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Comparison of monoclonal antibodies reactive with lymphocyte subsets in routinely fixed paraffin-embedded material: flow cytometric analyses, immunoperoxidase staining and influence of fixatives.

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Abstract

We have attempted to clarify the characteristics of monoclonal antibodies (MAbs) detecting lymphocyte subsets in fixed materials. We examined by means of flow cytometric technique influences of fixatives and reactivity with malignant lymphomas (MLs). Specific markers for T-cells were UCHL1 and OPD4, which reacted especially with helper/inducer T-cells. MT1 recognized almost all of T-cells from peripheral blood and tonsils, but reacted with a part of B-MLs. As for B-cell markers, L26 was the most reliable marker for B-MLs. L26 and MB1 antigens could not be detected on living cells flow cytometrically. LN1 reacted with a part of T-cells as well as B-cells, but fluorescent intensity of the former was apparently stronger than that of the latter. Although LN2 antigen was located mainly in the cytoplasm close to the nuclear membrane immunohistochemically, it could be detected on living cells flow cytometrically. LN2 positive cells belonged to B-cells in peripheral blood and tonsils. When fixed for relatively short time, B5 and buffered formalin were better for examining MAbs than non-buffered formalin and ethanol.

KEYWORDS: monoclonal antibodies, lymphocyte subset, flow cytometry

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Comparison of Monoclonal Antibodies Reactive with Lymphocyte Subsets in Routinely Fixed Paraffin-Embedded Material: Flow cytometric analyses, Immunoperoxidase Staining and Influence of Fixatives

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We have attempted to clarify the characteristics of monoclonal antibodies (MAbs) detecting lymphocyte subsets in fixed materials. We examined by means of flow cytometric technique influences of fixatives and reactivity with malignant lymphomas (MLs). Specific markers for T-cells were UCHL1 and OPD4, which reacted especially with helper/inducer T-cells. MT1 recognized almost all of T-cells from peripheral blood and tonsils, but reacted with a part of B-MLs. As for B-cell markers, L26 was the most reliable marker for B-MLs. L26 and MB1 antigens could not be detected on living cells flow cytometrically. LN1 reacted with a part of T-cells as well as B-cells, but fluorescent intensity of the former was apparently stronger than that of the latter. Although LN2 antigen was located mainly in the cytoplasm close to the nuclear membrane immunohistochemically, it could be detected on living cells flow cytometrically. LN2 positive cells belonged to B-cells in peripheral blood and tonsils. When fixed for relatively short time, B5 and buffered formalin were better for examining MAbs than non-buffered formalin and ethanol.

Key words : **monoclonal antibodies, lymphocyte subset, flow cytometry**

Many monoclonal antibodies (MAbs) have been produced against lymphocyte-membrane antigens to characterize the phenotype in connection with cell differentiation and function. Most of MAbs, however, can be used only on frozen sections or cell suspension because antigens detected by these MAbs are vulnerable to fixation and paraffin embedded procedure. Cryopreserved tissues, however, have a limitation in the practice of routine surgical pathology; the disadvantage of poor preservation of cell morpholoygy: Also biopsied specimens frequently arrive at our laboratories in fixed conditions.

Some commercially available MAbs can be used for detecting lymphocyte subsets in routinely fixed material. These include MAb to leukocyte common antigen (LCA) (CD45) (1,2) for a lymphoid origin, UCHL1 (CD45RO) (3,4) for T-cells, MB1 (CD45R) (5), LN1 (CDw75), LN2 (CD74) (6), and L26 (PanB) (7) for B-cells, and MT1 (CD43) (5) for activated lymphocytes. We have also prepared a MAb, OPD4, detecting helper/inducer (H/I) T-cells(8). These MAbs have been characterized by immunoperoxidase staining on tissue sections (1,3, 5-9), by flow cytometric analysis on cell lines and/or mononu-

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clear cells from peripheral blood (PB) (4,6–8), and by biochemical analysis of antigens (2,6–9). But the specificity of these reagents has not been well examined by flow cytometric analysis especially on peripheral lymphoid tissues in which most malignant lymphomas (MLs) occur. We have attempted to clarify the reactivity of these reagents with lymphocytes from tonsils as well as from PB. We also examined influence of fixatives and reactivity with well-characterized lymphoid malignancies.

Materials and Methods

Flow cytometric analysis. Lymphoid-cell suspension was prepared without enzyme treatment and by teasing palatine tonsils surgically resected from three individuals. Histologically, resected tonsils showed follicular hyperplasia. Mononuclear cells from PB were prepared by Ficoll-Hypaque density gradient centrifugation.

Approximately 10^6 mononuclear cells were incubated with 100 μl of diluted MAbs for 30 min at 4 °C. Dilution ratios and sources of examined MAbs are listed in Table 1. The cells were washed and incubated with fluorescein isothiocyanate (FITC)-conjugated F (ab′)$_2$ fragment of goat anti-mouse IgG diluted 1:20 (Tago Inc, Burlingame, CA, USA). After washing further, they were allowed to react with 50 μl of phycoerythrin (PE)-conjugated anti-T-cell and B-cell MAbs for 30 min at 4 °C. The PE-conjugated MAbs included CD3 (Leu4), CD4 (Leu3a), CD8 (Leu2a), CD20 (Leu16), and HLA-DR (Becton-Dickinson, Mountain View, CA, USA). The samples were analyzed by Epics 753 (Coulter Electrics, Inc., Hialeah FL) with simultaneous excitation using 488nm argon laser (300mW). The analyses were gated by using forward and 90° light scatter to exclude dead cells.

Table 1 Examined monoclonal antibodies reactive with routinely embedded materials

Monoclonal antibody	CD	Dilution	Commercial source[a]	References
LCA	45RB+CD45	1:25	D	1, 2
UCHL1	45RO	1:25	D	3, 4
MT1	43	1:20	B	5
OPD4	[b]	1:100	[c]	8
PanB (L26)	[b]	1:100	K	7
MB1	45R	1:20	B	5
LN1	$_w$75	Undiluted	T	6
LN2	74	Undiluted	T	6

a: Commercial sources of monoclonal antibodies. D = DAKOPATTS, Glostrup, Denmark; B = Bio-Science, Emmenbrucke, Switzerland; K = Kyowa Medics, Tokyo, Japan; T = Techniclone International, Santa Ana, CA.
b: Not clustered at the International Third Workshop.
c: In preparation

Influence of fixatives. To determine the optimal fixation conditions, tissue blocks of tonsils were fixed at room temperature for 1, 3, 6, 14, 21, 60 and 96 h in the following fixatives: 1) 10 % buffered formalin; 10 ml of 37 % formaldehyde solution and 90 ml of phosphate buffer ($NaH_2PO_4H_2O$ 0.4 g and Na_2HPO_4 0.65 g in 90 ml of aqua dest), pH 7.2. 2) 10 % non-buffered formalin: 10 ml of 37 % formaldehyde solution and 90 ml of tap water. 3) B5 fixative: 90 ml of aqua dest, 6 g of mercuric chloride, 2.074 g of sodium acetate ($CH_3COONa\ 3H_2O$), and 10 ml of 37 % formaldehyde solution, pH 5.7. 4. 100 % ethanol.

The intensity of immunoreactivity of MAbs was graded as good, weak, or very weak staining.

Immunoperoxidase staining. After deparaffinization, specimens were cut by 3 μm in thickness, and non-specific binding of immunoglobulin was blocked by a 10 min-preincubation with normal goat serum. After blocking endogenous peroxidase activity with 0.3 % H_2O_2-methanol, tissue sections were incubated with each MAb for 2 h at room temperature. They were washed with TBS (phosphate buffered saline with 0.05 % of tween 20) and were reacted with biotinylated anti-mouse immunoglobulins (Biogenex laboratories, San Ramon, CA, USA) for 30 min. After washing with TBS, peroxidase-conjugated streptavidin (Biogenex Laboratories, San Ramon, CA, USA) was applied for 30 min. Peroxidase was detected by 1.26 mM 3.3′-diaminobenzydine tetrahydrochloride and 0.005 % H_2O_2.

Lymphoma samples. We chose non-Hodgkin's lymphoma cases from the files of our laboratory. All the cases had been obtained within the last 2 years and fresh materials had been well characterized by immunohistological analysis for CD1, 3, 4, 5, 8, 10, 19, 20, 22, HLA-DR, and light chains of immunoglobulins, and by immuno-genotypical analysis of T-cell and B-cell receptor gene rearrangement.

Results

Cytofluorometric analysis of MAbs reactivity with tonsil and PB lymphocytes. The reactivity

of all reagents is summarized in Tables 2 and 3, and concrete flow cytometric patterns are shown in Fig. 1. L26 (Pan B) and MB1 failed to react with living cells.

Reactivity of reagents on mononuclear cells from tonsils: UCHL1, MT1 and OPD4 recognized 69 %, 88 % and 57 % of $CD3^+$ (T) cells, respectively. UCHL1 and OPD4 recognized a part of T-cells, a majority of which belonged to a helper/inducer T-cell subset; UCHL1 and OPD4 recognized 77 % and 67 % of $CD4^+$ cells, and 33 % and 20 % of $CD8^+$ cells, respectively (Figs 1-C, D, G, H). As $CD4^+$ cells occupied 81 % of T-cells in tonsils, 91 % of $UCHL1^+$ cells and 95 % of $OPD4^+$ cells were $CD4^+$, and only 9 % of $UCHL1^+$ and 5 % of $OPD4^+$ cells belonged to $CD8^+$ T-cells. On the other hand, MT1 recognized majority of T-cells (Fig. 1-K); 91 % of $CD4^+$ and 70 % of $CD8^+$ cells. LN1 reacted to 63 % of $CD20^+$ B-cells (Fig. 1-Q) and 8 % of $CD3^+$ cells. LN2 reacted with 43 % of $CD20^+$ cells but not reacted with T cells (Fig. 1-T).

Reactivity of reagents on PBLs: UCHL1, MT1, and OPD4 recognized 52 %, 98 % and 35 % of $CD3^+$ T-cells and 60 %, 98 % and 44 % of $CD4^+$ T-cells, respectively (Figs. 1-A, B, E, F, I, J). None of these reagents reacted with $CD20^+$ B-cells. As $CD4^+$ and $CD8^+$ cells occupied 46 %

Table 2 Flow cytometric analysis of reactivity of MAbs with mononuclear cells from tonsils; Relationship between the reactivity with examined MAbs and T and B-cell subsets

MAb	MAb+/CD3+[a]	MAb+/CD4+	MAb+/CD8+	MAb+/CD20+
UCHL1	69%	77%	33%	< 2%
OPDA4	57%	67%	20%	< 2%
MT1	88%	91%	70%	< 2%
LN1	8%	ND[b]	ND	63%
LN2	< 2%	ND	ND	43%

a: % of cells expressing both certain MAb (*e. g.* UCHL1)-related antigen and CD3 per CD3+ cells

b: Not done

Each value represents the average of triplicates. Average proportions of CD3, 4, 8, 20, and HLA-DR positive cells in mononuclear cells from tonsils were 27 %, 22 %, 5 %, 18 %, and 62 %, respectively.

Table 3 Flow cytometric analysis of reactivity of MAbs with PBLs; Relationship between the reactivity with MAbs and T and B-cell subsets

MAb	MAb+/CD3+[a]	MAb+/CD4+	MAb/CD8+	MAb+/CD20+
UCHL1	52%	60%	33%	< 2%
OPDA4	35%	44%	15%	< 2%
MT1	>98%	>98%	>98%	< 2%
LN1	11%	ND[b]	ND	70%
LN2	< 2%	ND	ND	74%

a: % of cells expressing both certain MAb (*e. g.* UCHL1)-related antigen and CD3 per CD3+ cells

b: ND means not done

Average of CD3, 4, 8, and 20 positive cells in PBLs were 66 %, 46 %, 20 %, and 18 %, respectively.

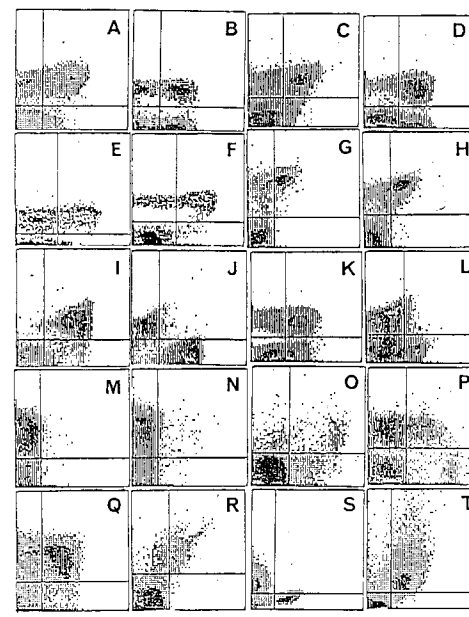

Fig. 1 Dual color flow cytometric analysis of peripheral blood lymphocytes and mononuclear cells from a tonsil. Examined MAbs (UCHL1, OPD4, MT1, L26, MB1, LN1, LN2)-FITC (green fluorescence) on the x-axis and other lymphocyte markers (Leu series)-PE (red fluorescence) on the y-axis plotted in logarithmic units. A: UCHL1, CD3 (Leu4), PBL; B: UCHL1, CD4 (Leu3a), PBL; C: UCHL1, CD3 (Leu4), tonsil; D: UCHL1, CD4 (Leu3a), tonsil; E: OPD4, CD3 (Leu4), PBL; F: OPD4, CD4 (Leu3a), PBL; G: OPD4, CD3 (Leu4), tonsil; H: OPD4, CD4 (Leu3a), tonsil; I: MT1, CD3 (Leu4), PBL; J: MT1, CD20 (Leu16), PBL; K: MT1, CD3 (Leu4), tonsil; L: MT1, CD20 (Leu16), tonsil; M: L26, CD20 (Leu16), tonsil; N: MB1, CD20 (Leu16), tonsil; O: LN1, CD20 (Leu16), PBL; P: LN1, CD3 (Leu4), PBL; Q: LN1, CD20 (Leu16), tonsil; R: LN2, CD20 (Leu16), PBL; S: LN2, CD3 (Leu4), PBL; T: LN2, CD20 (Leu16), tonsil.

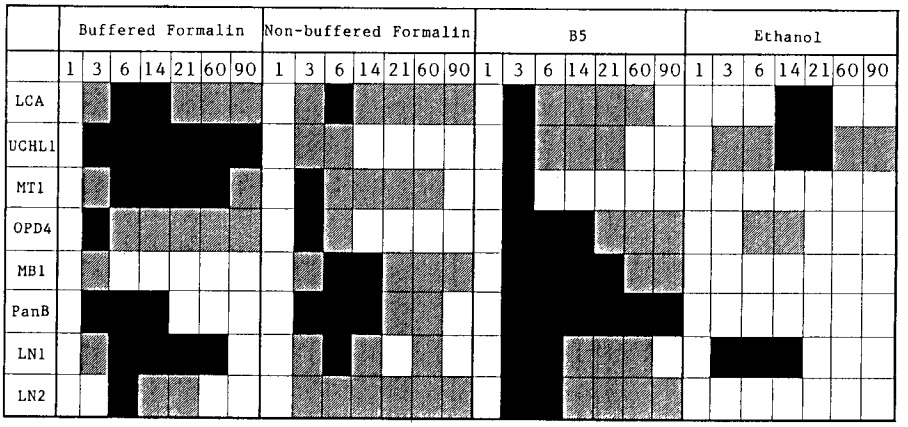

Fig. 2 Influence of fixation on the immunohistochemical reactivity of monorlonal antibodies (MAbs). Tissue blocks approximately 1 cm across of surgically resected tonsils were fixed in each fixative for 1 to 90 h, and paraffin-embedded sections were stained with MAbs. The stainability was evaluated as follows; black squares denote good staining, gray squares denote weak staining, and open squares denote very weak staining or poor morphological preservation. LCA : leukocyte common antigen.

Table 4 Immunophenotype and histological classification of examined non-Hodgkin's lymphomas[a]

	Number of Cases	
	B-cell phenotype	T-cell phenotype
Follicular Lymphomas	8	0
Diffuse lymphomas		
Diffuse small	3	0
Diffuse medium	3	7
Diffuse mixed	9	7
Diffuse large	35	4
Burkitt	2	0
Lymphoblastic	0	3
IBL-like T	0	3
CTL[b]	0	3
Others[c]	2	3
Number of total cases	62	30

a: Lymphomas were classified according to the LSG (Japanese lymphoma study group) classification (21) with modification.
b: Cutaneous T-cell lymphomas
c: Include 3 cases of adult T-cell lymphomas, 1 case of hairy cell leukemia, and 1 case of plasmacytoma.

and 20 % of PBLs, respectively, approximately 81 % of UCHL1$^+$ cells belonged to CD4$^+$ cells, and 87 % of OPD4$^+$ cells belonged to CD4$^+$ cells. LN1 reacted with 70 % of B-cells and 11 % of T-cells, but it reacted with CD20$^+$ cells more intensively than with CD3$^+$ cells (Figs. 1-O, P). LN2 reacted with 74 % of B-cells but not with T-cells (Figs. 1-R, S).

Influence of fixatives. The results are summarized in Fig. 2. One hour of fixation resulted in poor morphological preservation although immunohistochemical reactivity could be more or less detected. Thus, we omitted evaluation of the results in 1h-fixed materials. As a whole, intense stainability could be obtained in buffered formalin and B5 fixations. Ten percent of buffered formalin fixation for 3 to 14 h showed generally good reactivity, although it was relatively unsuitable for MB1. Non-buffered formalin was not satisfactory for UCHL1 and LN2 and decreased the reactivity of all the MAbs after fixation of more than 14 h. B5 fixation for 3 h resulted in excellent preservation of morphology and optimal immunohistochemical results. Ethanol fixation was as a whole unsuitable for immunohistochemical examination.

The fixatives affected not only intensity of reactivity but distribution of antigens. In B5-fixed sections, LN2 reacted with almost whole nuclear

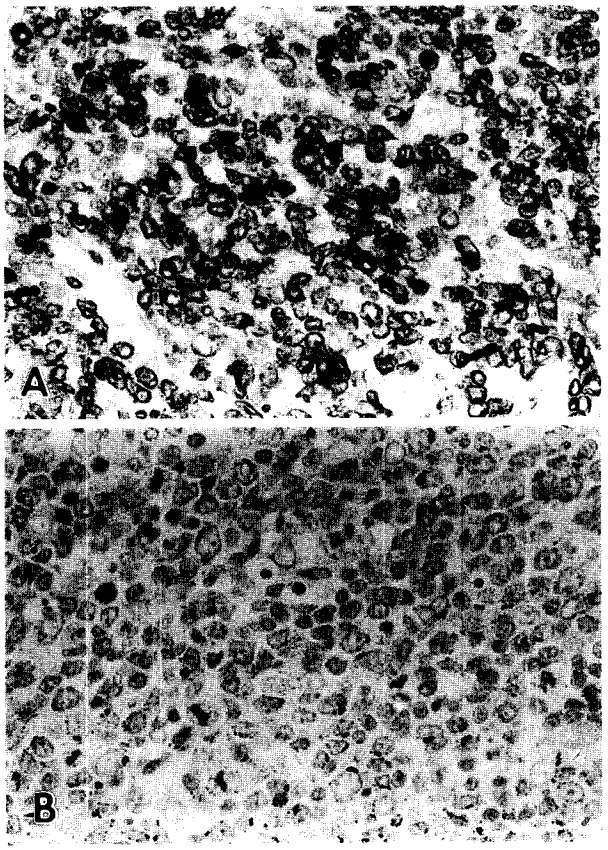

Fig. 3 Immunohistochemical localization of LN2 antigen. Germinal center cells of a tonsil fixed in B5 fixative for 3h (A) and non-buffered formalin for 6h (B). Streptavidin-biotin staining. ×450.

Table 5 Reactivity of MAbs with non-Hodgkin's lymphomas

Monoclonal antibody	B-cell lymphonas (n=62)		T-cell lymphonas (n=30)	
	Number of positive cases	Percent of positive cases	Number of positive cases	Percent of positive cases
LCA	59	95	23	77
UCHL1	2	3	20	67
OPD4	3	5	14	47
MT1	16	26	28	93
PanB(L26)	58	94	0	0
MB1	37	60	2	7
LN1[a]	29	81	2	7
LN2[b]	35	90	5	27

a: LN1 was examined in 36 B-MLs
b: LN2 was examined in 39 B-MLs and 18 T-MLs

membranes and perinuclear cytoplasm of B-cells. In formalin-fixed sections, on the other hand, LN2 antigen gathered to form a dot-like pattern near the nucleus (Fig. 3).

The reactivity of reagents with MLs. We have examined immunohistologically the reactivity of MAbs on 92 cases of non-Hodgkin's lymphomas(NHLs); 62 cases of B-cell and 30 cases of T-cell lymphomas. Histological classification of these cases were summarized in Table 4, and reactivity in Table 5.

LCA reacted with 89% of NHLs; 95% of B-MLs and 77% of T-MLs. UCHL1 and OPD4 reacted with 67% and 47% of T-MLs, respectively; whereas, only 3% and 5% of B-MLs were positive for UCHL1 and OPD4,

respectively. They were negative for 3 cases of $CD4^-/CD8^+$ (suppressor/cytotoxic) T-MLs in this series. MT1 recognized 93 % of T-MLs, but reacted with 26 % of B-MLs; the latter included 2 cases of follicular, one diffuse small, 3 diffuse mixed, and 10 diffuse large-cell lymphomas. L26 recognized 58 cases out of 62 B-MLs (94 %), but reacted with none of T-MLs. MB1 reacted with 60 % of B-MLs and 7 % of T-MLs. LN1 recognized 81 % of B-MLs and 7 % of T-MLs. LN2 reacted with 90 % of B- and 27 % of T-MLs.

Discussion

We have examined flow cytometrically the specificity of MAbs which can be applied to routinely fixed, paraffin-embedded materials. The effects of fixatives on reactivity of MAbs and of malignant lymphomas were also investigated.

UCHL1 and OPD4 recognized a part of T-cell subset. UCHL1 is a mouse IgG2a monoclonal antibody, recognized 180kDa antigen (3), and belongs to CD45RO (10). OPD4 has not yet been clustered at the International Workshop. This MAb is an IgG1 subclass and marks approximately 200kDa antigen (8). UCHL1 recognized 34 % of PBLs, 60 % of $CD4^+$ T-cells and 33 % of $CD8^+$ T-cells from peripheral blood. This result was essentially the same as that of Smith *et al.* (4). The majority of $OPD4^+$ cells also belonged to $CD4^+$ T-cells (8). On average, 87 % of $OPD4^+$ cells were $CD4^+$. The data obtained from flow cytometric analysis of tonsils were comparable with this result. UCHL1 and OPD4 reacted with approximately 60–70 % of all T-cells and 70–80 % of $CD4^+$ T-cells from tonsils. Therefore, UCHL1 and OPD4 are suitable markers for helper/inducer T-cells.

The percentages of $UCHL1^+$ and $OPD4^+$cells in $CD4^+$ cells from tonsils were higher than those from peripheral blood. Both $UCHL1^+$ and $OPD4^+$ cells have helper function for immunoglobulin synthesis (4, 8). Therefore, T-cells in examined tonsils may have higher helper function; in fact, the resected tonsils showed marked follicular hyperplasia.

UCHL1 and OPD4 recognized a part of T-MLs. In the previous reports, UCHL1 marked 63 to 83 % of T-MLs (3, 11, 12). In the present study, UCHL1 and OPD4 recognized approximately 70 % and 50 % of T-MLs, respectively. OPD4 marked about one half of T-MLs (8). Interestingly, in our series, 3 cases of $CD4^-$, 8^+ MLs were negative for UCHL1 and OPD4. Positivity rate of OPD4 in T-MLs was smaller than that of UCHL1; this may coincide with the reactivity of these reagents with normal T-lymphocytes. On the other hand, these reagents occasionally reacted with B-MLs as reported previously, suggesting an 'aberrant' expression (8, 11–15).

Concerning the effects of fixatives, Andrade (11) reported that B5 fixative was unsuitable for UCHL1, while Linder *et al.* (13) described UCHL1 reacted with most of T-MLs fixed in B5 (13). We found specimens B5-fixed for longer than 21 h diminished the reactivity of UCHL1; this accounts for the discrepancy between the observations made by Andrade and Linder *et al.*

MT1, which was formerly thought to belong to CD45, is currently classified as CD43 (16). The CD43 antigen has been cloned and is significant in acting lymphocytes. Flow cytometric analysis revealed that MT1 recognized almost all the T-cells from peripheral blood and tonsils, and that B-cells were negative for MT1. This result was compatible with that of immunoperoxidase staining on tissue sections described by Poppema *et al.* (9). Besides T-cells, MT1 reacted with 4 to 10 % of $CD3^-$ and $CD20^-$ cells from tonsils; these $MT1^+$ cells were considered macrophage-histiocytic cells.

In the present study, MT1 recognized over 90 % of T-MLs and approximately one-fourth of B-MLs although some authors (5, 17) reported that MT1 reacted with only a small part of B-MLs. This result is almost the same as the previous reports by Poppema, Andrade (11),

Dobson (18), and Norton (15). This discrepancy might be based on the difference in examined cases. From our results by flow cytometric and immunohistochemical methods, MT1 reacted with peripheral T-cells, but did not react with B-cells. The reason why MT1 recognized a part of B-MLs remains unclear. Ng (17) reported that MT1 reacted with most of T-MLs after short fixation, wheras it reacted with only about one half of T-MLs after long fixation. We found MT1 reactivity diminished when fixed in buffered formalin for longer than 60 h.

MB1 and L26 (PanB) are widely used and useful markers for detecting B cell phenotype. MB1 recognizes 200, 110, and 100kDa antigens and is classified as CD45R (16). L26 recognizes 32, 35 and 43 kDa antigens (7, 16). As Ishii (7) described, flow cytometric analysis revealed that these reagents failed to mark surface membrane of living cells. From our results, L26 recognized most of B-MLs and none of T-MLs. In the previous report (15), L26 marks sporadically T-MLs. In B-MLs, plasmacytic tumors were devoid of L26 immunoreactivity as stated by Cartun *et al.* (19). MB1 reacted with all the B-cells and about 50 % of T-cells (9), and centroblasts and immunoblasts were more weakly stained than smaller mature B-cells. It recognized 60 % of B-MLs, and most of the negative cases were large cell lymphomas and plasmacytic tumors. MB1 also reacted with 2 out of 30 T-MLs in our series, being compatible with the findings reported by Dobson *et al.* (18).

The results of flow cytometric analysis with LN1 and LN2 were unexpected. LN1 is an IgM antibody detecting 45-85kDa antigens and is now classified as CDw75 (10). It is well known that LN1 recognizes follicular center cells in non-neoplastic lymphoid tissues (6, 20) and that T-cells are negative for LN1 immunohistochemically. Flow cytometric analysis revealed that LN1 recognized a part of T-cells as well as B-cells in PB. It is interesting that the fluorescent intensity of $LN1^+/CD20^+$ cells was higher than that of $LN1^+/CD3^+$ cells. Examination of mononuclear cells from tonsils revealed that most of the $LN1^+$ cells were B-cells.

LN2 is classified as CD74, and recognizes 31 to 35kDa antigen (10). We had supposed that LN2 does not react with living lymphocytes flow cytometrically, because immunoreactive products were immunohistochemically observed at nuclear membrane or perinuclear areas (6). However, LN2 unexpectedly reacted with surface antigens of living B-cells from PB and tonsils. Localization of immuoreactive products was affected by fixatives (Fig. 3), although the exact reason for this remains unclear.

LN1 recognized 29 of 36 B-MLs and 2 of 30 T-MLs. This result is almost the same as the previously reported data (15, 20). LN1 reacted not only with centroblastic/centrocytic tumors but with centrocytic and immunoblastic B-MLs. Out of 8 follicular lymphomas, 7 cases were positive for LN1. LN2 reacted with 90 % of B-MLs and 27 % of T-MLs. Andrade (11) reported that none of 10 T-MLs was positive for LN2, whereas Okon *et al.* (20) stated that a part of T-MLs were positive for LN2, and Norton *et al.* (15) stated that LN2 reacted with 11 % of low grade and 22 % of high grade T-MLs. LN2 is believed to identify the cytoplasmic invariant chain of HLA complex (10). Therefore, a part of T-MLs expressing the HLA complex could be positive for LN2.

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References

1. Salter DM, Krajewski AS and Dewar AE: Immunohistochemical staining of non-Hodgkin's lymphoma with monoclonal antibodies specific for the leukocyte common antigen. J Pathol (1985) **146**, 345–353.
2. Warnke RA, Gatter KC, Falini B, Hildreth P, Woolston RE, Pulford K, Cordell JL, Cohen B, Wolf-Peeters CD, Mason DY: Diagnosis of human lymphoma with monoclonal antileukocyte antibodies. N Engl J Med (1983) **309**, 1275–1281.
3. Norton AJ, Ramsay AD, Smith SH, Beverley PCL and

Isaacson PG: Monoclonal antibody (UCHL 1) that recognises normal and neoplastic T-cells in routinely fixed tissues. J Clin Pathol (1986) **39**, 399–405.
4. Smith SH, Brown MH, Rowe D, Callard RE and Beverley PCL: Functional subsets of human helper-inducer cells defined by a new monoclonal antibody, UCHL 1. Immunology (1986) **58**, 63–70.
5. West KP, Warford A, Fray L, Allen M, Campbell AC and Lauder I: The demonstration of B-cell, T-cell and myeloid antigen in paraffin sections. J Pathol (1986) **150**, 89–101.
6. Epstein AL, Marder RJ, Winter JN and 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 Immuol (1984) **133**, 1028–1036.
7. Ishii Y, Takami T, Yuasa H, Takei T and Kikuchi K: Two distinct antigen system in human B-lymphocytes: Identification of cell surface and intracellular antigens using monoclonal antibodies. Clin Exp Immunol (1984) **58**, 183–192.
8. Yoshino T, Mukuzono H, Aoki H, Takahashi K, Takeuchi T, Kubonishi I, Ohtsuki Y, Motoi M, Akagi T: A novel monoclonal antibody (OPD4) recognizing a helper/inducer T-cell subset. Am J Pathol (1989) **134**, 1339–1346.
9. Poppema S, Hollema H, Visser L and Vos H: Monoclonal antibodies (MT1, MT2, MB1, MB2, MB3) reactive with leukocyte subsets in paraffin-embedded tissue sections. Am J Pathol (1987) **127**, 418–429.
10. Knapp W, Rieber P, Dorken B, Schmidt RE, Stein H and Borne AEG: Towards a better definition of human leukocyte surface molecules. Immunol Today (1989) **10**, 253–258.
11. Andrade RE, Wick MR, Frizzera G and Gajl-Peczalska KJ: Immunophenotyping of hematopoietic malignancies in paraffin sections. Hum Pathol (1988) **19**, 394–402.
12. Davey FR, Gatter KC, Ralfkiaer E, Pulford KAF, Krissansen GW and Mason DY: Immunophenotyping of non-Hodgkin's lymphomas using a panel of antibodies on paraffin-embedded tissues. Am J Pathol (1987) **129**, 54–63.
13. Linder J, Ye Y, Harrington DS, Armitage JO and Weisenburger DD: Monoclonal antibodies marking T-lymphocytes in paraffin-embedded tissue. Am J Pathol (1987) **127**, 1–8.
14. Norton AJ and Isaacson PG: Detailed phenotypic analysis of B-cell lymphoma using a panel of antibodies reactive in routinely fixed wax-embedded tissue. Am J Pathol (1987) **128**, 225–240.
15. Norton AJ and Isaacson PG: Lymphoma phenotyping in formalin-fixed and paraffin wax-embedded tissues: II. Profiles of reactivity in the various tumor types. Histopathology (1989) **14**, 557–579.
16. Norton AJ and Isaacson PG: Lymphoma phenotyping in formalin-fixed and paraffin wax-embedded tissues. I. Range of antibodies and staining patterns. Histopathology (1989) **14**, 437–446.
17. Ng CS, Chan JKC, Hui PK and Lo STH: Monoclonal antibodies reactive with normal and neoplastic T-cells in paraffin sections. Hum Pathol (1988) **19**, 295–303.
18. Dobson CM, Myskow MW, Krajewski AS, Carpenter FH and Horne HW: Immunohistochemical staining of non-Hodgkin's lymphoma in paraffin sections using the MB1 and MT1 monoclonal antibodies. J Pathol (1987) **153**, 203–212.
19. Cartun RW, Coles FB and Pastuszak WT: Utilization of monoclonal antibody L26 in the identification and confirmation of B-cell lymphomas. Am J Pathol (1987) **129**, 415–421.
20. Okon E, Felder B, Epstein A, Lukes RJ and Taylor CR: Monoclonal antibodies reactive with B-lymphocytes and histiocytes in paraffin sections. Cancer (1985) **56**, 95–104.
21. Suchi T, Tajima K, Nanba K, Wakasa H, Mikata A, Kikuchi M, Mori S, Watanabe S, Mohri N, Shamoto M, Harigaya K, Itagaki T, Matsuda M, Kirino Y, Takagi K, Fukunaga S: Some problems on the histopathological diagnosis of non-Hodgkin's malignant lymphoma; A proposal of a new type. Acta Pathol Jpn (1979) **29**, 755–776.

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