Springer TA, Sugamura K, Zola H. New York, Garland Publishing. 1998, pp 49-54
54) Izban KF, Hsi ED, Alkan S: Immunohistochemical analysis of mycosis fungoides on paraffin-embedded tissue sections. Mod Pathol **11**: 978-982, 1998
55) Nakauchi H: CD8: workshop panel report. *In* Leukocyte Typing VI White cell differentiation antigens, edited by Kishimoto T, Kikutani H, Vondem Borne AEG, Goyert SM, Mason DY, Miyasaka M, Moretta L, Okumura K, Shaw S, Springer TA, Sugamura K, Zola H. New York, Garland Publishing. 1998, pp 65-67
56) Mason DY, Cordell JL, Gaulard P, Tse AG, Brown MH: Immunohistological detection of human cytotoxic / suppressor T cells using antibodies to a CD8 peptide sequence. J

Clin Pathol **45** : 1084-1088, 1992
57) Edelman GM : Cell adhesion molecules in the regulation of animal form and tissue pattern. Annu Rev Cell Biol **2** :81-116, 1986
58) Tsang WY, Chan JK, Ng CS, Pau MY : Utility of a paraffin section-reactive CD56 antibody (123C3) for characterization and diagnosis of lymphomas. Am J Surg Pathol **20** : 202-210, 1996
59) Sadahira Y, Wada H, Nakamura E, Terayama K, Sugihara T, Yamada O, Mikami Y, Shirabe T: Nasal NK / T cell lymphoma presenting as transverse myelopathy. Virchows Archiv **436** : 393-397, 2000
60) Chan JK, Sin VC, Wong KF, Ng CS, Tsang WY, Chan CH, Cheung MM, Lau WH : Nonnasal lymphoma expressing the natural killer cell marker CD56 : a clinicopathologic study of 49 cases of an uncommon aggressive neoplasm. Blood **89** : 4501-4513, 1997
61) Hammer RD, Vnencak-Jones CL, Manning SS, Glick AD, Kinney MC : Microvillous lymphomas are B cell neoplasms that frequently express CD56. Mod Pathol **11** :239-246, 1998
62) Van Camp B, Durie BG, Spier C, De Waele M, Van Riet I, Vela E, Frutiger Y, Richter L, Grogan TM : Plasma cells in multiple myeloma express a natural killer cell-associated antigen : CD56 (NKH-1 ; Leu-19). Blood **76** : 377-382, 1990
63) Anderson P, Nagler-Anderson C, O'Brien C, Levine H, Watkins S, Slayter HS, Blue ML, Schlossman SF : A monoclonal antibody reactive with a 15-kDa cytoplasmic granule-associated protein defines a subpopulation of $CD8^+$ T lymphocytes. J Immunol **144** : 574-582, 1990
64) Felgar RE, Macon WR, Kinney MC, Roberts S, Pasha T, Salhany KE : TIA-1 expression in lymphoid neoplasms. Identification of subsets with cytotoxic T lymphocyte or natural killer cell differentiation. Am J Pathol **150** : 1893-1900, 1997
65) Sadahira Y, Ohmoto K, Yamamoto S, Nishihara H, Wada H, Yawata Y, Manabe T : Expression of cytotoxic molecule TIA-1 in malignant lymphomas mimicking fulminant hepatitis. Pathol Int **48** : 695-705, 1998
66) Kummer JA, Kamp AM, Tadema TM, Vos W, Meijer CJ, Hack CE : Localization and identification of granzymes A and B-expressing cells in normal human lymphoid tissue and peripheral blood. Clin Exp Immunol **100** : 164-172, 1995
67) de Bruin PC, Kummer JA, van der Valk P, van Heerde P, Kluin PM, Willemze R, Ossenkoppele GJ, Radaszkiewicz T, Meijer CJ : Granzyme B-expressing peripheral T cell lymphomas : neoplastic equivalents of activated cytotoxic T cells with preference for mucosa-associated lymphoid tissue localization. Blood **84** : 3785-3789, 1994
68) Jaffe ES, Krenacs L, Raffeld M : Classification of T cell and NK-cell neoplasms based on the REAL classification. Ann Oncol **8** (Suppl 2) : 17-24, 1997
69) Kannagi R, Nudelman E, Levery SB, Hakomori S : A series of human erythrocyte glycosphingolipids reacting to the monoclonal antibody directed to a developmentally regulated antigen SSEA-1. J Biol Chem **257** : 14865-14874, 1982
70) Sadahira Y, Sugihara T, Yawata Y, Manabe T : Cutaneous granulocytic sarcoma mimicking immunoblastic large cell lymphoma. Pathol Internat **49** : 347-353, 1999
71) Pinkus GS, Thomas P, Said JW : Leu-M1-a marker for Reed-Sternberg cells in Hodgkin's disease. An immunoperoxidase study of paraffin-embedded tissues. Am J Pathol **119** : 244-252, 1985
72) Rushin JM, Riordan GP, Heaton RB, Sharpe RW, Cotelingam JD, Jaffe ES : Cytomegalovirus-infected cells express Leu-M1 antigen. A potential source of diagnostic error. Am J Pathol **136** : 989-995, 1990
73) Sadahira Y, Nishihara H, Shimizu M, Hirokawa M, Wada H, Yamada O, Yawata Y, Manabe T : Epstein-Barr virus-associated Hodgkin's disease in HTLV-I seropositive patients : a report of two cases. Pathol Internat **48** : 65-71, 1998
74) Fukuda M : Lysosomal membrane glycoproteins. Structure, biosynthesis, and intracellular trafficking. J Biol Chem **266** : 21327-1330, 1991
75) Warnke RA, Pulford KA, Pallesen G, Ralfkiaer E, Brown DC, Gatter KC, Mason DY : Diagnosis of myelomonocytic and macrophage neoplasms in routinely processed tissue biopsies with monoclonal antibody KP1. Am J Pathol **135** : 1089-1095, 1989
76) Sadahira Y, Wada H, Manabe T, Yawata Y : Immunohistochemical assessment of human bone marrow macrophages in hematological disorders. Pathol Int **49** : 626-632, 1999
77) Falini B, Flenghi L, Pileri S, Gambacorta M, Bigerna B, Durkop H, Eitelbach F, Thiele J, Pacini R, Cavaliere A : PG-M1 : a new monoclonal antibody directed against a fixative-resistant epitope on the macrophage-restricted form of the CD68 molecule. Am J Pathol **142** : 1359-1372, 1993

78) Pinkus GS, Pinkus JL : Myeloperoxidase : a specific marker for myeloid cells in paraffin sections. Mod Pathol **4** : 733-741, 1991
79) Hudock J, Chatten J, Miettinen M : Immunohistochemical evaluation of myeloid leukemia infiltrates (granulocytic sarcomas) in formaldehyde-fixed, paraffin-embedded tissue. Am J Clin Pathol **102** : 55-60, 1994
80) Van Eyken P, De Wolf-Peeters C, Van den Oord J, Tricot G, Desmet V : Expression of leukocyte common antigen in lymphoblastic lymphoma and small noncleaved undifferentiated non-Burkitt's lymphoma : an immunohistochemical study. J Pathol **151** : 257-261, 1987
81) Giorno R, Sciotto CG : Use of monoclonal antibodies for analyzing the distribution of the intermediate filament protein vimentin in human non-Hodgkin's lymphomas. Am J Pathol **120** : 351-355, 1985
82) Delsol G, Gatter KC, Stein H, Erber WN, Pulford KA, Zinne K, Mason DY : Human lymphoid cells express epithelial membrane antigen. Implications for diagnosis of human neoplasms. Lancet **2 (8412)** : 1124-1129, 1984
83) Lasota J, Hyjek E, Koo CH, Blonski J, Miettinen M : Cytokeratin-positive large-cell lymphomas of B cell lineage. A study of five phenotypically unusual cases verified by polymerase chain reaction. Am J Surg Pathol **20** : 346-354, 1996
84) Chan JK : Gastrointestinal lymphomas : an overview with emphasis on new findings and diagnostic problems. Semin Diagn Pathol **13** : 260-296, 1996
85) Bakels V, van Oostveen JW, van der Putte SC, Meijer CJ, Willemze R : Immunophenotyping and gene rearrangement analysis provide additional criteria to differentiate between cutaneous T cell lymphomas and pseudo-T cell lymphomas. Am J Pathol **150** : 1941-1949, 1997
86) Picker LJ, Brenner MB, Weiss LM, Smith SD, Warnke RA : Discordant expression of CD3 and T cell receptor beta-chain antigen in T cell lymphoma. Am J Pathol **129** : 434 -440, 1987
87) Rudiger T, Ott G, Ott MM, Muller-Deubert SM, Müller-Hermelink HK : Differential diagnosis between classic Hodgkin's lymphoma, T cell-rich B cell lymphoma, and paragranuloma by paraffin immunohistochemisty. Am J Surg Pathol **22** : 1184-1191, 1998
88) Rudiger T, Jaffe ES, Delsol G, de Wolf-Peeters C, Gascoyne RD, Georgii A, Harris NL, Kadin ME, MacLennan KA, Poppema S, Stein H, Weiss LE, Müller-Hermelink HK : Workshop report on Hodgkin's disease and related diseases ('grey zone' lymphoma). Ann Oncol 9 Suppl **5** : S31-38, 1998
89) Chen CC, Raikow RB, Sonmez-Alpan E, Swerdlow SH : Classification of small B cell lymphoid neoplasms using a paraffin section immunohistochemical panel. Appl Immunohistochem Mol Morphol **8** : 1-11, 2000