Immunophenotypic Variations in Hairy Cell Leukemia

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Abstract

Hairy cell leukemia (HCL) exhibits a characteristic immunophenotypic profile that is strongly positive for pan–B-cell markers; positive for CD103, CD11c, and CD25; and usually negative for CD5, CD10, and CD23. We evaluated 35 HCL cases and identified atypical immunophenotypes in 12 cases (34%), including CD103– in 2 (6%), CD25– in 1 (3%), CD10+ in 5 (14%), and CD23+ in 6 (17%) cases. Among these cases one was CD103–/CD10+ and one was CD10+/CD23+. All available specimens from the 12 cases were reviewed and showed morphologic features characteristic for HCL. The initial clinical information was reviewed and showed no significant differences with that reported for typical HCL. Of the 12 cases, 11 patients received purine analogue therapy and achieved complete remissions. Our study indicates that it is not uncommon for HCL to display an unusual immunophenotype, including negativity for CD103 or CD25. Recognizing the variability of immunophenotype and correlating with morphologic and clinical features are essential for establishing an accurate diagnosis of HCL.

Hairy cell leukemia (HCL) is an uncommon but distinct form of chronic B-cell lymphoproliferative disorder, comprising about 2% of lymphoid leukemias. It affects primarily elderly men and is characterized by splenomegaly, pancytopenia, and monocytopenia.[1] The major sites of disease involvement are bone marrow (BM) and spleen with rare or only a small number of leukemic cells in the peripheral blood (PB).

The distinction between HCL and other chronic B-cell lymphoproliferative disorders is clinically important because patients with HCL do not respond well to conventional lymphoma chemotherapy but are highly sensitive to purine analogues such as cladribine (2-chlorodeoxyadenosine) and pentostatin (2'-deoxycoformycin). These drugs can induce long-term complete remission even in a significant portion of patients with relapsed disease.[2-7] Newer therapies such as BL22, a recombinant immunotoxin targeting CD22, also have been proven to be highly effective for patients with refractory HCL.[8,9] Therefore, accurate diagnosis of HCL is critical for appropriate treatment.

The pathologic diagnosis of HCL is based mainly on morphologic findings in PB and BM and flow cytometric immunophenotyping of leukemic cells. HCL has a characteristic immunophenotypic profile and light scatter characteristics. The tumor cells express B cell–associated markers, CD19, CD20, CD22, and CD79b, with typically brighter than normal CD20 staining, and are characteristically positive for CD103, CD11c, and CD25 and usually negative for CD10, CD5, and CD23.[1]

Coexpression of CD103, CD11c, and CD25 is considered unique for HCL and is often used as an absolute criterion for establishing the diagnosis of HCL. However, atypical immunophenotypes have been reported in otherwise typical HCL.[10-13] Several studies showed that CD10, a marker for B-cell

neoplasms of follicular center origin, was positive in HCL cases at frequencies ranging from 5% to 26%.[10,11,13] This seems to contrast with the findings of molecular studies that suggest its post–germinal center origin.[14-17] HCL negative for CD103 or CD25 also has been reported but is thought to be associated more often with HCL variant (HCL-V),[1,18-21] an aggressive disease with poor response to purine analogue therapy.

Atypical immunophenotypes, particularly when involving "unique" markers, can cause diagnostic confusion and may lead to false exclusion of the diagnosis of HCL. In the present study, HCL cases with any deviation from the "typical" immunophenotype were identified from our pathology database and evaluated further by correlating immunophenotypic results with morphologic findings and clinical features to address whether HCL cases with unusual immunophenotypes differ morphologically or clinically from HCL cases with a typical immunophenotype.

Materials and Methods

Case Selection and Evaluation

The study was approved by the institutional review board of Northwestern Memorial Hospital, Chicago, IL. The pathology database in our institution was searched for cases of HCL from January 1997 to December 2004 that had been evaluated by morphologic examination and flow cytometry. A total of 35 cases of HCL were identified, and no cases with a diagnosis of HCL-V were found during this time. The flow cytometric data for all 35 cases were reviewed. Cases with any deviation from an immunophenotype characteristic of HCL (bright CD20+, CD19+, CD79b+, CD103+, CD11c+, CD25+, CD10–, CD5–, CD23–) were selected for reevaluation of morphologic features and correlation with clinical features. Materials for review of morphologic features included all initial and follow-up PB, BM aspirate, and core biopsy specimens and tissue specimens, if available. Clinical information included laboratory data at diagnosis, clinical manifestations, treatment received, and response to treatment.

Flow Cytometric Immunophenotyping

The sample processing, staining, and data interpretation were performed as previously described.[22] The data collection and analysis were performed on a standard 4-color Beckman-Coulter XL flow cytometer and EXPO32 software (Beckman Coulter, Miami, FL). Gates were selected to identify a CD45+/CD19+ population with slightly greater side-scattered light intensity and brighter CD19+ staining than that of normal lymphocytes. The following antibodies were from Beckman Coulter/Immunotech: monoclonal antibodies against CD19 (J4.119), CD20 (B9E9), FMC7 (FMC7),

CD103 (2G5), CD11c (BU15), CD5 (SFCI24T6612), CD10 (ALB1), and CD23 (9P25) and polyclonal antibodies against immunoglobulin κ and λ light chains. The reactive or nonreactive normal cell populations for the relevant antigen within the sample were used as positive or negative control samples, respectively. Cases with negative staining of CD103 or CD25 were restained for the corresponding marker using an independent monoclonal antibody against CD103 (Ber-ACT8, DAKO, Carpinteria, CA) or CD25 (B1.49.9, Becton Dickinson Biosciences, San Jose, CA) to rule out the possibility of false-negative staining resulting from epitope deletion or masking.

Results

Flow Cytometric Immunophenotyping

All 35 cases of HCL showed surface immunoglobulin light chain restriction (17 λ and 18 κ) and were positive for pan–B-cell markers, including CD20, CD19, CD79b, and FMC7. CD20 staining was brighter than that of normal B cells. All cases were bright positive for CD11c and negative for CD5. The results of CD103 and CD25 staining in the 35 cases showed distinct clustering of cases with positive staining in more than 70% or less than 10% of monoclonal B cells. The staining patterns of CD10 and CD23 were relatively more heterogeneous. Therefore, an arbitrary cutoff of 30% was used to define positive or negative staining for these 2 markers in this study.

Of the 35 HCL cases, 12 (34%) were found to have variations in 1 or 2 markers compared with the typical immunophenotype of HCL. The types of the specimens and detailed flow cytometric immunophenotyping results for the 12 cases are listed in ▮Table 1▮. In the 12 cases, λ and κ light chain restriction was found in 7 and 5 cases, respectively. The frequencies of single antigen variation were as follows: CD103–, 2 (6%); CD25–, 1 (3%); CD10+, 5 (14%); and CD23+, 6 (17%). Two cases showed variations in 2 markers, 1 was CD103–/CD10+ and 1 was CD10+/CD23+. The negative staining of CD103 or CD25 was confirmed by repeated staining with a second independent monoclonal antibody against a different epitope on the same antigen. Representative dot-plot histograms for CD103–, CD25–, CD10+, and CD23+ HCL cases are shown in ▮Image 1A▮, ▮Image 2A▮, ▮Image 3A▮, and ▮Image 4A▮.

Clinical Information at Diagnosis and Morphologic Findings

Among the 35 patients with HCL, 32 were men and 3 were women and ages ranged from 27 to 85 years with a mean age of 55.5 years. The 12 patients with unusual immunophenotypes

▌Table 1▐
Types of Specimens and Flow Cytometric Results in Cases With Unusual Immunophenotypes

Case No.	Specimen Type	Light Chain Restriction	CD103 (%)*	CD25 (%)*	CD11c (%)	CD5 (%)	CD10 (%)*	CD23 (%)*
1	BM	λ	**10**	91	91	<1	<1	21
2	BM	κ	**4**	100	100	10	**95**	5
3	Spleen	κ	100	**<1**	100	<1	<1	1
4	PB	λ	100	97	100	2	**82**	**36**
5	PB	κ	86	83	97	1	**87**	21
6	BM	κ	92	95	100	<1	**94**	—
7	PB	λ	100	90	65	10	**95**	10
8	PB	λ	100	95	91	1	<1	**90**
9	BM	λ	100	68	100	1	<1	**100**
10	BM	λ	100	83	100	4	<1	**100**
11	PB	λ	99	88	96	11	<1	**66**
12	BM	κ	100	100	100	1	10	**73**

BM, bone marrow; PB, peripheral blood.
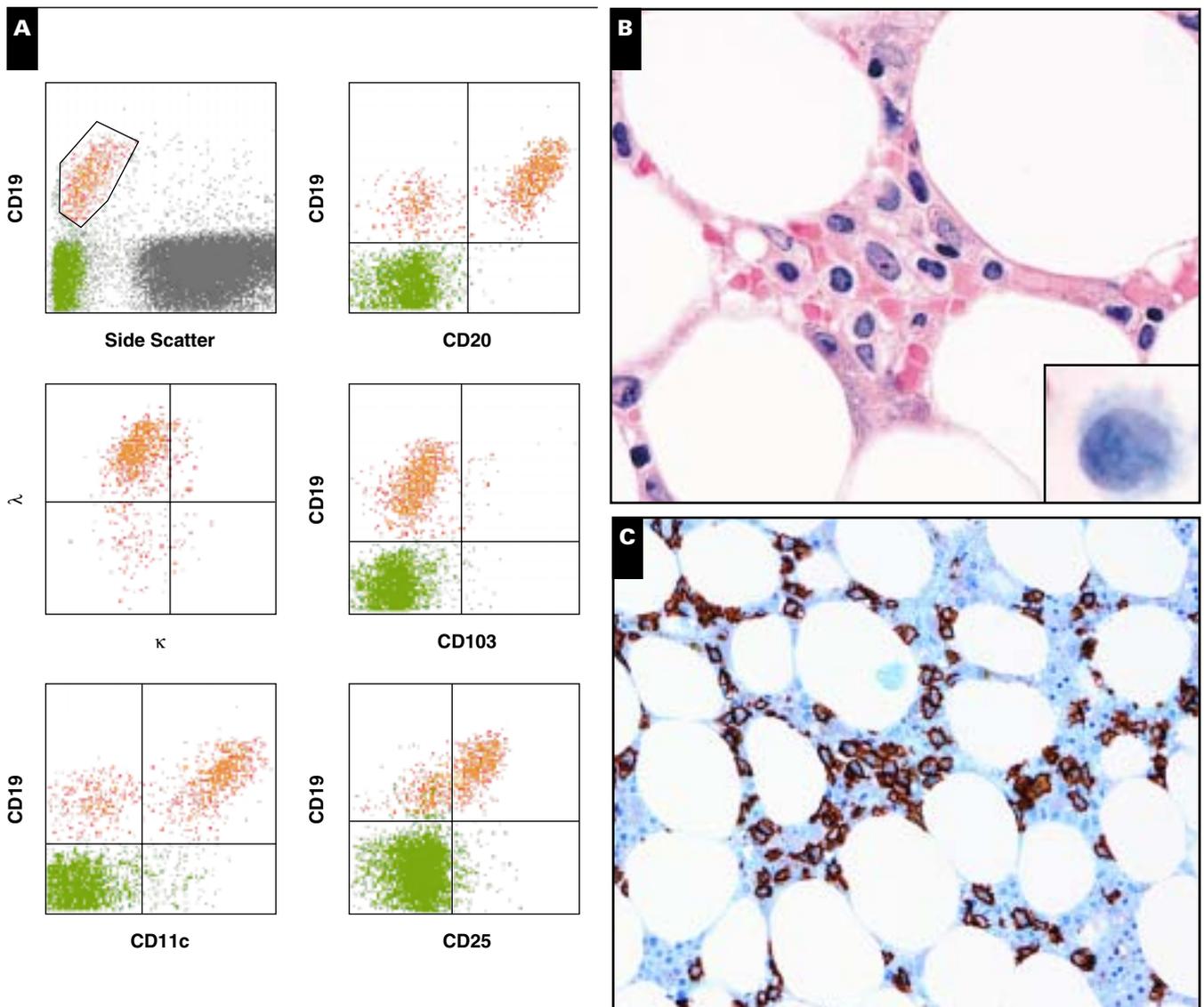* Unusual immunophenotypes are in bold type.

▌Image 1▐ (Case 1) A CD103– hairy cell leukemia. **A**, Selected immunophenotypes showing a λ-restricted B-cell population bright positive for CD20, CD11c, and CD25 but negative for CD103. Green, all lymphocytes; orange, dominant color for CD19+ B cells; black, ungated events. **B**, Bone marrow morphologic features (H&E, ×600) and leukemia cell in the peripheral blood (inset, Wright-Giemsa, ×1,000). **C**, Bone marrow with immunostain for CD20 (×200).
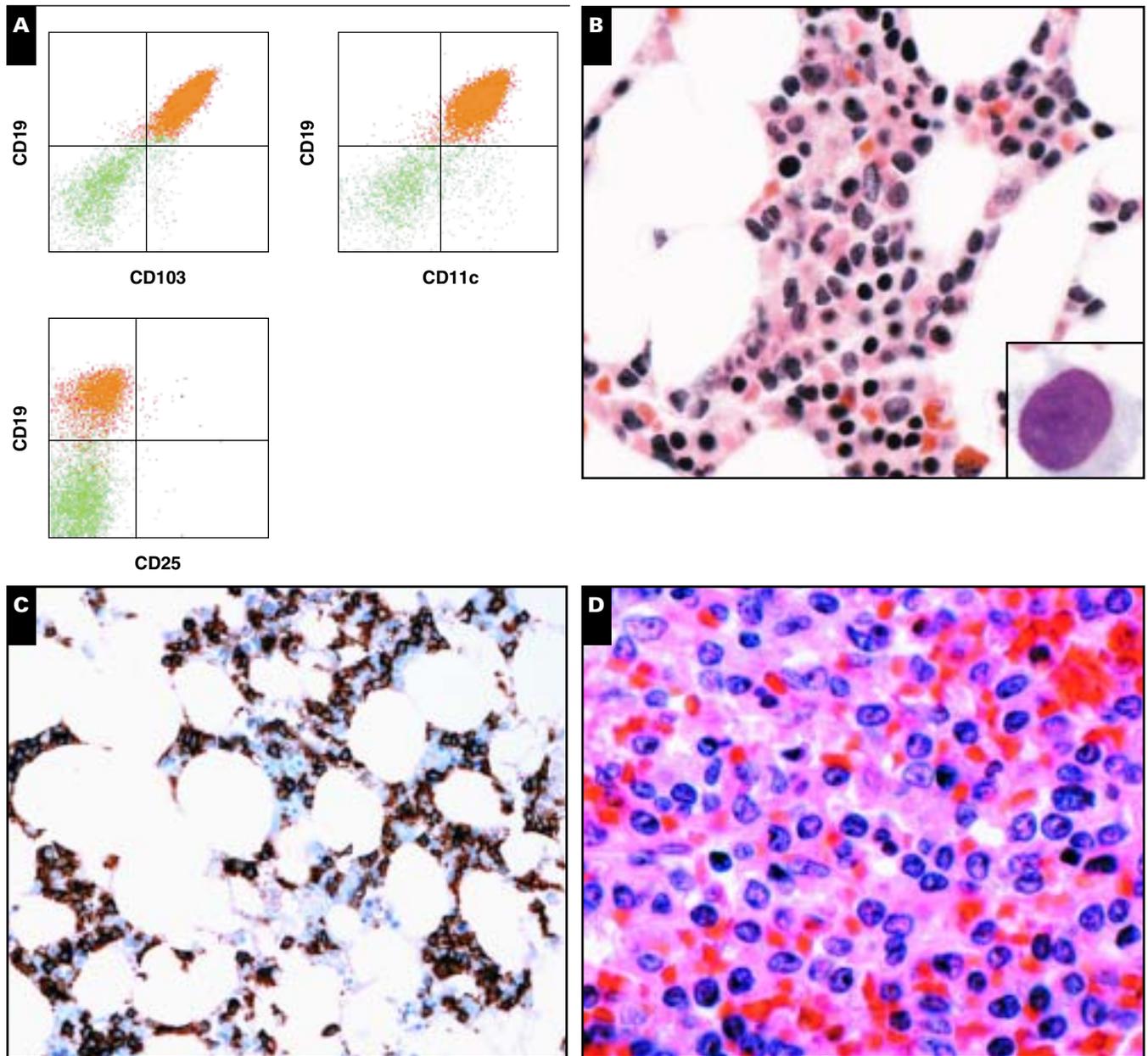
❚**Image 2**❚ (Case 3) A CD25– hairy cell leukemia. **A**, Selected immunophenotypes showing a clonal B-cell population positive for CD103 and CD11c but negative for CD25. Green, all lymphocytes; orange, dominant color for CD19+ B cells. **B**, Bone marrow morphologic features (H&E, ×600) and leukemia cell in the peripheral blood (inset, Wright-Giemsa, ×1,000). **C**, Bone marrow with immunostain for CD20 (×200). **D**, Spleen section showing red pulp infiltration by leukemic cells (H&E, ×600).

were all men whose ages ranged from 35 to 85 years with a mean age of 56.9 years. Clinical and laboratory information at diagnosis for the 12 patients is given in ❚**Table 2**❚. Anemia, leukopenia, absolute monocytopenia, thrombocytopenia, and splenomegaly were present in 64%, 91%, 91%, 73%, and 100% of patients, respectively. It is interesting that case 8 had absolute lymphocytosis and absence of monocytopenia, which is unusual for typical HCL.

The initial and follow-up BM biopsy specimens and PB smears for the 12 cases and a splenectomy specimen from 1

case were reviewed. The PB of all cases except case 8 showed leukopenia with rare to occasional circulating hairy cells. The hairy cells exhibited abundant cytoplasm and "hairy" projections and round to slightly indented nuclei with reticular chromatin and indistinct nucleoli, characteristic for blood involvement by HCL. Neoplastic cells with similar morphologic features also were identified in BM aspirates in all cases. Although an elevated WBC count was present in case 8, the hairy cells in PB and BM showed the typical morphologic features of HCL ❚**Image 4B**❚ and ❚**Image 4C**❚. The BM sections of
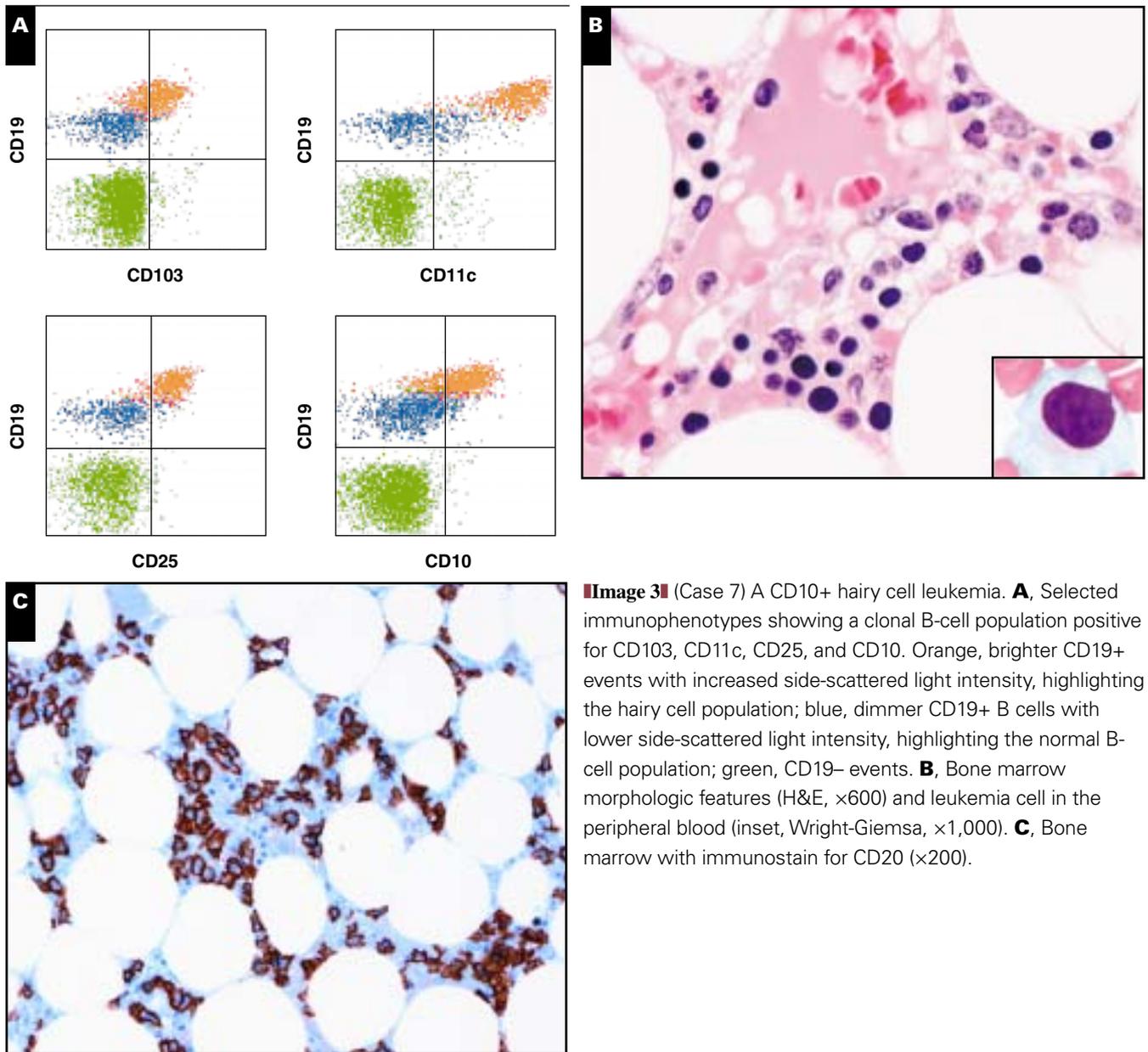
Image 3 (Case 7) A CD10+ hairy cell leukemia. **A**, Selected immunophenotypes showing a clonal B-cell population positive for CD103, CD11c, CD25, and CD10. Orange, brighter CD19+ events with increased side-scattered light intensity, highlighting the hairy cell population; blue, dimmer CD19+ B cells with lower side-scattered light intensity, highlighting the normal B-cell population; green, CD19– events. **B**, Bone marrow morphologic features (H&E, ×600) and leukemia cell in the peripheral blood (inset, Wright-Giemsa, ×1,000). **C**, Bone marrow with immunostain for CD20 (×200).

the 12 cases except case 4 showed varying degrees of interstitial infiltration by hairy cells with round to oval nuclei, indistinct nucleoli, moderate cytoplasm, and frequent extravasated RBCs ▐**Image 1B**▌, ▐**Image 1C**▌, ▐**Image 2B**▌, ▐**Image 2C**▌, ▐**Image 3B**▌, ▐**Image 3C**▌, Image 4B, and Image 4C. The infiltrates were subtle in most cases. However, a CD10+/CD23+ HCL (case 4) demonstrated extensive infiltration (90%) of BM by hairy cells.

The splenectomy specimen of a CD25– HCL (case 3) showed markedly extended red pulp infiltrated by cells with morphologic features consistent with hairy cells. Numerous "RBC lakes" also were noted, which are characteristic of (although not pathognomonic for) spleen involvement by HCL ▐**Image 2D**▌.

Morphologic features suggestive of HCL-V such as prominent nucleoli were not identified in any case, including CD103– and CD25– cases Image 1B and Image 2B. Tartrate-resistant acid phosphatase (TRAP) stain was performed on the 3 cases with negativity for CD103 or CD25 (cases 1-3), and all showed positive staining in neoplastic cells.

Treatment and Follow-up

Of the 12 patients with an unusual immunophenotype, 11 received purine analogue therapy and 1 (case 3) was treated with splenectomy alone. The follow-up ranged from 5 to 198 months. The information on treatment and follow-up is summarized in ▐**Table 3**▌. All 11 patients achieved a complete remission after initial purine analogue therapy. Two patients (CD103–/CD10+,
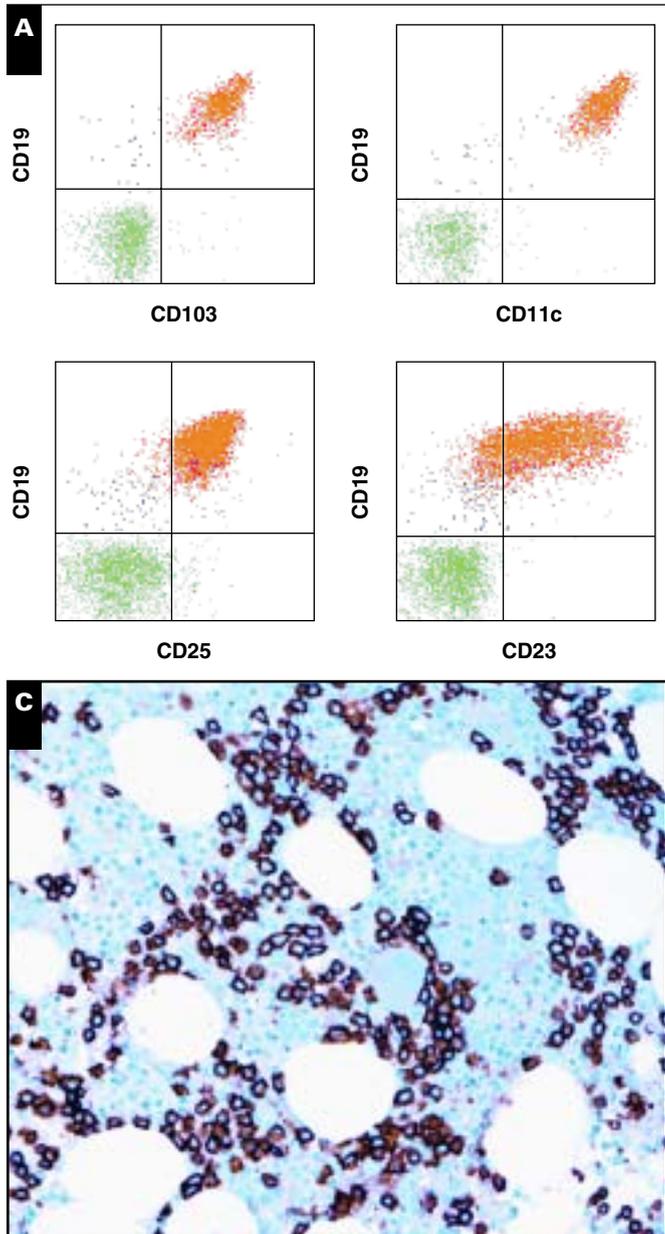
❚**Image 4**❚ (Case 8) A CD23+ hairy cell leukemia. **A**, Selected immunophenotypes showing a clonal B-cell population positive for CD103, CD11c, CD25, and CD23. The cell populations highlighted by different colors are the same as indicated in Image 3. **B**, Bone marrow morphologic features (H&E, ×600) and leukemia cells in the peripheral blood (inset, Wright-Giemsa, ×1,000). **C**, Bone marrow with immunostain for CD20 (×200).

case 2; and CD23+, case 12) experienced relapse after long-term complete remission (8 and 9 years, respectively) but were successfully treated again with purine analogues and achieved a second complete remission. One CD10+ patient (case 7) experienced relapse after 2 years of complete remission and subsequently received BL22 therapy and achieved a second complete remission. The CD25– patient (case 3), an 85-year-old man, achieved partial remission with splenectomy; we do not have further follow-up information on this patient.

Discussion

Accurate diagnosis of HCL is critical because therapy with purine analogues is associated with high complete response rates and long relapse-free survival in patients with HCL but is less effective in patients with other chronic B-cell leukemias or lymphomas.[2-7] The diagnosis of HCL usually is made by examining the morphologic features of the PB and BM in conjunction with the characteristic immunophenotype as determined by flow cytometric analysis.

Many studies on immunophenotype define HCL as a monoclonal B-cell disease with coexpression of CD103, CD11c, and CD25.[13,23,24] Therefore, it is not surprising that cases that lack CD103 or CD25 could be excluded from consideration of a diagnosis of HCL. The major differential diagnoses would include other CD5– lymphoproliferative disorders such as HCL-V and splenic marginal zone lymphoma. Negativity for CD25 is frequently seen in HCL-V, and lack of CD103 also has been reported in this disease.[1,12,18-21] Unlike typical HCL, patients with HCL-V commonly present with a

▌Table 2▐
Laboratory Results and Clinical Information for Cases With Unusual Immunophenotypes

Case No./ Sex/Age (y)	Unusual Phenotype	Laboratory Results at Diagnosis*				Splenomegaly
		Hemoglobin, g/dL (g/L)	WBC Count, /μL (× 10⁹/L)	Monocyte Count, /μL (× 10⁹/L)	Platelet Count, × 10³/μL (× 10⁹/L)	
1/M/81	CD103–	8.3 (83)	800 (0.8)	0 (0.00)	212 (212)	Yes
2/M/78	CD103–/CD10+	NA	NA	NA	NA	Yes
3/M/85	CD25–	10.9 (109)	2,600 (2.6)	0 (0.00)	132 (132)	Yes
4/M/44	CD10+/CD23+	7.2 (72)	1,300 (1.3)	0 (0.00)	22 (22)	Yes
5/M/55	CD10+	12.9 (129)	2,100 (2.1)	0 (0.00)	69 (69)	Yes
6/M/57	CD10+	15.1 (151)	2,400 (2.4)	0 (0.00)	72 (72)	Yes
7/M/46	CD10+	13.5 (135)	3,300 (3.3)	60 (0.06)	58 (58)	Yes
8/M/35	CD23+	15.2 (152)	14,100 (14.1)	280 (0.28)	83 (83)	Yes
9/M/59	CD23+	12.2 (152)	2,800 (2.8)	100 (0.1)	112 (112)	Yes
10/M/44	CD23+	12.9 (129)	2,500 (2.5)	100 (0.1)	160 (160)	Yes
11/M/46	CD23+	11.9 (119)	700 (0.7)	0 (0.00)	198 (198)	Yes
12/M/53	CD23+	14.4 (144)	3,100 (3.1)	30 (0.03)	103 (103)	Yes

NA, not available.
* Reference ranges: hemoglobin, 13.0-17.5 g/dL (130-175 g/L); WBC count, 3,500-10.500/μL (3.5-10.5 × 10⁹/L); monocyte count, 200-1,000/μL (0.2-1.0 × 10⁹/L); platelet count, 140-390 × 10³/μL (140-390 × 10⁹/L).

▌Table 3▐
Therapy and Follow-up of Cases With Unusual Immunophenotypes

Case No.	Unusual Phenotype	Initial Therapy	Response (mo)	Relapse	Secondary Treatment	Response (mo)
1	CD103–	2-CdA	CR (24)	No	No	—
2	CD103–/CD10+	2-dCF	CR (96)	Yes	2-CdA	CR (102)
3	CD25–	Splenectomy	PR (?)	NA	NA	NA
4	CD10+/CD23+	2-CdA	CR (24)	No	No	—
5	CD10+	2-CdA	CR (45)	No	No	—
6	CD10+	2-CdA	CR (5)	No	No	—
7	CD10+	2-CdA	CR (24)	Yes	BL22	CR (36)
8	CD23+	2-CdA	CR (27)	No	No	—
9	CD23+	2-CdA	CR (43)	No	No	—
10	CD23+	2-CdA	CR (40)	No	No	—
11	CD23+	2-CdA	CR (56)	No	No	—
12	CD23+	2-CdA	CR (108)	Yes	2-CdA	CR (60)

2-CdA, cladribine (2-chlorodeoxyadenosine); 2-dCF, pentostatin (2'-deoxycoformycin); BL22, a recombinant immunotoxin targeting CD22; CR, complete remission; NA, not available; PR, partial remission.

high WBC count and an absence of monocytopenia and have a poor response to purine analogue therapy.[1,4,18,20,21,25] Morphologically the neoplastic cells have prominent nucleoli and usually are negative for TRAP.[1,20]

Splenic marginal zone lymphoma may show morphologic features and immunophenotypes similar to HCL. In our series, we identified 3 HCL cases that were negative for CD103 or CD25. Review of the PB and BM specimens and the spleen in these cases demonstrated characteristic features for typical HCL, and all 3 cases were positive for TRAP. Clinically, 2 cases negative for CD103 showed excellent response to cladribine therapy, and a case negative for CD25 was treated with splenectomy alone and achieved partial remission. Therefore, these cases were morphologically and clinically similar to those reported as typical HCL.[1-3,6,7]

The cell of origin of HCL is not clear. Phenotypically, HCL cells do not resemble any normal B-cell subpopulations. However, studies of immunoglobulin heavy chain variable (*IgVH*) genes revealed that the majority of HCL cases bear somatic mutations, indicating that cells giving rise to HCL have passed through the germinal center.[14-16] Gene expression profile analysis also revealed that HCL has a phenotype more related to post–germinal center B cells.[17] It is interesting that CD10, a marker for lymphoid cells with germinal center origin, has been reported positive in HCL in a relatively higher percentage compared with other small B-cell leukemias or lymphomas of non–germinal center origin. The frequencies of CD10 expression in HCL in the reported studies ranged from 5% to 26%.[10,11,13] Molecular studies on this subset of cases may give insight to whether these cases represent a subset of HCL with possible germinal center origin. In our 35 HCL cases, we identified 5 with expression of CD10 (14%). We did not find any morphologic or clinical features in CD10+ cases that differed from those reported for HCL with a typical immunophenotype. Our findings are in agreement with the results reported by Jasionowski et al.[13]

CD23 positivity has been reported in approximately 20% of HCL cases in some studies.[11,26] However, to the best of our

knowledge, there have been no reported studies on whether CD23+ and CD23– HCLs differ morphologically or clinically. Lack of data on CD23 in HCL probably is related to the fact that the status of CD23 does not pose diagnostic difficulties for an otherwise typical HCL, and it is considered an activation marker for B lymphocytes.

Our interest in CD23+ HCL was triggered by a study by Roman et al,[27] who found that a functional inducible nitric oxide synthase was expressed constitutively in ESKOL, a CD23+ HCL cell line. Ligation of CD23 increased inducible nitric oxide synthase expression and decreased the percentage of cells undergoing apoptosis. Similar results also were observed in leukemic cells from patients with HCL. In our study, CD23 positivity was seen in 6 of 35 cases of HCL in a relatively high percentage of leukemic cells, 3 more than 90% and 3 between 35% and 75%. One case had unusual manifestations, leukocytosis with absolute lymphocytosis and absence of monocytopenia, and 2 cases lacked thrombocytopenia. However, these cases had morphologic findings typical for HCL, and all had an excellent response to purine analogue therapy. Whether the relatively low frequency of cytopenias in CD23+ HCL cases in our study was related to the CD23 positivity is unclear. A larger number of cases and more in-depth studies would be needed to address the issue.

Several new markers have shown value in distinguishing HCL from other small B-cell malignancies in recent studies. Expression of annexin A1 detected by immunohistochemical analysis or positivity of CD123 or negativity of CD27 determined by flow cytometry demonstrated high sensitivity and specificity for HCL.[28-30] Expression of CD123 or annexin A1 also was characteristic for typical HCL and allowed differentiating it from its variant forms.[28,29] Expression of T-cell intracellular antigen-1 has been found in about 55% of HCLs but not in other forms of B-cell malignant neoplasms. Therefore, expression of T-cell intracellular antigen-1 in neoplastic cells of low-grade B-cell lymphomas may be a good diagnostic marker for HCL.[31] These markers will provide additional valuable complementary tools for the evaluation of HCL.

We report 12 of 35 HCL cases with unusual immunophenotype that was variably negative for CD103 or CD25 or positive for CD10 or CD23. If the immunophenotype is interpreted in isolation, the diagnosis of HCL could be missed. However, these cases showed similar morphologic and clinical features compared with typical HCL. Our findings indicate that it is not uncommon for HCL to display immunophenotypic variation, and recognition of these variations and correlation with morphologic findings and clinical information are essential for accurate diagnosis of HCL.

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