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Immunohistologic Analysis of Malignant Lymphoma 1. Accuracy of Histological Diagnosis and Usefulness of Monoclonal Antibodies

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ABSTRACT. In order to test whether B cell or T cell origin of malignant lymphomas can be reliably suspected on histological basis alone, and to know the usefulness of some antibodies to lymphocytic cells which are currently available commercially, we reviewed 30 cases of malignant lymphomas both histologically and immunohistochemically. Hematoxylin-eosin stained sections from those cases were reclassified using Lukes and Collins' classification with minor modification. Then, monoclonal antibodies; LCA, MT-1, and MB-1 have been applied to paraffin sections from same cases. In addition, four cases of plasmacytoma and a case of metastatic carcinoma which resembled malignant lymphoma were studied immunohistochemically. Our results indicate that (1) in most cases, T and B cell origin of lymphoma may be accurately classified by H-E stained section although further study with more cases is definitely necessary to reach this conclusion. (2) LCA immunoreactivity seemed to be stronger in lymphomas of T cell system. (3) MT-1 and MB-1 immunoreactivity did not correlate perfectly with LCA positivity. And (4) MT-1 and MB-1 provide useful tools to dictate markers for T and B cell lymphomas in routinely fixed and paraffin-embedded tissue.

Key words : immunohistochemistry — malignant lymphoma — LCA — MT-1 — MB-1

Malignant lymphoma is a group of diseases in which heterogeneous groups of neoplastic lymphocytes are included.[1-3] Each neoplastic lymphocyte is now believed to pursue functions and cytologic appearance of normal counterpart to some extent, and to represent a phase in the transformation process of T or B lymphocytes.[1-3] These days, immunophenotypic analysis of those lymphoid neoplasms made it possible to define the malignant lymphoma according to the surface marker.[4] Most techniques here, however, were limited their usefulness by necessitating fresh and unfixed materials. The commercially available monoclonal antibodies; LCA, MT-1, and MB-1 were promoted initially for use in frozen sections but have also been shown to identify T and B cells in fixed tissues.[5,6]

The aims of this study were to evaluate (1) how accurately the speculation of T or B cell origin in lymphomas can be done, (2) how often LCA, MT-1

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and MB-1 react with lymphoma cells, and (3) what type of lymphomas are reactive with those antibodies.

MATERIALS AND METHODS

Thirty cases of malignant lymphomas, diagnosed during 3 years from July 1983 to June 1986 at our hospital were used for this study. During this period, not only histological but also immunohistochemical, flow-cytometric, and electron microscopic examination were performed when they were feasible. All the materials were lymph nodes taken surgically at their early time of presentation before any treatment.

I. Histological study

Lymph node biopsy samples had been fixed in 20% buffered formalin as well as in B-5 fixative. The original solution of B-5 fixative consisted of 60 gm of mercuric chloride and 12.5 gm of sodium acetate (anhydrous) dissolved in 900 ml of distilled water. It was diluted with 40% formaldehyde (9:1) before use. For reclassification according to Lukes-Collins' scheme,[1,2,7,8] only H-E stained sections were reviewed three times by two of the authors. NCI working formulation,[9] and LSG classification[10] were also utilized for the comparison.

II. Immunohistochemical study

Materials fixed in 20% buffered formalin and B-5 fixative were utilized for this study. Tissue had been routinely processed and embedded in paraffin. Blocks were sectioned at 4 μm. Each tissue section was deparaffinized with xylene. ABC (Avidin-biotin complex) immunoperoxidase technique was used for the immunohistochemical study. Intrinsic peroxidase activity was blocked by 10% H_2O_2 in methanol for 30 min. Then, the sections were incubated with primary antiserum; LCA (Dakopatts, Denmark), MT-1, and MB-1 (Bio-Science, Emmenbrucke, Switzerland) at the dilution of 1:50, 1:10 and 1:10 respectively. Bridge antisera, and ABC reagent, purchased from Vector Company (Vector Laboratories, Burlingame, CA), were applied. Finally, sections were stained with diaminobenzidine (0.02%; Sigma Chemical Co., St. Louis), and counterstained with hematoxylin. The presence of the brown stain of diaminobenzidine was considered a positive reaction.

III. Flow cytometric study

A small piece of fresh tissues was minced manually with scissors and was put in fetal calf serum (FCS)-added phosphate buffered saline (PBS) until use (4°C). This free cell suspension was then passed through a millipore filter (300 mesh) and cell pellet was collected. Using OKT 3,4,8 and Leu 1,2,3 antibodies, lymphocyte surface markers were detected in Spectrum III. Highest percentage of the lymphocyte types was regarded as main tumor cells except for cases of IBL-like T cell lymphoma.

For plasmacytoma and metastatic carcinoma cases, tissues were fixed in 20% buffered formalin. Immunoperoxidase method was applied exactly in the same manner as described before.

RESULTS

I. Accuracy of histological diagnosis

Cases were classified according to the scheme of Lukes and Collins. They were composed of a case of malignant lymphoma, follicular center cell (FCC), small cleaved cell type, 3 of FCC, large cleaved cell type, one of FCC, small non-cleaved cell type, 9 of FCC, large non-cleaved cell type, 3 of B immunoblastic sarcoma, 7 of small T lymphocytic type in which two cases of so-called IBL-like T cell lymphoma were included, 6 cases of T immunoblastic sarcoma in which pleomorphic T cell lymphomas were also included, and 4 of plasmacytoma. As shown in Table 1, histological speculation of B or T cell derivation of malignant lymphomas seems to fairly well correlate with the result of immunohistochemical study. Only one case which we initially considered to be a malignant lymphoma, large non-cleaved FCC type (B cell origin) was of T cell malignancy immunohistochemically, and tumor cells were positive for MT-1 but negative for MB-1. Retrospectively, this case was rediagnosed as T immunoblastic sarcoma.

TABLE 1. 30 cases of malignant lymphomas classified according to Lukes and Collins' scheme

Diagnosis	Number of cases	
	Histological diagnosis before immunohistochemistry	Histological diagnosis after immunohistochemistry
Malignant lymphoma		
(B cell type)		
Follicular center cell		
small cleaved	1	1
large cleaved	3	3
small noncleaved	1	1
large noncleaved	9	8
B immunoblastic	3	3
Subtotal	17	16
(T cell type)		
Small T (incl. IBL-like T)	7	7
T immunoblastic	6	7
Subtotal	13	14
Total	30	30

II. Immunohistochemical study and characteristics of each antiserum

The result is tabulated in Table 2. Only cases with the presence of immunoreactive cells which we considered neoplastic were expressed as positive with the exception of cases labeled as IBL-like T cell lymphoma in which neoplastic cells were difficult to identify with certainty in immunostained sections. The number within parentheses indicates materials fixed in B-5 fixative.

Leukocyte common antigen

All types of malignant lymphomas were stained positively for the antibody against LCA (Fig. 1) except for cases of small cleaved FCC lymphoma and

TABLE 2. Immunohistochemical results: Positivity of each antiserum

Diagnosis \ Antiserum	LCA	MB-1	MT-1
Malignant lymphoma			
Follicular center cell			
small cleaved	0/1 (0/1)	1/1	0/1
large cleaved	3/3 (1/1)	3/3	0/3
small noncleaved	1/1 (1/1)	1/1	0/1
large noncleaved	6/8 (3/3)	8/8	0/8
B immunoblastic	1/3 (0/0)	2/3	0/3
Subtotal	11/16	15/16	
%	68.8	93.8	
Small T (incl. IBL-like T)	6/7 (6/6)	2/7	3/7
T immunoblastic	5/7 (2/2)	0/7	5/7
Subtotal	11/14	2/14	8/14
%	78.6	14.2	57.1
Total	21/30		
%	67.7		
Epithelial tumor	0/1 (0/0)	0/1	0/1
Thymoma	3/3 (1/1)	0/3	1/3
Plasmacytoma	0/4 (0/2)	0/4	0/4

plasmacytoma. Overall positivity was 67.7%. There was no evidence of specific immunoreactivity according to lymphoma cell types and/or differentiational levels if indeed the morphology of lymphomas corresponds to the differentiational

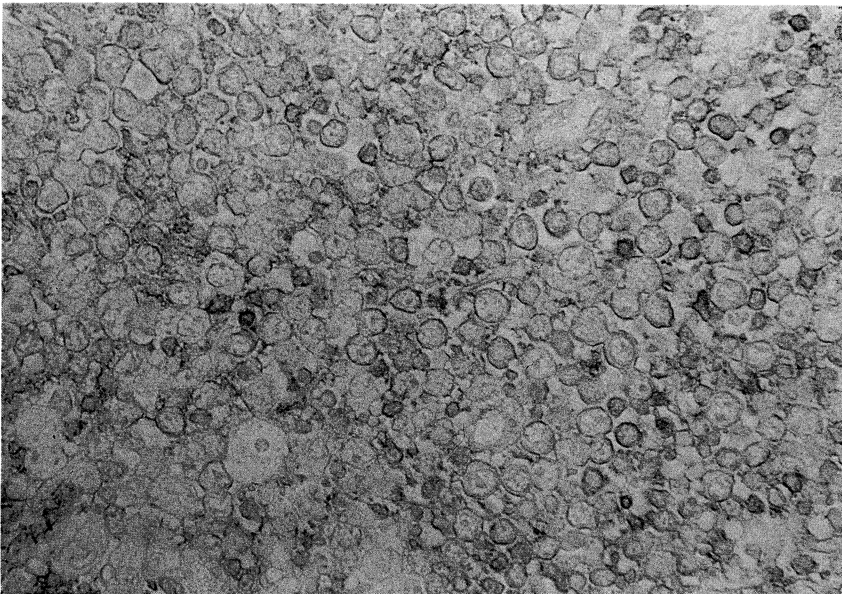

Fig. 1. LCA in a case of malignant lymphoma, follicular center cell, diffuse, non-cleaved cell type. Majority of cells are immunoreactive. (Immunoperoxidase-ABC)

level. Positivity was slightly greater in T cell lymphomas (78.6%) than B Cell lymphomas (68.8%). With regard to the fixative, neither fixatives gave different result in positivity but the intensity of the immunoreactive endproducts was much stronger in B-5 fixative.

MB-1

We expressed some cases of small T cell lymphomas as MB-1 positive. These were the cases of IBL-like T cell lymphoma. We interpreted those cells with positive MB-1 were intermixed reactive cells in various differentiational levels. Therefore, MB-1 was entirely non-reactive with lymphoma cells of T cell differentiation, and was specific to B cell neoplasia (Fig. 2). Positivity did not change according to the level of differentiation. Overall positivity using this antiserum in B cell neoplasm was 93.8% which was much higher than that of LCA (68.8%). In addition, there were five MB-1 positive cases among LCA negative B cell lymphomas. No difference in positivity was noted between formalin-fixed and B-5 fixed materials.

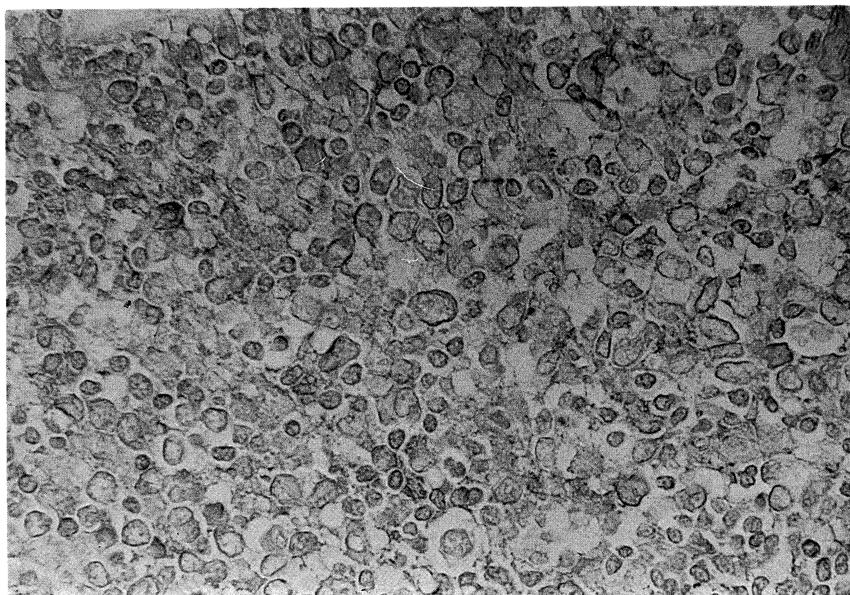

Fig. 2. MB-1 immunoreactivity in the same case as Fig. 1. Majority of cells here are also immunoreactive. (Immunoperoxidase-ABC)

MT-1

MT-1 was positive in eight cases of 14 T cell lymphomas, representing positivity of 57.1% and was considered quite specific to T cell neoplasia. MT-1 positivity among T cell lymphomas was lower than LCA positivity among the same disease group (78.6%). MT-1 and MB-1 were mutually exclusive in entire areas. There were two cases in which LCA was negative but MT-1 was positive. Difference in fixation method; i.e., formalin or B-5 did not change either their positivity or intensity.

Other remarks

There were some cases in which the results were somewhat peculiar to us.

It is not unusual that there is a mixture of B lymphocytes in T cell lymphomas because some neoplastic T cells may pursue its normal function to call a variety of B cells into the tumor mass. Such mixture was prominent in cases of IBL-like T cell lymphoma. In one case of T cell lymphomas (Figs. 3, 4), even scattered immunoblastic cells were positive for MB-1 (Fig. 5) and negative for

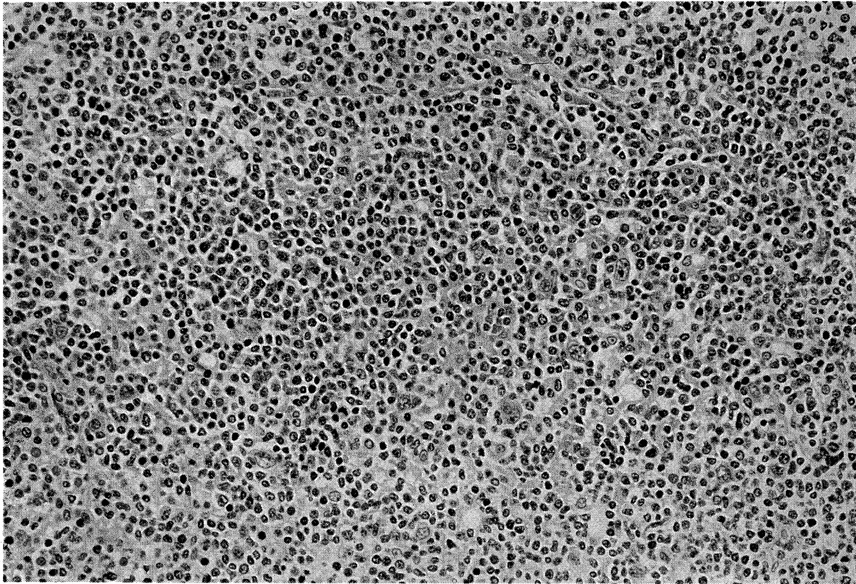

Fig. 3. Malignant lymphoma, diffuse, small T cell type. (H-E)

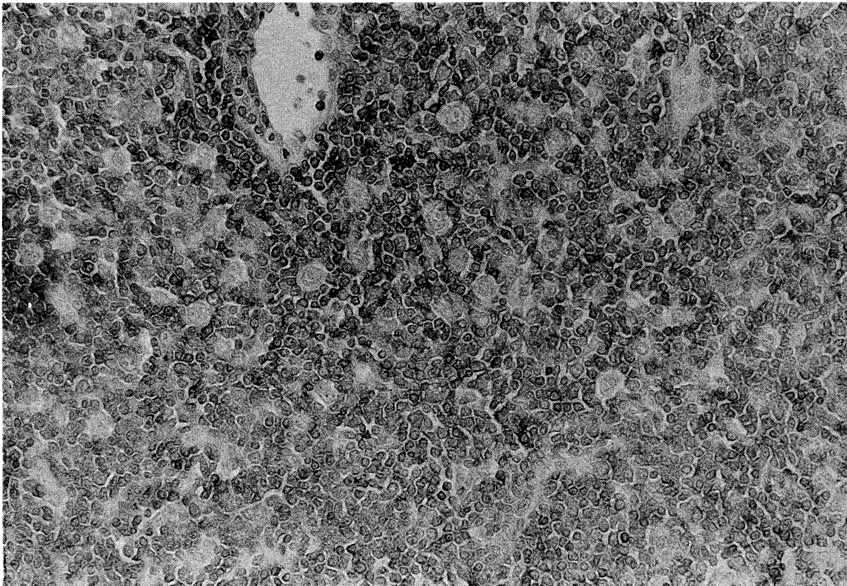

Fig. 4. LCA (the same case as Fig. 3). Note that small lymphocytic cells are immunoreactive while large cells are non-reactive. (Immunoperoxidase-ABC)

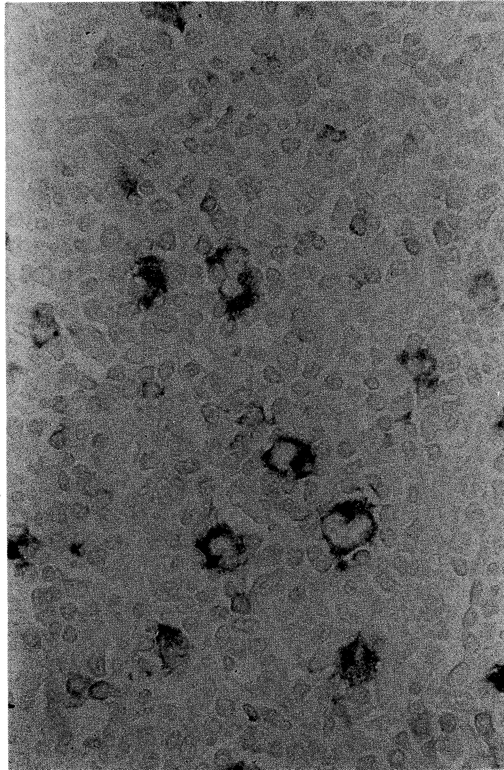

Fig. 5. MB-1 (the same case as Fig. 3). Note that small lymphocytic cells are not immunoreactive whereas large cells are positive for this antiserum. (Immunoperoxidase-ABC)

MT-1 (Fig. 6), whereas numerous neoplastic small lymphocytes were positive for MT-1. We interpreted that such immunoblastic cells were probably reactive cells called by neoplastic cells. Unexpectedly, in addition, B cell neoplasms were also intermingled by small T lymphocytes. In some cases, leukocytes and smooth muscle cells were stained positively for either MB-1 or MT-1.

III. Flow cytometric study

The results of flow cytometric study will be described in detail separately. Overall features of T and B cell phenotypic appearance were in good agreement with the results of immunohistochemistry.

DISCUSSION

Leukocyte common antigen (LCA) is a glycoprotein restricted to the cell membrane surface of leukocytes, including B and T lymphocytes, thymocytes, macrophages and granulocytes. Evidences suggest that LCA represents not a single molecule but rather a group of structurally related molecules with different functions.[11,12] Anti-LCA antibody purchased from Dako is allegedly a mixture of the monoclonal antibody 2B11 which reacts with cells in T cell zone, and another monoclonal antibody PD7/26 which reacts with cells in

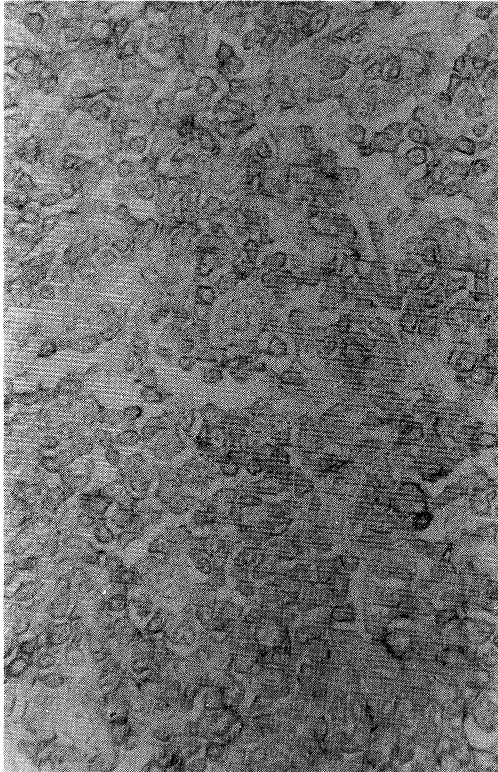

Fig. 6. MT-1 (the same case as Fig. 3). Small lymphocytic cells are immunoreactive but large cells are negative for this antiserum.

follicular mantle zone, a small number of cells in the follicles and some B cells in the interfollicular zone.[5] In addition to those lymphoid cells, they weakly crossreact with macrophages, histiocytes and polymorphonuclear leukocytes.

According to the manufactures, MB-1 was raised against a Hodgkin's derived B cell line and MT-1 against human lymph node cells.[6] These antibodies can be applied not only to the fresh frozen material but also to the formalin-fixed and paraffin-embedded material, and are now commercially available. MT-1 reacts with T lymphocytes but it also does react weakly with hematopoietic cells other than B lymphocytes. MB-1 strongly reacts with B lymphocytes but it is weakly immunoreactive with other hematopoietic cells as well. A few reports have appeared concerning to their cell specificity and their application to malignant lymphoma. In normal tissues, tissue distribution of immunoreactive cells in our hands (unpublished observation) was in accordance with that previously reported.[6]

To date, a variety of classification system have been proposed as to malignant lymphomas. These days, NCI working formulation[9] tends to be used worldwide, and LSG classification[10] in this country. These two classification schemes are mainly based on the distribution pattern, cell size and nuclear morphology of tumor cells, but do not specify its cell origin; namely, T or B cell origin, except for follicular lymphomas which are considered to be of B cell origin. Biologically, in contrast, Lukes and Collins' classification,[8] and

Kiel classification[3] seems to be more accurate. They separate lymphomas according to the currently believed differentiational schemes and base their rationale of morphological classification on the fact that differentiational levels are reflected by the corresponding morphologies. Anybody who wishes to know about malignant lymphomas has to study about their scheme,[1,2,7,8] at least once, whether they are really true or not, and no matter how immature their schemes are yet. For these reasons, we had learned Lukes' scheme of classification by reading their publications, by attending their teaching courses and by consulting our cases for the past seven years. Fortunately, results of the present study indicate that T cell or B cell origin of malignant lymphomas may be suspected rather accurately in H-E stained sections by their classification scheme. In our hands, only one case of T cell lymphoma was misinterpreted as B cell lymphoma. The case number we dealt, however, is too small to reach the conclusion, and probably the correspondence rate will be reduced when many more cases are studied. Therefore, accumulation of cases with further study is definitely necessary, and this should be accompanied by immunological study.

LCA is a useful marker in differentiating lymphomas from other malignant tumors such as carcinomas. However, LCA is not always positive for malignant lymphomas. Twenty-one cases of 30 malignant lymphomas (67.7%) were positive for LCA in our study. Needless to say, this antiserum does not differentiate T cells or B cells. In contrast, MT-1 and MB-1 seems rather specific to the T cell and the B cell respectively. MT-1 was positive in 57.1% of T lymphoma cases, and MB-1 in 94% of B lymphomas. This result seems to imply two things. Firstly, MB-1 is more reliable antibody to identify B cells than MT-1 to recognize T cells. Secondly, a mixture (cocktail) of MB-1 and MT-1 would become better antisera than LCA which is a cocktail of 2B11 and PD7/26 in order to know whether cells under problem are lymphocyte or not. In this case, however, it should be taken into consideration that carcinoma cells may be stained positively with MB-1 even though they are poorly differentiated, because certain normal epithelial cells react with this antiserum. All the antisera we tested this time seem not to tell the differentiational level of the lymphocyte. Other antisera should be sought or produced to probe it in paraffin-embedded materials. It should be also noted that plasma cells and plasmacytoid lymphocytes are non-reactive with these antibodies.

Lastly, unexpected unique findings in our study should be mentioned. The principle of recent oncology tells that the neoplasia shows monoclonality. In other words, neoplastic cells in a certain tumor should be phenotypically monoclonal. In our study of malignant lymphomas, however, many of B cell lymphomas were immunohistochemically polyclonal as are the majority of T cell lymphomas. Those intermingling cells with different phenotype are considered not to be a tumor element but a reactive component against the tumor cell. It was also a surprise for us that even immunoblastic cells in certain lymphomas were of reacting cells with an opposite phenotype to the tumor.

In summary, our study indicates that histological differentiation of T or B cell derivation in malignant lymphomas seems to be possible to certain extent. LCA, MT-1, MB-1 and possibly a mixture of MT-1 and MB-1 provide reliable and useful tools for identfying lymphocytes, and furthermore T or B cell derivation.

Acknowledgment

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