Analysis of CD10+ Hairy Cell Leukemia

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Abstract

Hairy cell leukemia (HCL) has been reported to sometimes express CD10. However, the reported frequencies have been quite variable and the significance of CD10 expression has not been addressed. Cases of HCL submitted to our flow cytometry service during a 2-year period were evaluated for CD10 expression. Information regarding demographics, clinical manifestations, tissue morphologic features, and response to treatment was reviewed. Of the 97 HCL cases identified, 10 expressed CD10. The level of CD10 staining was typically well above control levels and also could be detected easily by immunohistochemical analysis. All cases analyzed were negative for bcl-6. Our study suggests that approximately 10% of otherwise typical cases of HCL show aberrant CD10 expression. CD10+ HCL cases seem to be morphologically and clinically similar to CD10– HCL cases. Appreciating that HCL can express CD10 may be especially important when evaluating specimens with suboptimal morphologic features and/or limited immunophenotyping panels.

Hairy cell leukemia (HCL) is a distinct type of mature B-cell lymphoproliferative disorder that affects primarily older adults (median age at onset, 55 years) and shows a male predominance (male/female ratio of approximately 5:1).[1] The major sites of disease involvement usually are the spleen and bone marrow, and most patients have pancytopenia and splenomegaly at initial examination.[1,2]

HCL has a characteristic immunophenotype with almost all cases being positive for surface CD103, bright CD22, CD11c, and CD25.[3-5] This immunophenotype is not observed in other mature small B-cell neoplasms and, therefore, generally can be used to establish a definitive diagnosis.[4,6,7] Although HCL is classically thought to be negative for CD10 expression, several studies have reported finding CD10+ HCL cases,[3,5,8] with one reporting a frequency of approximately 25%.[3] CD10 is an important phenotypic marker used typically to identify mature B-cell neoplasms of follicular center cell origin, ie, follicular lymphoma, Burkitt lymphoma, and a subset of diffuse large B-cell lymphomas.[9,10] Reports of relatively high numbers of CD10+ HCL cases raise the possibility that this neoplasm also may be of follicular center cell origin or represent a subset that differs pathologically and/or clinically from more typical CD10– HCL cases.

We report on 11 cases of CD10+ HCL derived from the database of a large reference flow cytometry laboratory. Although other studies have identified CD10+ HCL cases, none have specifically addressed whether CD10+ HCL cases might differ morphologically, clinically, or in other ways from CD10– HCL cases or whether CD10 expression was stable or specimen type–dependent. In addition, we wanted to take advantage of the large number of cases seen in our

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reference flow cytometry laboratory, as the frequency of CD10+ HCL cases reported in other relative large studies has been quite variable, ranging from 5% to 26%.[3,5]

Materials and Methods

Case Selection and Patient Information

The ARUP flow cytometry database (Salt Lake City, UT) was searched for records from January 1999 to October 2002 of cases of monoclonal B-cell lymphoproliferative disorders with a characteristic HCL phenotype (CD11c+, CD22+, CD25+, and CD103+). Specimens submitted for phenotyping included peripheral blood, bone marrow, spleen, and fine-needle aspirates of tissues.

Flow Cytometric Immunophenotyping

Specimen processing and antibody staining were performed as previously described.[11] Antibodies against CD3 (SK7), CD5 (L17F12), CD7 (4H9), CD11c (S-HCL-3), CD20 (L27), CD22 (S-HCL-1), CD25 (2A3), κ (TB28-2), and λ (1-155-2) were obtained from BD Bioscience (San Jose, CA). Antibodies against CD2 (SCFC13PT2H9), CD4 (SFCI12T4D11), CD8 (SCFCI21thy3D3), CD10 (J5), CD19 (J4.119, HD237, 89B), and CD45 (J.33) were obtained from Beckman Coulter (Miami, FL). Antibodies against CD10 (SS2/36), CD103 (Ber-ACT8), and polyclonal κ and λ were obtained from DAKO (Carpinteria, CA), while an antibody against CD45 (HI30) was obtained from Caltag (Burlingame, CA). All antibodies were conjugated directly with fluorescein isothiocyanate, phycoerythrin (PE), PE–Texas red, or PE–cyanin 5.1.

Data acquisition and analysis were performed with an EPICS XL cytometer and EXPO32 software (Beckman Coulter). Both 3- and 4-color analyses were performed using CD45 and light scatter gating to identify cell populations and exclude debris. HCL cases were considered positive for CD10 expression if CD10 was present on at least 20% of the monoclonal B cells relative to isotype controls and formed a distinct population. Some of the flow cytometric results also were repeated on cryopreserved viable cells, which gave values similar to the initial studies that are reported.

Morphologic Studies

Morphologic features were reviewed in each CD10+ HCL case to assess for cytologic and histologic features typically associated with HCL.[1,12] When available, the original diagnostic material, including paraffin-embedded, H&E-stained sections of bone marrow cores, bone marrow aspirates, peripheral blood smears, tissues, and cell blocks prepared from flow cytometry specimens, was reviewed. Wright stains of bone marrow aspirate and peripheral blood smears prepared from flow cytometry specimens were also examined.

Immunohistochemical Studies

Paraffin-embedded bone marrow biopsy sections were stained with monoclonal antibodies against CD20 (clone L-26; dilution 1:2,000; DAKO), CD10 (clone 56C6; dilution 1:80; Novocastra, Newcastle upon Tyne, England) and bcl-6 (polyclonal; dilution 1:250; Santa Cruz Biotechnology, Santa Cruz, CA) using an avidin-biotin peroxidase detection on an automated immunostainer (Ventana, Tucson AZ) following antigen retrieval. Also, when sufficient material was available, cell blocks were prepared from banked flow cytometry specimens for additional immunohistochemical studies. Cell pellets were prepared using plasma and thromboplastin to enhance clot formation and then were fixed in Hollande fixative (picric acid, 40 g; copper acetate, 25 g; acetic acid, 15 mL; formaldehyde, 100 mL; and distilled water, 400 mL) and embedded in paraffin. Four-micrometer-thick sections were stained with monoclonal antibodies against CD20, CD10, and bcl-6 as described for paraffin-embedded bone marrow biopsy sections. Expression of each of the markers was evaluated and correlated with hairy cell morphologic features (lymphoid cells with moderately abundant cytoplasm and smooth, oval nuclear contours).

Results

Clinical Manifestations

Ten small B-cell lymphoproliferative disorders with a characteristic HCL phenotype (CD11c+, CD22+, CD25+, and CD103+) along with expression of CD10 were identified in the ARUP flow cytometry database. These represented approximately 10% (10/97) of all cases that expressed a characteristic HCL phenotype during a 2-year period. In addition, 1 case of CD10+ HCL seen in consultation from an outside institution also was studied. The demographic and initial clinical information for the CD10+ HCL cases is summarized in **Table 1**. Age at the time of initial assessment ranged from 36 to 80 years (median, 51 years) and 10 of 11 patients were men. Initial peripheral WBC counts ranged from 600 to 7,700/μL (0.6-7.7 × 10^9/L) with 73% of the patients (8/11) being leukopenic at initial examination. Absolute neutrophil counts ranged from 300 to 2,200/μL (0.3-2.2 × 10^9/L), with 73% of patients (8/11) having absolute neutropenia. Absolute monocyte counts also seemed to be low in all but 1 of the 10 cases for which data were available. Hemoglobin values range from 9.1 to 14.8 g/dL (91-148 g/L), with 73% (8/11) of patients being anemic. Platelet counts ranged from 23 to

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Table 1
Patient Characteristics and Clinical Information*

Case No./Sex/ Age (y)	Initial Laboratory Results: WBC Count (ANC; AMC)	Initial Laboratory Results: Hemoglobin Concentration	Initial Laboratory Results: Platelet Count	Reported Splenomegaly†	Previous Therapy‡
1/M/36	3,900 (1,400; 100)	13.9	59	Yes	None
2/F/41	7,500 (900; NA)	10.5	57	Yes	None
3/M/51	3,500 (1,000; 140)	10.2	58	Yes	None
4/M/56	600 (300; 20)	12.9	69	Yes	None
5/M/39	4,400 (1,000; 90)	12.4	50	Yes	None
6/M/58	3,800 (2,200; 110)	14.8	105	No	None
7/M/41	1,500 (700; 30)	9.6	98	Yes	Interferon, cladribine
8/M/61	3,400 (1,700; 510)	9.7	155	Yes	None
9/M/80	1,900 (700; 100)	11.7	69	Yes	Interferon, cladribine
10/M/52	3,600 (1,300; 40)	13.7	115	Yes	None
11/M/50	7,700 (500; 80)	9.1	23	Yes	None

AMC, absolute monocyte count; ANC, absolute neutrophil count; NA, not available.
* Laboratory values are given in conventional units (WBC counts, /μL; hemoglobin concentration, g/dL; platelet count, × 10^3/μL); conversions to Système International units are as follows: WBC counts (× 10^9/μL), multiply by 0.001; hemoglobin concentration (g/L), multiply by 10.0; platelet count (× 10^9/L), multiply by 1.0.
† Splenomegaly was reported based on physical examination in all cases except 6 and 8. In cases 1 and 8, splenomegaly was reported after a computerized tomography scan. In case 7, splenomegaly also was noted on a plain abdominal radiograph.
‡ Any chemotherapy received before specimen evaluation in our laboratories.

155 × 10^3/μL (23-155 × 10^9/L), and all patients except 1 (case 8) were thrombocytopenic. Splenomegaly was reported in all cases except case 6.

Flow Cytometric Findings

Results of the flow cytometric immunophenotyping studies are summarized in **Table 2**. All cases expressed a characteristic HCL phenotype—CD19+, CD20+, CD22+, CD103+, CD25+, and CD11c+. The intensity of CD20 and/or CD22 expression also seemed to be increased above normal B-cell levels in many cases similar to CD10– HCL. In case 10, the initial specimen examined was not stained for CD22, CD103, CD25, and CD11c because a diagnosis of HCL was not suspected initially, but the monoclonal B-cells were CD10+. Six cases were κ light chain restricted, and 5 cases were λ restricted. Two different monoclonal antibodies were used to detect CD10 (J5 and SS2/36). Although the SS2/36 appeared somewhat brighter than J5 in the 3 specimens stained

Table 2
Flow Cytometric Immunophenotypic Results

Case No.	Specimen*	HCL Gate (% of Total Leukocytes)	CD22 (%)†	CD103 (%)†	CD25 (%)†	CD11c (%)†	Light Chain	CD10 (%)†: J5	CD10 (%)†: SS2/36
1	Spleen	83	92	29	82	93	κ	61	ND
	BM	31	64	37	52	72	κ	53	ND
	Mediastinal mass (35)	58	94	74	92	87	κ	83	ND
2	BM	57	99	89	72	97	λ	ND	50
3	BM	8	80	80	73	90	κ	61	72
4	BM	24	93	76	88	98	κ	ND	32
5	BM	36	96	93	90	97	κ	31	ND
	PB	23	96	93	86	96	κ	47	ND
	PB (0.25)	45	96	95	93	94	κ	58	ND
6	PB	4	53	35	45	94	λ	33	22
7	BM	16	56	38	2‡	50	λ	‡	ND
	BM (24)	7	68	64	74	94	λ	86	ND
	BM (39)	8	61	55	70	92	λ	70	ND
8	BM	31	98	96	87	98	κ	29	46
9	BM	68	98	93	95	98	κ	97	ND
10	PB	5	ND	ND	ND	ND	λ	ND	48
	BM (1)	51	77	40	68	93	λ	ND	78
11	PB	76	91	89	78	90	λ	ND	89

BM, bone marrow; HCL, hairy cell leukemia; ND, not done; PB, peripheral blood.
* The interval between specimens is given in parentheses, in months.
† Percentage of positive cells in the HCL gate (all cases CD19+ and CD20+, with percentages similar to those given for CD22).
‡ See text under "Flow Cytometric Findings."

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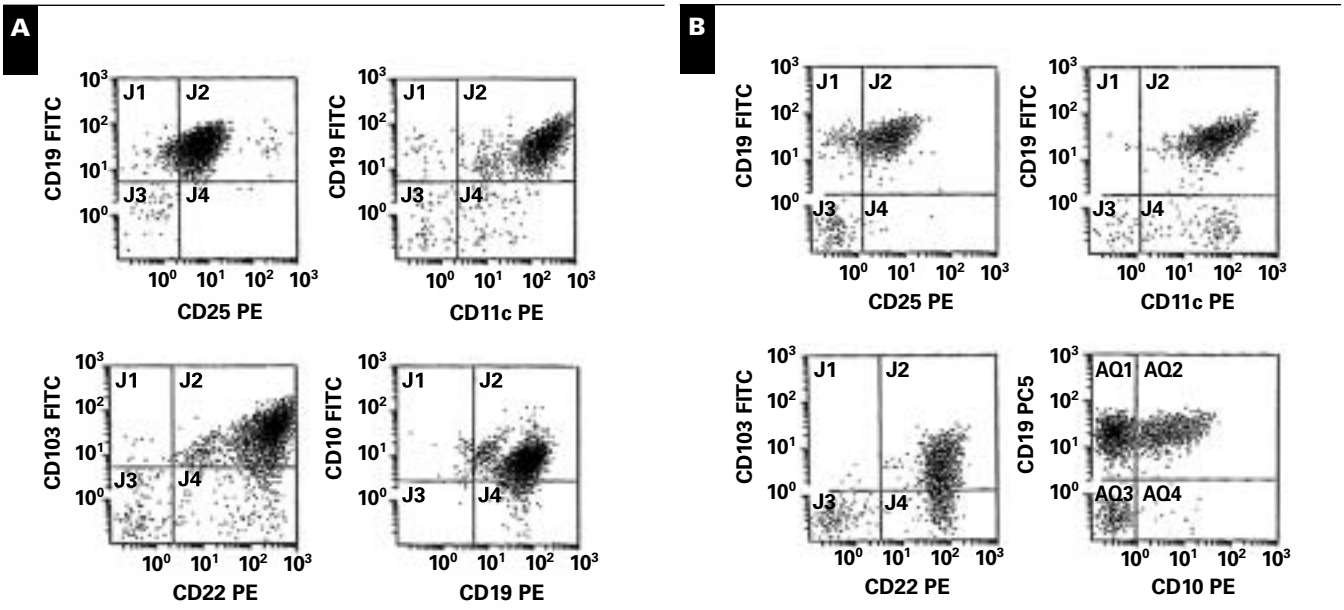

Image 1 Scatter plots from case 10 (**A**) and case 4 (**B**) with different levels of CD10 expression. Most cells in the hairy cell gates (shown) are CD19+ B cells that express CD25, bright CD11c, bright CD22, and CD103. In case 10, the majority of cells express CD10, while in case 4, approximately one third of the B cells express CD10 (bottom right panels). FITC, fluorescein isothiocyanate; PE, phycoerythrin; PC5, PE–cyanin 5.1.

with both, the ranges of percentage of positivity were roughly comparable (29%-97% for the J5 clone and 33%-95% for the SS2/36 clone). Three cases were studied on several different occasions and showed the same CD10+ phenotype. Although bone marrow from case 7 also was studied on 3 occasions, it is unclear whether the monoclonal B cells in the initial specimen were CD10+ owing to apparent technical problems (staining with other antibodies looked abnormal, suggesting instrument problems or insufficient amounts of antibodies).

Representative scatter plots from 2 cases (4 and 10) are shown in **Image 1**. In cases with lower overall percentages of CD10+ cells, such as case 4, 2 populations appeared to be present, one that was completely negative and the other with clear positive staining for CD10. This differential expression of CD10 also could be noted in follow-up immunohistochemical staining (see "Immunohistochemical Results").

Table 3
Specimens Available for Morphologic Review

Case No.	Peripheral Blood	Bone Marrow			Spleen	Other Tissue
		Core	Aspirate	Clot		
1	X	X*	X		X	X†
2		X	X	X		
3		X	X			
4		X	X	X		
5	X	X	X		X	
6	X	X		X		
7	X	X	X			
8		X	X			
9		X	X	X		
10	X	X	X			
11	X	X	X			

* A touch preparation of the core biopsy specimen was reviewed.
† Perisplenic lymph nodes and mediastinal mass.

Morphologic Findings

Types of specimens from the CD10+ HCL phenotype cases that were available for morphologic review are summarized in **Table 3**. In each of the 6 cases in which peripheral blood samples were available, atypical lymphocytes were identified with morphologic features consistent with HCL, ie, moderate to abundant cytoplasm with hair-like cytoplasmic projections and round to reniform nuclei with moderately clumped chromatin. Similar lymphoid cells, consistent with involvement by HCL, also were identified in bone marrow aspirate smears (available from 10 cases). Bone marrow core sections, which were available from 11 cases, showed a diffuse, interstitial infiltrate of atypical lymphoid cells without nodularity or aggregates. Representative bone marrow morphologic features are shown in **Image 2** for case 2. Spleens were examined in 2 cases (1 and 5). In case 1, the spleen showed a diffuse infiltrate of atypical lymphoid cells primarily within the red pulp, consistent with HCL. However, HCL could not be confirmed morphologically in the spleen from case 5, probably because it was removed after completion of cladribine chemotherapy.

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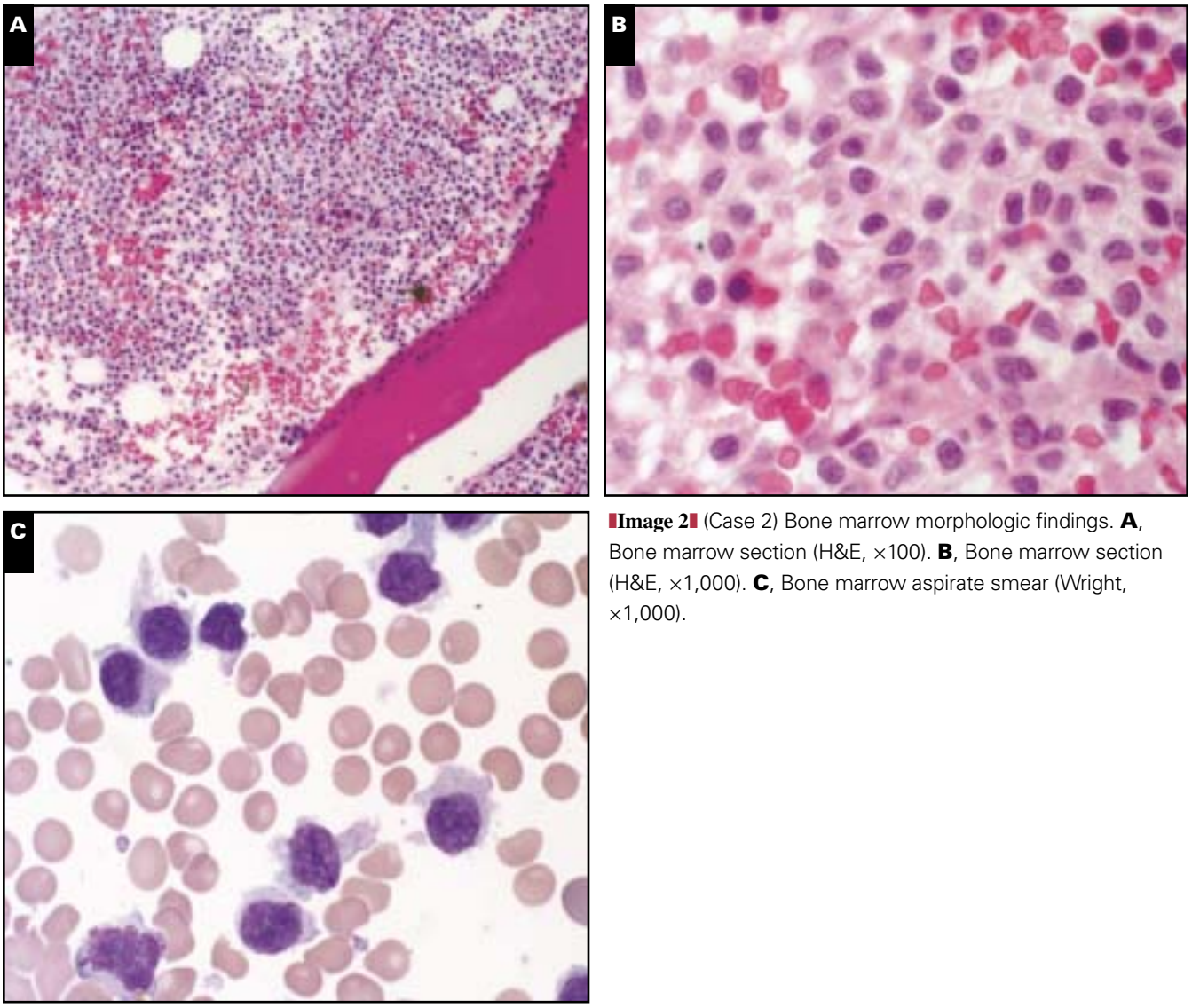

Image 2 (Case 2) Bone marrow morphologic findings. **A**, Bone marrow section (H&E, ×100). **B**, Bone marrow section (H&E, ×1,000). **C**, Bone marrow aspirate smear (Wright, ×1,000).

Immunohistochemical Results

Sufficient material for CD20, CD10, and bcl-6 immunohistochemical analysis was available in 8 cases (2-7, 10, 11). In all cases examined, the atypical lymphoid cells displayed distinct membrane staining for CD10 and CD20 and did not show nuclear labeling with bcl-6. **Image 3** shows representative staining in case 4, in which approximately one third of the atypical lymphoid cells labeled with CD10, which correlated well with the differential CD10 expression levels detected by flow cytometric immunophenotyping.

Clinical Follow-up

Information on the therapy and subsequent clinical course for each patient is summarized in **Table 4**. Total follow-up times ranged from 1 to 96 months. The intervals for assessment of clinical remission status ranged from 1 to 8 months. Eight patients achieved complete remission after 1 cycle of cladribine chemotherapy (cases 2-6, 8, 10, 11). Three patients (cases 1, 7, 9) experienced relapses after initial treatment and required further therapy. Two patients (cases 1 and 7) achieved complete remission after their most recent course of cladribine. One patient (case 9) achieved only a partial response after the most recent course of pentostatin.

Discussion

We report 11 cases of HCL that show aberrant expression of surface CD10. The CD10+ cases we describe demonstrated an otherwise typical HCL phenotype (CD103+, CD25+, CD22+, and CD11c+) and had morphologic features also characteristic of HCL. Thus, there is little doubt about the diagnosis of HCL in these cases. Expression of

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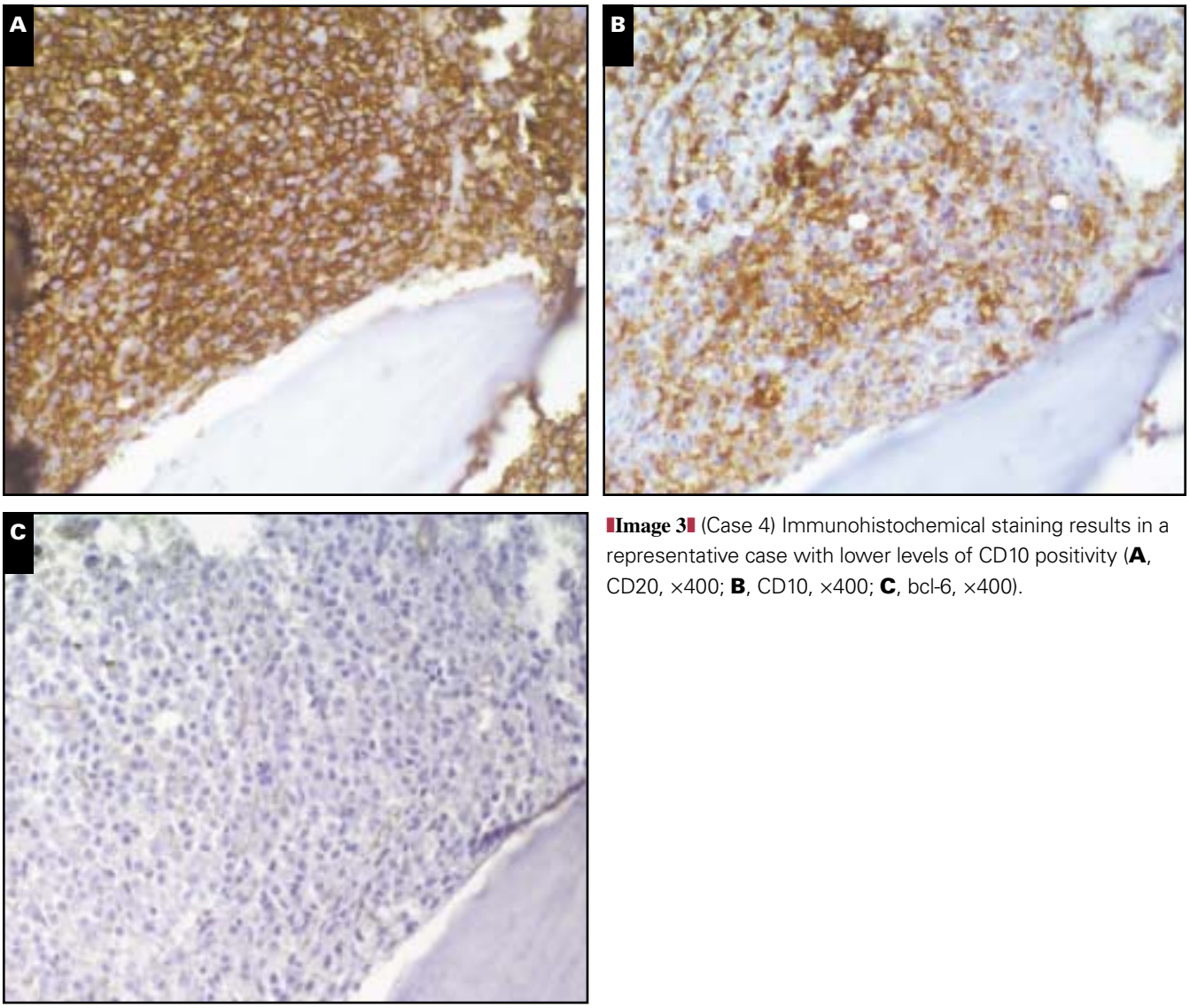

Image 3 (Case 4) Immunohistochemical staining results in a representative case with lower levels of CD10 positivity (**A**, CD20, ×400; **B**, CD10, ×400; **C**, bcl-6, ×400).

CD10 on HCL cells detected by flow cytometry was typically well above negative control values and also was not clone-dependent, as we were able to demonstrate CD10 expression using 2 different antibody clones with roughly equivalent results. Moreover, we also were able to easily detect CD10 expression using standard immunohistochemical methods in tested bone marrow biopsy specimens or cell suspensions. Therefore, detecting CD10 expression in HCL does not seem to be problematic or dependent on using a particular method.

Although only 3 cases were studied with multiple specimens obtained from different anatomic sites, all showed similar levels of CD10 positivity, suggesting that CD10 expression in HCL is not dependent on a specific location or cellular environment. In addition, analysis of the 4 CD10+ HCL cases with multiple specimens obtained at different times suggested that CD10 expression in HCL is not lost or modulated during the course of the disease. However, additional CD10+ HCL cases will have to be studied further to more fully validate these findings.

Ten of the reported cases of CD10+ HCL represent approximately 10% of the total HCL cases seen in our laboratory during a 2-year period (total of 97). CD10 expression in HCL also has been reported in other studies with variable frequencies. Kaleem et al[8] identified a similar percentage of CD10+ HCL cases as we report herein, but the number of cases evaluated was substantially lower than in our study (2/16 cases positive [13%]). The study by Juliusson et al[5] included more cases, but only 4% (3/68) were CD10+ using a 20% cutoff. Although the reason for this lower value is unclear, some of the difference may be related to gate contamination by non-HCL cells, greater problems interpreting single-color flow cytometry data, and/or including some non-HCL cases. Robbins et al[3] reported finding CD10

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Table 4
Therapy and Clinical Course

Case No.	Therapy*	Clinical Impression†	Posttherapy Laboratory Results‡		
			WBC Count (ANC)	Hemoglobin Concentration	Platelet Count
1	Splenectomy	Relapse	—	—	—
	Cladribine (35)	CR (1)	10,800 (8,100)	14.4	883
2	Cladribine	CR (1)	6,300 (4,500)	12.9	234
3	Cladribine	CR (5)	4,100 (2,800)	14.7	112
4	Cladribine	CR (1)	1,300 (1,200)	10.7	153
5	Cladribine	CR (8)	12,000 (5,900)	14.6	487
6	Cladribine	CR (1)	5,500 (4,200)	14.5	118
7	Interferon	Relapse	—	—	—
	Cladribine (12)	Relapse	—	—	—
	Interferon (36)	Relapse	—	—	—
	Interferon (72)	Relapse	—	—	—
	Cladribine (89)	CR (6)	3,000 (1,900)	15.3	181
8	Cladribine	CR (1)	5,300 (3,900)	13.9	236
9	Interferon	Relapse	—	—	—
	Cladribine (24)	Relapse	—	—	—
	Cladribine (48)	Relapse	—	—	—
	Cladribine (72)	Relapse	—	—	—
	Pentostatin (96)	Partial response (3)	3,900 (2,600)	11.4	116
10	Cladribine	CR (1)	8,000 (2,900)	12	200
11	Cladribine	CR (3)	8,200 (7,700)	12.2	53

ANC, absolute neutrophil count; CR, complete remission.
* The interval between treatments is given in parentheses, in months.
† Clinical impression as determined by patients' oncologists. Interval between treatment and assessment of response is given in parentheses, in months.
‡ Obtained after completion of the most recent chemotherapy course. Laboratory values are given in conventional units (WBC counts, /μL; hemoglobin concentration, g/dL; platelet count, × 10^3/μL); conversions to Système International units are as follows: WBC counts (× 10^9/μL), multiply by 0.001; hemoglobin concentration (g/L), multiply by 10.0; platelet count (× 10^9/L), multiply by 1.0.

expression on 26% of the large number of HCL cases they studied (34/133). Although their cases seemed to be well characterized and the same 20% positivity cutoff was used, neither the staining intensities nor the percentages of CD10+ HCL cells were given. Since the majority of our cases showed relatively strong CD10 staining on the positive cells, the higher percentage of CD10+ cases reported by Robbins et al[3] may be related to including some with apparent weak positivity on a low percentage of cells, characteristics that would have led to exclusion in our study. Moreover, all of the specimens immunophenotyped by Robbins et al[3] were peripheral blood specimens, some of which may have had low numbers of HCL cells, which could have complicated the determination of accurate CD10 cell percentages and/or identification of possible low-level, nonspecific staining artifacts.

The cell of origin for HCL is thought to be a post–germinal center B cell.[1] This proposal is based in part on studies of immunoglobulin heavy chain variable genes expressed by HCL that have found somatic mutations in most cases and some evidence for ongoing mutation.[13,14] CD10 expression in normal mature B cells and B-cell lymphomas is largely restricted to those of the germinal center type.[9,10] Although expression of CD10 in HCL raises the possibility that these also may be of germinal center origin, the lack of bcl-6 expression in all cases we studied, which represents another perhaps more specific marker of germinal center B cells,[9,15] argues against this possibility.

Aberrant expression of CD10 also has been reported in other non–follicular center cell small B-cell neoplasms, although generally at a lower frequency than we observed for HCL. For example, Berger et al[16] reported CD10 expression in 2 of 124 marginal zone lymphomas, while Xu et al[17] identified 1 of 17 marginal zone lymphomas and 1 of 10 mantle cell lymphomas that expressed CD10. Approximately 6% of multiple myelomas have been reported to be CD10+.[18] Therefore, finding aberrant CD10 positivity in otherwise typical HCL should not be unexpected, but it is unclear why it seems to be more frequent than reported for other non–follicular center cell–derived B-cell malignant neoplasms.

Consistent with aberrant expression, our study suggested that CD10+ HCL is not otherwise different morphologically or clinically from CD10– HCL. The morphologic and clinical features we observed in our cases were similar to those reported for typical HCL.[2,12] Recent reports suggest that expression of CD10 may have prognostic significance in B-cell neoplasms such as diffuse large B-cell lymphoma.[19,20] However, this does not seem to be the case with HCL since our CD10+ cases showed typical responses to cladribine therapy.[2] Larger numbers of patients and continued clinical follow-up may be necessary to more

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fully assess the possibility of prognostic implications of CD10 expression in HCL. Moreover, our study does not rule out the possibility of other differences between CD10+ and CD10– HCL that we have not addressed.

We report a series of HCL cases that express CD10 but otherwise appear morphologically and clinically like more typical CD10– HCL. Although we found no differences between CD10– and CD10+ HCL, recognition that HCL may sometimes be CD10+ is still important diagnostically. Specifically, knowing that CD10 expression can occur in HCL with some regularity could help ensure that proper diagnoses are made when evaluating specimens with suboptimal morphologic features or those in which only limited immunophenotyping panels are used. Indeed, the initial specimen for 1 case included in the present study was not evaluated for HCL markers because of the CD10 positivity.

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