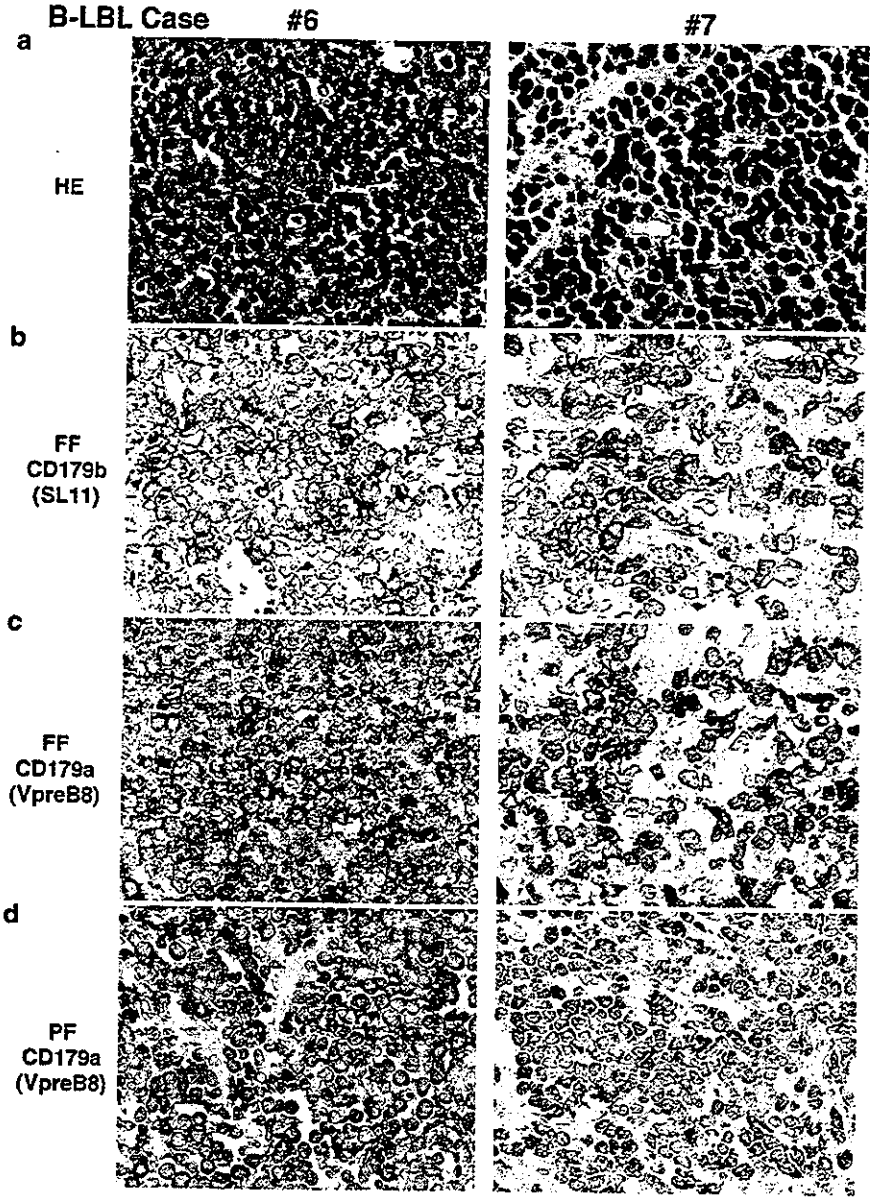

Figure 2 Immunohistochemical detection of CD179a and CD179b. CD179a and CD179b were detected in B-lymphoblastic lymphoma tissues using immunohistochemical staining on acetone-fixed fresh frozen sections ((b), (c), FF) and formalin-fixed, paraffin-embedded tissue sections ((d), PF) from biopsy tissues. The H&E-staining of formalin-fixed and paraffin-embdded tissues is also shown ((a), HE).

Immunohistochemical Staining of CD179a in Formalin-fixed, Paraffin-embedded Tissues

Next, we examined whether mAbs against CD179a and CD179b could be used in formalin-fixed, paraffin-embedded tissues. When paraffin-embedded tissues prepared from clinical specimens obtained from B-LBL patients were examined using immunohistochemical staining with the heat-induced epitope retrieval treatment, only VpreB8 reacted with the tissue. The staining results were consistent with those obtained from the immunostaining of acetone-fixed frozen sections. None of the other mAbs reacted with the B-LBL samples. Since higher concentrations of VpreB8 resulted in nonspecific nuclear staining in paraffin sections of Burkitt lymphomas, care must be taken when deciding the appropriate conditions for the use of this mAb.

Discussion

In the current study, we clearly presented that both VpreB8 and HSL11 are useful for the immunohistochemical detection of CD179a and CD179b, respectively, in acetone-fixed B-LBL tissues. Furthermore, VpreB8 can also be used in paraffin-embedded sections. The reactivities of these Abs were highly specific for B-LBL. Reactivity was not seen in tissues of Burkitt lymphoma, diffuse large B-cell

Table 1 Detection of CD179a and CD179b in B-lineage lymphoma tissues using immunohistochemical staining in acetone-fixed fresh frozen sections

Case no.	*Age (years)*	*Sex*	*Origin*	*CD179a*	*CD179b*	*TdT*	*CD34*	*CD19*	*CD79a*	*DR*	*CD20*	μ	*LC*	*CD10*	*CD77*
B-LBL															
1	4	M	Bil-CL	+	+	+	−	+	+	+	+P	−	−	+	−
2[a]	9	M	R-testis	+	+	+	−	+	+	+	+M	NT	NT	+	−
3	7	M	L-CL	+	+	+	−	+	+	+	−	+	−	+	−
4	5	F	L-CL	+	+	+	−	+	+	+	−	−	−	+	−
5	7	F	L-CL	+	+	+	+	+	+	+	−	+	−	+	−
6	1	F	R-CL	+	+	+	−	+	+	+	−	−	−	+	−
7	12	M	AT	+	+	−	−	+	+	+	−	−	−	+	−
8	5	F	L-upper arm	+	−	−	−	+	+	+	+M	−	−	+	NT
9	7	M	L-CL	−	−	+	−	+	+	+	+P	−	−	+	NT
10	4	F	R-radius	+	−	−	+	+	+	+	−	−	−	+	−
11	9	M	CNS	NT	+	+	NT	+	+	NT	NT	+	NT	NT	NT
Burkitt															
1	6	F	AT	−	−	−	−	+	+	+	+	+	−	−	+
2	7	M	AT	−	−	−	−	+	+	+	+	+	Lamda	+	+
3	15	M	AT	−	−	−	−	+	+	+	+	+	Lamda	+	+
4	4	M	AT	−	−	−	−	+	+	+	+	+	Kappa	+	+
5	6	M	AT	−	−	−	−	+	+	+	+	+	Kappa	+	−
6	5	M	AT	−	−	−	−	+	+	+	+	+	Kappa	+	+
7	4	M	AT	−	−	−	−	+	+	+	+	+	Lamda	+	+
B-DL															
1	7	F	R-CL	−	−	−	−	+	+	+	+	+	Lamda	−	−
2	6	M	AT	−	−	−	−	+	+	+	+	+	Lamda	−	−
3	8	M	R-CL	−	−	−	−	+	+	+	+	+	Lamda	+	−

B-LBL, precursor B-cell lymphoblastic lymphoma; DL, diffuse large cell lymphoma; Bil, bilateral; L, left; R, right; CL, cervical lymph nodes; AT, abdominal tumor; LC, light chains; NT, not tested; P, patchy staining pattern; M, membranous staining pattern.
[a]Testicular relapse of precursor B acute lymphoblastic leukemia.

Table 2 Immunohistochemical staining of CD179a and CD179b on acetone-fixed fresh frozen sections of non-B-cell lineage neoplasm tissues

	Positivity	
	CD179a	*CD179b*
T-LBL	0/7	0/7
Extramedullary myeloid tumors		
Granulocytic sarcoma	0/2	0/2
AMoL, skin infiltration	0/1	0/1
Ewing sarcoma	0/2	0/2

T-LBL, precursor T-cell lymphoblastic lymphoma; AMoL, acute monocytic leukemia.

lymphoma, T-LBL, extramedullary myeloid tumors, and Ewing sarcoma.

In pediatrics, the three major types of B-cell lymphoma are B-LBL, Burkitt lymphoma, and diffuse large B-cell lymphoma; the latter two types must be distinguished from B-LBL since the therapeutic protocols for these diseases are quite different from that for B-LBL. In the Berlin Frankfurt Munster (BFM) study group, for example, B-LBL cases were treated using ALL-type protocol with a total therapy duration of at least 24 months.[17] In contrast, mature B-cell lymphoma cases, including Burkitt lymphoma and diffuse large B-cell lymphoma, are treated using a short course of treatment that is usually completed within a year.[18] Each type of B-lineage lymphoma is morphologically unique and distinctive upon histological examination. In the practical pathological diagnosis of lymphomas, however, pathologists may experience difficulties in differentiating B-LBL from other B-lineage lymphomas, especially when only poor-quality biopsy specimens are available.[12] Unfortunately, pathologists are not always familiar with B-LBL because of its rarity among childhood lymphomas; as a result, patients with B-LBL may be misdiagnosed as having mature B-cell lymphoma, such as Burkitt lymphoma. The similarity in marker expression patterns for B-LBL and Burkitt lymphoma is also partly responsible for the risk of misdiagnosis.[11,12]

TdT is considered to be a reliable marker for the diagnosis of cases of precursor lymphocyte origin,[11,12] but TdT is not always positive in B-LBL cases as reported by several different groups.[19–22] For example, Mertelsmann *et al*[20] reported that TdT was absent in approximately 5% of ALL and LBL cases. Orazi *et al*[21] also reported that 6% (two out of 35) of LBL cases was TdT-negative assessed by immunohistochemical staining. On the other hand, CD34 is expressed on human bone marrow progenitor cells and leukemic blasts, and is considered to be an immature marker. Although the expression of CD34 on B-lineage lymphomas suggests their precursor B-cell origin, the positivity of CD34 among the B-LBL cases is approximately 50%. In addition, both TdT

and CD34 are not restricted to the precursor of B cells. In contrast, CD20 is a B-cell-specific marker and its expression increases with B-cell maturation. Therefore, the absence of CD20 expression in B-lineage lymphomas suggests their precursor B-cell origin. However, CD20 expression is variable among cases of B-LBL and approximately 50% of B-LBL cases are CD20-positive, exhibiting sometimes a strong membranous staining pattern.[11] Therefore, it is difficult to specify a B-precursor origin using CD20 expression alone. Based on the above evidences, the development of other markers capable of revealing a precursor B-cell origin is urgently required; in this regard, the results described here are expected to assist in the proper diagnosis of B-LBL among B-cell lymphomas in childhood.

CD179a and CD179b are essential for the development of precursor B cells. Although their biological significance is not fully understood, they are believed to serve as surrogate LCs expressed with μ HCs in pre-BCR to determine whether the clone should survive or die. After subsequent rearrangements in κ or λ LC genes, the expression of the surrogate LCs is suppressed.[6–9] The utilization of such functional molecules in the diagnosis of precursor B-cell lymphomas is appropriate if the expression is conserved even in tumor cells. In precursor B-ALL cells, we previously reported that CD179a, CD179b, and the complete form of pre-BCR were detected by HSL96, HSL11, and HSL2, respectively, and were expressed in most of the CD10-positive precursor B-ALL cases,[10] suggesting that these markers may be useful for the further classification of this disease. Consistent with this observation, CD179a and CD179b, detected by VpreB8 and HSL11, respectively, were frequently expressed in B-LBL cases, whose origin is comparable to that of precursor B-ALL. Thus, the successful employment of these functional molecules in the diagnosis of B-cell lymphomas is another important aspect emphasized in this study.

As shown here, CD179a and CD179b immunohistochemistry can identify more than 90% of B-LBL cases. In our series, the positivity of TdT among the B-LBL cases examined was lower (73%) than that of previous reports.[19–22] The reason for this discrepancy is not known, but it is noteworthy that three TdT-negative cases were positive for either CD179a or CD179b or both. Thus, by combining the TdT and CD179 markers, we believe that virtually all B-LBL cases can be properly judged as having a precursor B-cell origin. The absence of CD179a/b reactivity in Burkitt and diffuse large B-cell-type lymphomas further supports the reliability of this marker.

Occasionally, B-LBL may be misdiagnosed as Ewing sarcoma, since these two diseases have similar morphologies and immunostaining patterns.[23] CD99 (MIC2) was previously considered to be a specific marker for Ewing sarcoma, but this molecule has now been shown to be frequently expressed in B-LBL. Bone tumors with a blastic morphology and a CD45−, CD20−, MIC2+ phenotype can be diagnosed as Ewing sarcoma. In such cases, immunostaining for CD179a/b along with TdT and CD79a will lead to a proper diagnosis. In addition, immunostaining for CD179a/b is also useful for distinguishing B-LBL from either T-LBL or extramedullary myeloid tumors, both of which are included in frequent differential for B-LBL.

Diagnostic markers must be usable in paraffin sections for practical diagnostic procedures. In this regard, the utilization of mAb VpreB8 in paraffin sections, as demonstrated in this report, should facilitate its use in daily diagnostics. Caution must be exercised, however, when using VpreB8 because this antibody may produce nonspecific binding. After careful examination, we selected a concentration of 1.25 μg/ml for our system; however, this value should be evaluated for each laboratory in which the mAb is used, since differences in detection systems may affect the results. Other than VpreB8, unfortunately, none of the mAbs against CD179a/b tested in this study was useful for immunohistochemical detection in paraffin-embedded sections. Since the expression of CD179b was always accompanied by that of CD179a in our cases assessed using fresh frozen section staining (Table 1), paraffin section staining with VpreB8 may be sufficient for the diagnosis of B-LBL. However, the generation of novel mAbs against CD179a/b and preBCR that can react in paraffin sections would be useful and may provide more convincing results.

In conclusion, we have demonstrated that mAbs against CD179a/b specifically detect B-LBL tissues. Although an examination of a larger number of lymphoma tissues is required to confirm their reliability, the application of these mAbs in the immunohistochemical examination of lymphoma tissues should contribute to a precise diagnosis of B-lineage lymphomas.

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References

1 Sakaguchi N, Melchers F. λ5, a new light-chain-related locus selectively expressed in pre-B lymphocytes. Nature 1986;324:579–582.
2 Kudo A, Melchers F. A second gene, VpreB in the λ5 locus of the mouse, which appears to be selectively expressed in pre-B lymphocytes. EMBO J 1987;6: 2267–2272.
3 Bauer SR, Kudo A, Melchers F. Structure and pre-B lymphocyte restricted expression of the VpreB in humans and conservation of its structure in other mammalian species. EMBO J 1988;7:111–116.
4 Hollis GF, Evans RJ, Stafford-Hollis JM, *et al.* Immunoglobulin λ light-chain-related genes 14.1 and 16.1 are expressed in pre-B cells and may encode the human immunoglobulin ω light-chain protein. Proc Natl Acad Sci USA 1989;86:5552–5556.
5 Schiff C, Bensmanna M, Gulglielmi P, *et al.* The immunoglobulin λ-like gene cluster (14.1, 16.1 and Fl1) contains gene(s) selectively expressed in pre-B cells and is the human counterpart of the mouse λ5 gene. Int Immunol 1990;2:201–207.
6 Karasuyama H, Kudo A, Melchers F. The proteins encoded by the VpreB and λ5 pre-B cell specific genes can associate with each other and with μ heavy chain. J Exp Med 1990;172:969–972.
7 Tsubata T, Reth M. The products of pre-B cell-specific genes (λ5 and VpreB) and the immunoglobulin μ chain form a complex that is transported onto the cell surface. J Exp Med 1990;172:973–976.
8 Karasuyama H, Rolink A, Shinkai Y, *et al.* The expression of VpreB/λ5 surrogate light chain in early bone marrow precursor B cells of normal and B cell-deficient mutant mice. Cell 1994;77:133–143.
9 Lassoued K, Nunez CA, Billips L, *et al.* Expression of surrogate light chain receptors is restricted to a late stage in pre-B cell differentiation. Cell 1993;73:73–86.
10 Tsuganezawa K, Kiyokawa N, Matsuo M, *et al.* Flow cytometric diagnosis of the cell lineage and developmental stage of acute lymphoblastic leukemia by novel monoclonal antibodies specific to human preB cell receptor. Blood 1998;92:4317–4324.
11 Brunning RD, Borowitz M, Matutes E, *et al.* Precursor B lymphoblastic leukaemia/lymphoblastic lymphoma (precursor B-cell acute lymphoblastic leukaemia). In: Jaffe ES, Harris NL, Stein H, Vardiman JW (eds). Pathology & Genetics: Tumours of Haematopoietic and Lymphoid Tissues. IARC Press: Lyon, 2001, pp 111–114.
12 Gatter K, Delsol G. B-cell lymphoblastic lymphoma. In: Gatter K, Delsol G (eds). The Diagnosis of Lymphoproliferative Diseases. Oxford University Press: Oxford, 2002, pp 59–63.
13 Ishii Y, Takami T, Yuasa H, *et al.* Two distinct antigen systems in human B lymphocytes: identification of cell surface and intracellular antigens using monoclonal antibody. Clin Exp Immunol 1984;58:183–192.
14 Ishii Y, Kon S, Takei T, *et al.* Four distinct antigen systems on human thymus and T cells defined by monoclonal antibodies: immunohistological and immunochemical studies. Clin Exp Immunol 1983;53: 31–40.
15 Fujimoto J, Ishimoto K, Kiyokawa N, *et al.* Immunocytological and immunochemical analysis on the common acute lymphoblastic leukemia antigen (CALLA): evidence that CALLA on ALL cells and granulocytes are structurally related. Hybridoma 1988;7:227–236.
16 Ishii E, Fujimoto J, Tanaka S, *et al.* Immunohistochemical analysis on normal nephorogenesis and Wilms' tumor using monoclonal antibodies reactive with lymphohaematopoietic antigens. Virchows Arch A Pathol Anat Histopathol 1987;411:315–322.
17 Neth O, Seidemann K, Jansen P, *et al.* Precursor B-cell lymphoblastic lymphoma in childhood and adolescence: clinical features, treatment, and results in trials NHL-BFM 86 and 90. Med Pediatr Oncol 2000;35: 20–27.
18 Kavan P, Kabickova E, Gajdos P, *et al.* Treatment of children and adolescents with non-Hodgkin's lymphoma (results based on the NHL Berlin–Frankfurt–Munster 90 protocols). Cas Lek Cesk 1999;138:40–46.
19 Kung PC, Long JC, McCaffrey RP, *et al.* Terminal deoxynucleotidyl transferase in the diagnosis of leukemia and malignant lymphoma. Am J Pathol 1978;64:788–794.
20 Mertelsmann R, Moore MA, Clarkson B. Methods and clinical relevance of terminal deoxynucleotidyl transferase determination in leukemic cells. Haematol Blood Transfus 1981;26:68–72.
21 Orazi A, Cattoretti G, Jphn K, *et al.* Terminal deoxynucleotidyl transferase staining of malignant lymphomas in paraffin sections. Mod Pathol 1994;7: 582–586.
22 Kaleem Z, Crawford E, Pathan MH, *et al.* Flow cytometric analysis of acute leukemias. Diagnostic utility and critical analysis of data. Arch Pathol Lab Med 2003;127:42–48.
23 Hsiao CH, Su IJ. Primary cutaneous pre-B lymphoblastic lymphoma immunohistologically mimics Ewing sarcoma/primitive neuroectodermal tumor. J Formos Med Assoc 2003;102:193–197.