

Case Study

# CD20-Positive Cytotoxic T Cell Lymphoma : Report of Two Cases and Review of the Literature

Atsuko Kitamura, Yoriko Yamashita and Naoyoshi Mori

We explored two cases of CD20-positive cytotoxic T cell lymphoma, a gastric lymphoma and an intestinal lymphoma. Neoplastic cells of the gastric lesion, possessing small cleaved-like nuclei with dense nuclear chromatin, infiltrated the mucosa in association with disappearance of the gastric glands. Neoplastic cells of the intestinal case had large, round nuclei with conspicuous nucleoli, with ulceration of intestinal surface membrane. In both cases, the neoplastic cells exhibited CD3<sup>+</sup>, CD4<sup>-</sup>, CD8<sup>+</sup>, CD20<sup>+</sup>, CD79a<sup>-</sup>, perforin<sup>+</sup>, granzyme B<sup>+</sup>, TIA-1<sup>+</sup> phenotypes. Neoplastic cells of the intestinal lesion also demonstrated positive reactivity for CD56. The gastric lymphoma was initially diagnosed as an extranodal marginal zone B-cell lymphoma, due to morphological similarity to gastric MALT lymphomas and CD20 positivity. Thus, CD20 expression on neoplastic cells in T cell lymphoma may complicate accurate diagnosis. In this report, we review previously reported CD20-positive T cell lymphoma cases, reinforcing our suggestion that recognition of this type of CD20+ lymphoma is important in making a correct diagnosis and providing adequate therapy for patients.

**Key words** CD20, CD3, T cell lymphoma, cytotoxic T cell

## INTRODUCTION

Certain lymphomas express the markers of more than one lineage of cells, such as T, B, and myeloid lineages. Two common lymphomas exhibiting biphenotypic features are small lymphocytic lymphoma (SLL)/chronic lymphocytic leukemia (CLL) and mantle cell lymphoma, which usually express the T cell-specific antigen CD5 in combination with various B-cell markers<sup>1,2</sup>. The normal counterpart to this lymphoma is thought to be a naive lymph node-resident B cell, which typically expresses CD5 antigen in addition to B cell markers such as CD20, CD79a. CD5 expression is almost invariably observed in SLL/CLL, an essential finding for diagnosis of this lymphoma. Precursor T cell lymphoblastic lymphoma (T-LBL)/T cell acute lymphoblastic leukemia (T-ALL), was recently shown to express the B cell antigen CD79a in 40% of all T-LBL/T-ALL cases<sup>3-5</sup>. The high frequency of CD79a expression makes it unlikely that the presence of this B cell marker will result in misdiagnosis of this lymphoma. As CD20 expression in T cell lymphoma is quite rare, obtaining the correct diagnosis of this type of CD20+ lymphoma can be difficult. To treat patients with such disease adequately, we believe that recognition of this type of

lymphoma should be stressed, because misdiagnosis will have a large impact on therapeutic strategy. We recently evaluated two such CD20-positive T cell lymphomas. We present these cases herein and review those previously reported<sup>6-16</sup>.

## CASE REPORT

### Case 1.

A 73-year-old female was indicated to have a gastric lesion at a health check in the Spring of 2002. The patient was diagnosed with a MALT lymphoma by endoscopic biopsy, with positive reactivity for *Helicobacter pylori* (*H. pylori*) infection. Although she completed eradication therapy for *H. pylori*, her condition did not improve. Endoscopic examinations made in March and September again gave a diagnosis of MALT lymphoma. Upon endoscopic examination in April 2004, positive reactivity for CD3 by immunohistochemistry and the presence of T cell receptor rearrangement by PCR analysis suggested a diagnosis of T cell lymphoma. Biopsy specimens were sent to our laboratory for consultation.

### Case 2.

A 51-year-old male was admitted to the hospital with chronic pancreatitis in October 2001. Despite improvement of the pancreatitis, the patient exhibited a high fever and increased levels of LDH. A large tumor mass in the pancreatic head, a thickening of the small intestinal wall, and the accumulation of suppurative fluid in the abdomen were also

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Department of Pathology of Biological Response, Nagoya University Graduate School of Medicine, Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

Address correspondence and reprint request to Naoyoshi Mori, Department of Pathology of Biological Response, Nagoya University Graduate School of Medicine, Tsurumai-cho, showa-ku, Nagoya 466-8550, Japan

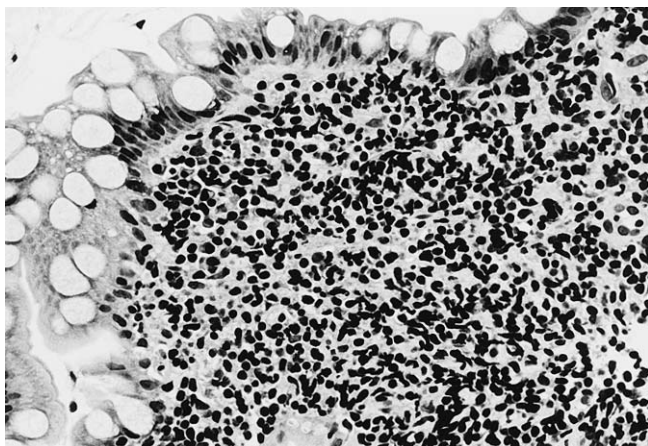
observed. In February 2002, small intestinal resection revealed a pathological diagnosis of B cell lymphoma, MALT-type. The tissue specimens were sent to our laboratory for consultation. In this patient, a liver biopsy was also performed.

## MATERIALS AND METHODS

Tissues were fixed in 10% formaldehyde and embedded in paraffin. Sections were prepared at 2-4  $\mu\text{m}$  thick and stained with hematoxylin and eosin (H & E). Antibody staining was detected by the avidin-biotin-peroxidase complex method using antibodies specific for CD8, CD20 (L26), CD79a (mb-1), perforin (DAKO, Copenhagen, Denmark), CD3, CD4, CD5 (Novocastra Laboratories, Newcastle, UK), CD56 (123C3) (Zymed Laboratories, South San Francisco, CA, USA), TIA-1 (Coulter, Miami, FL, USA), granzyme B (Monozan, Ueden, Netherlands), CD43 (MT1) (Bio-Science Products AG, Emmerbrucke, Switzerland), and CD45RO (UCHL-1) (Nichirei Corp., Tokyo, Japan).

## RESULTS

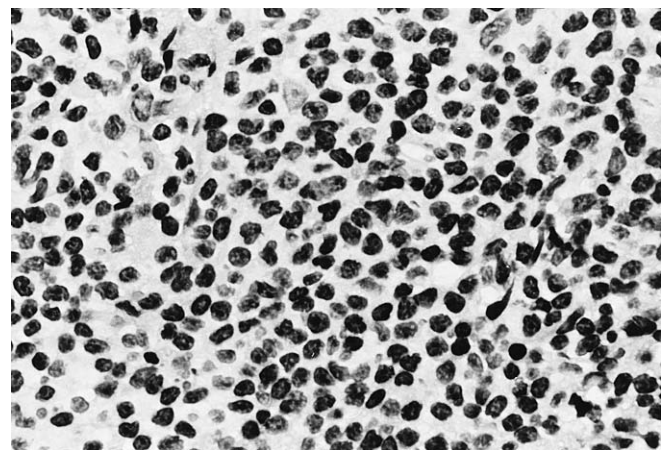
For the first case, endoscopic biopsy was performed six times from August 2002 to April 2004. The morphologic features of each sample were similar in each biopsy. The neoplastic cells, exhibiting a centrocyte-like morphology, possessed medium-sized nuclei of irregular shape containing dense nuclear chromatin (Figs. 1, 2). These cells infiltrated the mucosa, resulting in the disappearance of some glands; no conspicuous lymphoepithelial lesions, however, were visible. These atypical cells existed in small clusters within mucosal tissues.



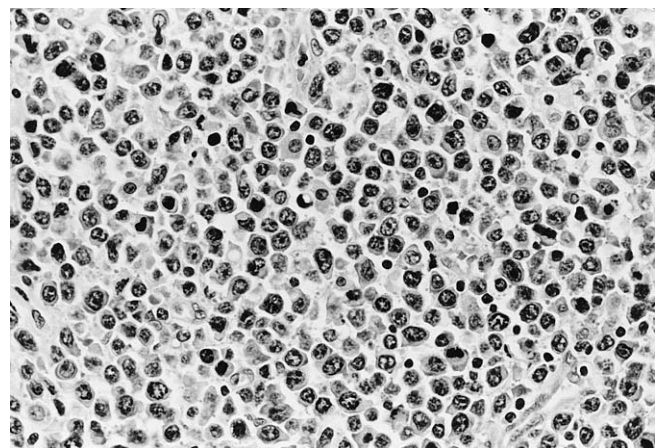
**Fig. 1.** H & E-stained section of a gastric specimen (Case 1). Numerous medium-sized atypical cells with dense nuclear chromatin are observed in conjunction with the disappearance of the gastric glands.  $\times 300$ .

The neoplastic cells of the second case contained large, round nuclei with conspicuous nucleoli (Fig. 3). These cells were disseminated throughout the mucosa and submucosa, creating a surface membrane ulcer. Lymphoepithelial lesions were not conspicuous in this case. We observed a marked thickening of the entire small intestine wall. H & E staining of biopsied liver tissue from this patient revealed numerous large atypical lymphoid cells with round nuclei infiltrating the portal tracts and surrounding tissues.

Immunohistochemical studies revealed that neoplastic cells from the first patient were CD20<sup>+</sup>, CD79a<sup>-</sup>, CD3<sup>+</sup>, CD43<sup>+</sup>, CD45RO<sup>+</sup>, CD5<sup>+</sup>, CD4<sup>-</sup>, CD8<sup>+</sup>, perforin<sup>+</sup>, granzyme B<sup>+</sup>, and TIA-1<sup>+</sup> (Table 1, Figs. 4-9). Cells from the patient with a small intestinal lymphoma exhibited a similar phenotype, but also demonstrated positive reactivity for CD56. Immunostaining of the liver identified neoplastic cells that were positive for CD3, but negative for CD20. These cells



**Fig. 2.** Higher magnification of the specimen in Fig. 1. The atypical cells exhibit small cleaved-like nuclei.  $\times 600$ .



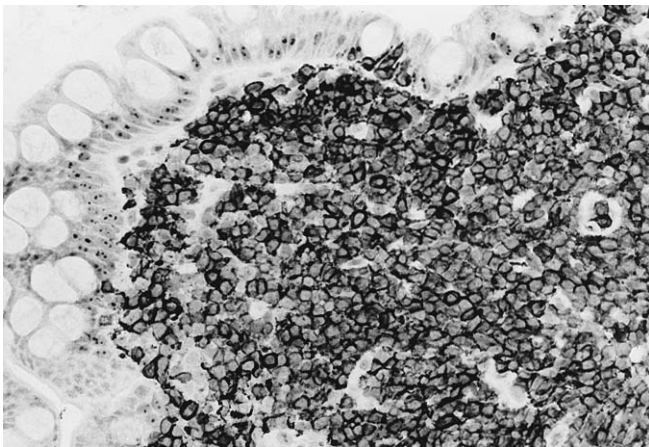
**Fig. 3.** H & E-stained section of an intestinal specimen (Case 2). The atypical cells have large, round nuclei with conspicuous nucleoli.  $\times 600$ .



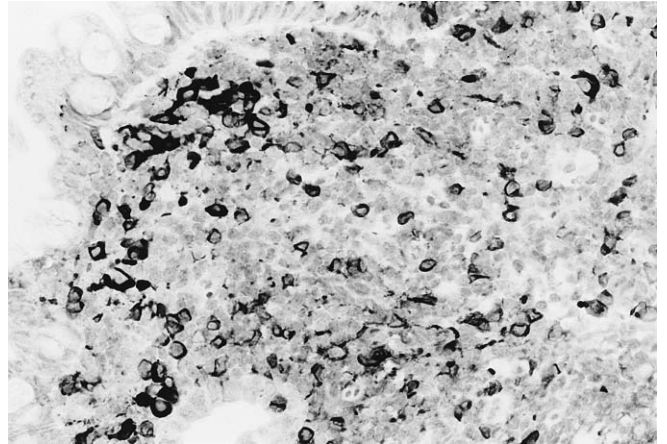
**Table 1.** Immunohistochemical findings

Antibody	Case 1	Case 2	
	Stomach	Small intestine	Liver
CD20	+	+	-
CD79a	-	-	-
CD3	+	+	+
CD43	+	+	+
CD45RO	+	-	-
CD5	+	-	-
CD4	-	-	-
CD8	+	+	+
CD56	-	+	-
Pf	+	+	-
GrB	+	+	+
TIA-1	+	+	-

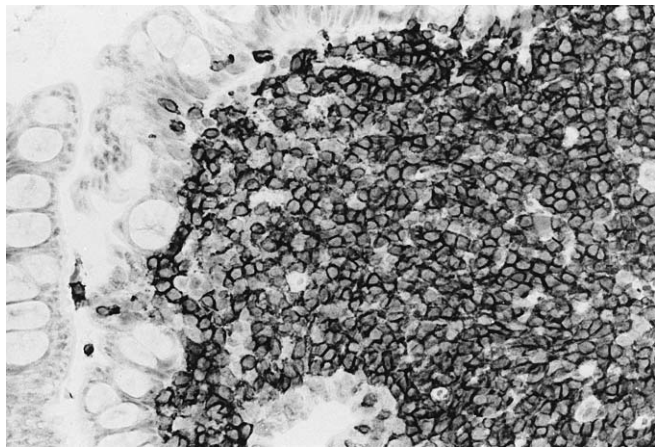
Pf, perforin ; GrB, granzyme B ; +, positive ; -, negative



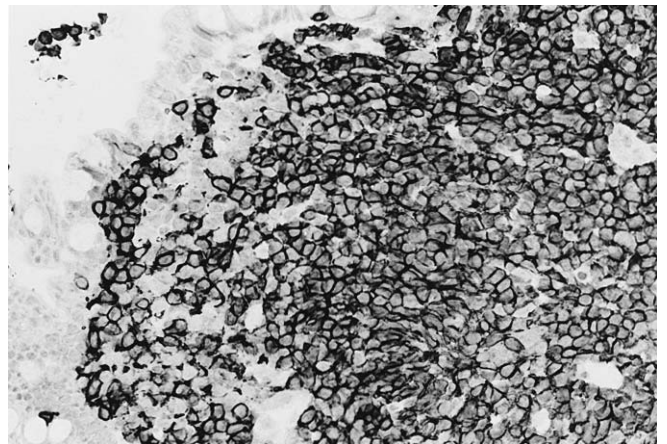
**Fig. 4.** Immunostaining for CD20 (L26) revealed positive reactivity in almost all neoplastic cells (Case 1). × 300.



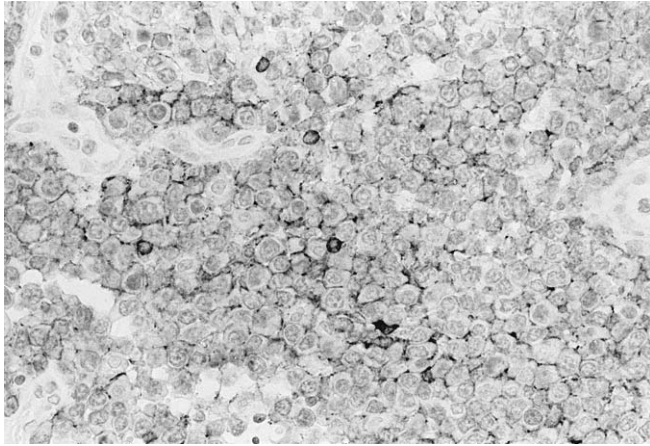
**Fig. 5.** Immunostaining for CD79a (mb-1) was negative in most of the neoplastic cells (Case 1). × 300.



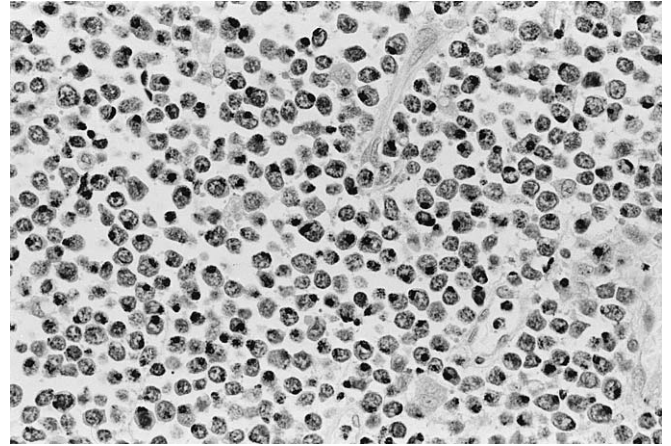
**Fig. 6.** Immunostaining for CD3 demonstrated positive reactivity in the majority of neoplastic cells (Case 1). × 300.



**Fig. 7.** Immunostaining for CD8 was positive in most neoplastic cells (Case 1). × 300.



**Fig. 8.** Immunostaining for CD20 (L26) exhibited weak positive reactivity in the neoplastic cells (Case 2).  $\times 600$ .



**Fig. 9.** Immunostaining with TIA-1 revealed positive cytoplasmic reactivity in the neoplastic cells (Case 2).  $\times 600$ .

were also CD43<sup>+</sup>, CD4<sup>-</sup>, CD8<sup>+</sup>, and TIA-1<sup>+</sup>.

## DISCUSSION

Algino *et al.*<sup>17</sup> reported that a small population of cells coexpressing CD3 (Leu4) and CD20dim (Leu16) could be identified in 94% of normal bone marrow specimens, with this population representing 0% to 11% of the mononuclear cells and 0% to 22% of all marrow lymphoid cells. Hultin *et al.*<sup>18</sup> confirmed that CD20 is expressed at low levels on a subpopulation of human T lymphocytes.

More than 10 cases of CD20-positive T cell lymphoma have been reported<sup>6, 8, 9, 11-16</sup> (Table 2). Given that as high a proportion as 12% of normal peripheral blood can coexpress CD20dim and CD3, Quintanilla-Martinez *et al.*<sup>9</sup> concluded that the CD20-positive T cell lymphoma phenotype was not aberrant, but instead represented the neoplastic counterpart of a small subpopulation of normal peripheral blood T cells.

Blakolmer *et al.*<sup>8</sup> investigated 94 extranodal non-B cell lymphoma cases, including 52 enteropathy-type intestinal T cell lymphoma, 11 cases of nasal NK/T cell lymphoma, 31 patients with primary cutaneous peripheral T cell lymphoma, and 17 instances of nodal peripheral T cell lymphoma, unspecified. Four of these cases, including three cases of enteropathy-type intestinal T cell lymphoma and one of NK/T cell lymphoma, expressed both T and B cell-specific surface markers. Three were positive for CD79a, but negative for CD20, while the remaining sample was positive for CD20 and negative for CD79a. The authors therefore concluded that use of more than one B cell marker was necessary to determine the cell lineage. Yao *et al.*<sup>6</sup> reported a case of T cell lymphoma expressing both CD79a and CD20 that also expressed CD3, CD8, CD43, and TIA-1. While non-neoplastic cells were positive for  $\beta$ F1 staining, the neoplastic cells were negative. Thus, the neoplastic cells of this particular case

were likely of  $\gamma\delta$  T cell origin.

Certain lymphomas express both T and B cell antigens; up to 40% of T-LBLs express the B cell-associated antigen CD79a (mb-1)<sup>3-5</sup>. Our research suggests that this antigen is expressed in the early stages of T cell development<sup>5</sup>. CD79a is frequently expressed in T-LBL with double negative phenotypes, but rarely seen in double positive phenotypes. We also demonstrated that CD79a is expressed in normal thymocytes. Thus, CD79a is not expressed aberrantly but is present on lymphoma cells following the neoplastic transformation of normal CD79a-expressing thymic T cells. This expression pattern conforms to the concept of Quintanilla-Martinez *et al.*<sup>9</sup> that CD20 is not aberrantly expressed, but is expressed by the neoplastic counterparts of normal CD20<sup>+</sup>T cells in the peripheral blood.

Certain cases of lymphoma, however, aberrantly express biphenotypic features<sup>19, 20</sup>. The cells of a pyothorax-associated lymphoma<sup>19</sup> were reported to exhibit three phenotypes, both B and T cell, B cell only, and T cell only phenotypes. Although all three types of neoplastic cells exhibited a similar morphology, immunohistochemical staining revealed that the neoplastic cells in certain regions express both B cell (L26<sup>+</sup>, B1<sup>+</sup>) and T cell markers (CD3<sup>+</sup>, CD43<sup>+</sup>, CD45RO<sup>+</sup>, Leu4<sup>+</sup>), while the neoplastic cells in other regions exhibited either a T cell or B cell phenotype alone. These results were confirmed by staining of sequential specimens, suggesting that these neoplastic cells aberrantly expressed a variety of phenotypes. Petitjean *et al.*<sup>20</sup> reported that pyothorax-associated lymphomas originate from B cells at a late stage of differentiation, occasionally exhibiting an aberrant dual B/T-phenotype. As B cell lymphomas have not been reported to express CD3, both of the cases reported herein are of T cell origin. In addition, both cases were definitively positive for CD3, and, at least for the first case, PCR analysis confirmed the presence of T cell receptor gene



**Table 2.** Summary of case reports of CD20-positive T cell lymphoma

Author (ref. no.)	Case no.	Site of tumor	Morphology	Immunophenotype	TCR gene rearrangement	Ig gene rearrangement
Norton <i>et al.</i> (12)	1	Lymph node	Medium/large	CD20 <sup>+</sup> (IH, P) CD2 <sup>+</sup> , CD3 <sup>+</sup> , CD5 <sup>+</sup> , CD7 <sup>+</sup> , CD8 <sup>+</sup> (IH, F)	TCR $\beta$	Germline
Linder <i>et al.</i> (13)	2	Lymph node	large	CD20 <sup>+</sup> (IH, P), CD3 <sup>+</sup> , CD22 <sup>-</sup> (IH, F)	ND	ND
Hamilton-Dntoit <i>et al.</i> (14)	4	Lymph node	Medium/large(3) anaplastic large cell(1)	CD20 <sup>+</sup> (IH, P), T cell markers <sup>+</sup> (IH, F)	ND	ND
Quintanilla-Martinez <i>et al.</i> (9)	1	Lymph node	Medium/large	CD20 <sup>+</sup> , CD43 <sup>+</sup> , CD45RO <sup>+</sup> (IH, P) CD20 <sup>+</sup> , CD2 <sup>+</sup> , CD3 <sup>+</sup> , CD4 <sup>+</sup> , CD5 <sup>+</sup> , CD6 <sup>+</sup> , CD7 <sup>+</sup> , CD8 <sup>-</sup> , CD19 <sup>-</sup> , CD22 <sup>-</sup> , Ig <sup>-</sup> , $\beta$ F1 <sup>+</sup> (IH, F)	TCR $\beta$	Germline
Yao <i>et al.</i> (6)	1	Adrenal	Large	CD20 <sup>+</sup> , CD79a <sup>+</sup> , CD3 <sup>+</sup> , CD4 <sup>-</sup> , CD5 <sup>-</sup> , CD8 <sup>+</sup> , CD43 <sup>+</sup> , CD45RO <sup>-</sup> , CD56 <sup>+</sup> , TIA-1 <sup>+</sup> , Ig <sup>-</sup> , $\beta$ F1 <sup>-</sup> (IH, P)	TCR $\gamma$	Germline
Blakolmer <i>et al.</i> (8)	1	Skin	Medium	CD20 <sup>+</sup> , CD79a <sup>-</sup> , CD2 <sup>-</sup> , CD3 <sup>+</sup> , CD4 <sup>-</sup> , CD5 <sup>+</sup> , CD7 <sup>+/-</sup> , CD8 <sup>-</sup> , CD56 <sup>-</sup> , TIA-1 <sup>+/-</sup> , GrB <sup>+</sup> (partially), $\beta$ F1 <sup>+</sup> (IH, P), CD19 <sup>-</sup> (IH, F)	TCR $\gamma$	Germline
Mohrmann <i>et al.</i> (15)	1	Lymph node	Large	CD20 <sup>+</sup> , CD79a <sup>-</sup> , CD2 <sup>+</sup> , CD3 <sup>+</sup> , CD4 <sup>+</sup> (weakly), CD5 <sup>+</sup> , CD8 <sup>-</sup> , CD30 <sup>+</sup> (focally), CD43 <sup>+</sup> , CD45RO <sup>+</sup> , CD56 <sup>-</sup> , GrB <sup>-</sup> , Pf <sup>-</sup> , TIA-1 <sup>-</sup> (IH, P) CD20 <sup>bright</sup> CD5 <sup>dim</sup> /CD20 <sup>dim/neg</sup> CD5 <sup>bright</sup> , CD2 <sup>+</sup> , CD7 <sup>+</sup> (FC)	TCR $\beta$ , TCR $\gamma$	Germline
Yokose <i>et al.</i> (16)	1	Lymph node bone marrow	Medium/large	CD20 <sup>+</sup> , CD3 <sup>+</sup> , CD4 <sup>+</sup> , CD5 <sup>+</sup> , CD45RO <sup>+</sup> (IH, P) CD3 <sup>-</sup> , CD19 <sup>-</sup> , CD22 <sup>-</sup> , Ig <sup>-</sup> (FC)	TCR $\gamma$	Germline
Sun <i>et al.</i> (11)	1	Lymph node	Large	CD20 <sup>+</sup> , CD79a <sup>-</sup> , CD3 <sup>+</sup> , CD4 <sup>+</sup> , CD5 <sup>+</sup> , CD7 <sup>-</sup> , CD8 <sup>+</sup> (weakly), CD45RO <sup>+</sup> , TIA-1 <sup>-</sup> , Gr <sup>-</sup> (IH, P) CD20 <sup>+</sup> , CD79a <sup>-</sup> , CD2 <sup>+</sup> , CD3 <sup>+</sup> , CD4 <sup>+</sup> , CD5 <sup>+</sup> , CD7 <sup>-</sup> , CD8 <sup>+</sup> , Ig <sup>-</sup> (FC)	TCR $\beta$ , TCR $\gamma$	Germline
		Skin	Large	CD20 <sup>-</sup> , CD3 <sup>+</sup> , CD45RO <sup>+</sup> (IH, P)	TCR $\gamma$	ND

IH, P, immunohistochemistry on paraffin section ; IH, F, immunohistochemistry on frozen section ; FC, flow cytometry ; ND, not done

rearrangement.

For the case of gastric lymphoma, the patient was diagnosed as having a MALT-type gastric lymphoma, due to morphological appearance and positive reactivity for CD20. The patient was treated with antibiotic therapy to eradicate *H. pylori*. After the patient's condition did not improve, a diagnosis of T cell lymphoma was made. In the case of small intestinal lymphoma, the neoplastic cells were CD3<sup>+</sup> and CD20<sup>+</sup> (weak) ; the cells identified within the liver, however, were CD3<sup>+</sup>, but CD20<sup>-</sup>. Sun *et al.*<sup>11</sup> reported a case of CD20-positive T cell lymphoma, in which the lymph node neoplastic cells were CD20<sup>+</sup> and CD3<sup>+</sup>, while the lymphoma cells resident in the skin were CD3<sup>+</sup>, but CD20<sup>-</sup>. These results indicate the instability of CD20 expression in this neoplasm, correlating well with our results. Expression of CD20 by neoplastic cells was weaker in the small intestinal lymphoma than that seen in the first case. Therefore, the difference between CD20 expression in each of the two specimens iso-

lated from the second patient may be attributable to differences in fixation conditions and detection sensitivity. In addition, the liver biopsy was performed thirteen months after the initial operation, raising the possibility that the characteristics of the neoplastic cells might have changed in that time. In two of four CD79a-positive peripheral T cell lymphomas reported by Blakolmer *et al.*<sup>8</sup>, biopsies each from two different sites were obtained ; the neoplastic cells were positive for CD79a, while the lymphomatous tissue at the site of the second biopsy was negative. The authors questioned the legitimacy of this result, hypothesizing that the observed expression of CD79a might result from cross-reactivity with an unknown epitope.

Some cases previously reported as CD20+T cell lymphoma expressed a cytotoxic T cell phenotype, exhibiting expression of CD8, TIA-1, granzymes, or perforin (Table 2). Six of thirteen reported cases, including those detailed here, revealed a CD8 phenotype. As CD8-positive lymphomas

account for only 10% of the total peripheral T cell lymphomas reported, the incidence of CD8-positivity among CD20-positive T cell lymphomas was quite high. As the number of reported cases is still small, this issue will need to be addressed in the future.

In conclusion, recognition of these cases of CD20-positive cytotoxic T cell lymphoma is important to provide adequate therapy to patients, hopefully improving prognosis.

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