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Characteristic CD103 and CD123 Expression Pattern Defines Hairy Cell Leukemia:

Usefulness of CD123 and CD103 in the Diagnosis of Mature B-Cell Lymphoproliferative Disorders

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Abstract

By using flow cytometry, we studied CD103 and CD123 expression by the malignant cells in 300 B-cell lymphoproliferative disorder (BC-LPD) cases, including 114 hairy cell leukemia (HCL), 20 CL variant (HCLv), 9 splenic marginal zone lymphoma (SMZL; in 5, only CD103 was evaluated), 133 chronic lymphocytic leukemia (CLL), 3 follicular lymphoma (FL), and 21 mantle cell lymphoma (MCL). All HCLs expressed uniform CD103 and bright CD123. Among the 20 HCLv cases, 20 (100%) were CD 103+ and 8 (40%) were CD123+ (partied or dim). CD103 was negative in all MCL, FL, CLL, and SMZL cases. CD123 was positive in 1 (25%) of 4 SMZL, 3.8% of CLL (5/133), 7 (33%) of 21 MCL, and 1 (33%) of 3 FL cases. CD103 is specific for HCL and HCLv. CD123 expression is more widespread in BC-LPDs but is useful in conjunction with CD25 to differentiate HCLv from HCL. These findings support the usefulness of CD123 and CD103 to aid in the differential diagnosis of BC-LPDs.

Keywords

CD123; Flow cytometry; Non-Hodgkin lymphoma; CD103; B-cell lymphoproliferative disorders

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Flow cytometric (FC) immunophenotyping is vital in the diagnosis of B-cell lymphoproliferative disorders (BC-LPDs), including B-cell chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), and hairy cell leukemia (HCL). One marker that has universally gained acceptance as being highly useful for the diagnosis of HCL is CD123.[1–3] CD123 is a monoclonal antibody that binds specifically to the α subunit of the interleukin-3 receptor.[4] CD123 is expressed in acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), and natural killer cell and dendritic cell malignancies; however, it is rarely expressed in mature B-cell disorders.[3,5] The coexpression of CD123, in conjunction with bright CD11c, bright CD20, bright CD22, CD25, and CD103, is the immunophenotypic sine qua non in diagnosing HCL.[1,6] However, most laboratories relegate CD103 and CD123 to "special" B-cell panels that are used only when the diagnosis of HCL or HCL variant (HCLv) is considered. Consequently, the extent of expression, specificity, and usefulness of CD103 and CD123 outside the realm of HCL and related disorders (splenic marginal zone lymphoma [SMZL] and HCLv) is unclear. Therefore, we sought to evaluate CD103 and CD123 expression in a variety of BC-LPDs (follicular lymphoma [FL], MCL, and CLL) besides HCL and related disorders (HCL, HCLv, and SMZL) and to correlate the extent, intensity, and specificity of expression using multiparametric FC immunophenotyping.

Materials and Methods

Case Selection and Patient Information

From our FC database, we identified 300 cases of BC-LPDs from 2008 to 2010, in which CD103 and CD123 were done as part of a routine FC panel. CD103 and CD123 were evaluated in all cases during this period in which a diagnosis of BC-LPD was considered and sufficient cells were available. All cases in which a BC-LPD was diagnosed were included in the study. Cases included HCL (n = 114), HCLv (n = 20), SMZL (n = % CLL (n = 133), FL (n = 3), and MCL (n = 21). CD103 was evaluated in an additional 5 cases of SMZL (cases received before CD123 was added to diagnostic panels). Diagnoses were made by a hematopathologist (C.M.Y. or M.S.-S.), based on morphologic features, immunophenotype, clinical history, cytogenetics, and molecular diagnostic testing according to the World Health Organization classification.[7] All specimens were submitted as part of routine diagnostic evaluation and screening for protocol eligibility to the Flow Cytometry Unit, Laboratory of Pathology, National Cancer Institute, Bethesda, MD. All patients signed institutional review board-approved informed consents to be screened.

FC Immunophenotyping

Specimens were stained within 24 hours of collection with a panel of antibodies. Erythrocytes were lysed by incubating with lysing solution (150 mmol/L NH_4C1,10 mmol/L $KHCO_3$, and 0.1 mmol/L EDTA) for 10 minutes at room temperamre (maintained at 21°C-23°C) at a ratio of 1:9 (volume of sample/ volume of lysing solution). Specimens were then washed with phosphate-buffered saline to remove cytophilic antibodies before determining cell number. Specimens were stained for 30 minutes at room temperature (maintained at 21°C-23°C) with a cocktail of 4 to 8 antibodies (antibody concentrations used per manufacturer's recommendations) according to Clinical Laboratory Standards Institute document H43-A2 recommendations.[8,9] Antibody combinations were chosen based on the

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number of cells, diagnosis, and previous immunophenotypic data Table 1. All cells were fixed in 1.0% paraformaldehyde after staining and stored at 4°C for up to 12 hours before acquisition.

Specimens were acquired with 6-parameter, 4-color flow cytometry on the FACSCalibur (BD Biosciences, San Jose, CA) using CellQuest Pro software (BD Biosciences) before September 2009, and from September 2009 onward, with 10-parameter, 8-color flow cytometry on the FACSCanto (BD Biosciences) using FACSDiva software (BD Biosciences) (sensitivity of fluorescent detectors monitored using standard beads according to the manufacturer's recommendations). At least 5,000 lymphocytes were acquired per tube, and as many as 500,000 total events were acquired in cases of minimal residual disease. Data (collected in list mode) were analyzed with FCS Express (De Novo Software, Los Angeles, CA).

For analysis, cell populations were gated according to characteristic forward and side scatter properties, in conjunction with antigen back-gating. Expression of CD103 and CD123 was determined by gating on neoplastic cells (eg, CD20+/CD11c+bright B cells in HCL and CD19+/CD5+ B-cell populations for MCL and CLL). Normal lymphoid cells within specimens served as internal positive and negative controls (eg, B cells served as negative controls for T celldirected antibodies) and for antibody binding intensity. Bright expression was defined as higher than normal B cells, and dim expression was defined as lower than that observed in normal B cells, in concordance with the 1997 US-Canadian consensus guidelines[10] and 2006 Bethesda International Consensus Guidelines.[11] Partial expression was defined as a subpopulation of neoplastic cells staining more intensely with an antibody than observed in the internal negative control (eg, subpopulation of neoplastic B cells staining more intensely with CD123 than the CD123− T cells).

Results

The diagnoses made on the 300 specimens were as follows: 114 cases of HCL, 20 of HCLv, 9 of SMZL (CD123 data available for only 4 cases), 133 of CLL, 3 of FL, and 21 of MCL. Diagnoses of the neoplasms were determined by a hematopathologist (C.M.Y. or M.S.-S.) according to the World Health Organization classification[7] based on clinical history, morphologic features, immunophenotype, cytogenetics, and molecular studies.

CD103 was positive in all HCL and HCLv cases but negative in all MCL, FL, CLL, and SMZL cases studied. Among the HCLv cases, 2 (10%) of 20 had partial, and 2 (10%) had dim CD103 expression. CD123 was positive in the CD5+/CD19+ monoclonal B cells in 33% of MCL cases (7/21 total CD123+ cases; 2/21 dim CD123; 4/21 partial dim CD123; 1 partial bright CD123), and 3.8% of CLLs (5/133, partial dim CD123) including 1 atypical CLL. The CLL cases with CD123 expression exhibited virtually diagnostic expression of dim CD20, dim CD22, CD5, CD23, and partial dim CD11c Table 2. Image 1 illustrates 2 representative cases of CLL, one negative for CD123 (Image 1A) and the other with partial dim CD123 expression (Image 1B). The CD123+ MCL cases had typical diagnostic immunophenotypes (Table 2), ie, positive for CD5, CD25, and CD38 but negative for CD10, CD11c, and CD23 and cyclin D1 positivity in immunohistochemical studies. Partial dim

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CD123 expression in 1 case of MCL is shown in Image 1C. Partial dim CD123 expression was observed in the CD19+ and CD10+ monoclonal B cells in 1 of 3 FLs (Image 1D). Among the 4 SMZL cases in which CD123 was evaluated, 1 showed partial dim CD123 expression (Image 1E) by the CD20+ monoclonal B cells, whereas the remaining cases were negative. All HCLs demonstrated bright homogeneous CD123 expression by the CD11c, CD25, and CD20 bright positive HCL cells (Image 1F). Of the 20 HCLv cases, 8 (40%) were CD123 dim positive (Image 1G), with 2 of 8 demonstrating only partial CD123. Bright homogeneous CD123 expression was observed only in HCL cases and was not detected in HCLv, MCL, CLL, or FL.

In the diagnostic evaluation of low-grade B-cell lymphoproliferative processes, demonstration of CD103 positivity indicated a diagnosis of HCL or HCLv Table 3. In the present study, CD103 was 100% sensitive and specific for the diagnosis of HCL or HCLv. If simply considering CD123 positivity, it has 100% sensitivity and 87%. specificity for HCL. If, however, CD123 antigen intensity is also considered, bright homogeneous CD123 expression is 100% sensitive and specific for HCL. Coexpression of CD103 and bright CD123 was specific for HCL, whereas CD103+ and CD123−/ dim was diagnostic of HCLv. The CD103-/CD123dim immunophenotype was nonspecific; it was observed in a number of other B-cell malignancies, including CLL, MCL, SMZL, and FL.

Discussion

Few studies have assessed the diagnostic value of CD103 and CD123 in HCL, HCLv, and non-HCL BC-LPDs; furthermore, those few reports have limited numbers of cases.[3,5] In the present study, we determined the sensitivity and specificity of CD103 and CD123 in the diagnosis of HCL and HCLv and evaluated the frequency and extent of expression of CD123 and CD103 in a variety of non-HCL BC-LPDs.

In the present study, CD103 was 100% sensitive and specific for HCL and HCLv. These results differ from those of previous studies,[1,3] which found that 98% to 100% of HCL and 36% to 47% of HCLv cases were CD103+ (B-ly-7). This difference may be because of technical or interpretation differences. In the present study, a whole blood lysis technique was used for RBC removal, and directly conjugated antibodies were used compared with density gradient separation of WBCs and an indirect staining method.[1] Furthermore, previous studies interpreted more than 30% of the cells staining greater than controls as positive, eliminating dim and some partial CD103 expression from consideration. Another study looking at CD103+ BC-LPDs found that 78.6% of such cases coexpressed CD25 and were thought to be consistent with classic HCL. All but 1.4% (3 cases) of the CD103+ and CD25+ cases were composed of small lymphocytes (consistent with HCLv or SMZL, definitive diagnostic classification of each case not provided). Of the 3 cases with larger cells, 1 was in a patient with a history of HCL and was thought to be consistent with progression of HCL to diffuse large B-cell lymphoma and 2 were of splenic origin with morphologic features thought to be consistent with prolymphocytes or paraimmunoblasts.[12] This raises the possibility that CD103 may be expressed in occasional non-HCL or HCLv malignancies and indicates the importance of inclusion of an expanded panel of B-cell antigens in any initial diagnostic evaluation.

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We notably identified that uniform bright CD123 staining is 100% specific for HCL and can help to distinguish HCL from HCLv, although dim CD123 staining can be observed in other disorders, including HCLv, SMZL, MCL, CLL, and FL. Few studies have evaluated the expression of CD123 in non-HCL BC-LPDs.[3,5] In an early study conducted by Munoz et al, [5] CD123 expression was evaluated in a variety of hematopoietic conditions. Although 93% of AML and 100% of ALL cases were CD123+, the frequency of expression was low in the mature BC-LPDs. They noted strong expression of CD123 in 6 of 7 HCL cases (the CD123− case was also CD25−, consistent with HCLv) and did not observe this pattern of bright expression in other BC-LPDs, which is in concurrence with the findings in our study. They noted CD123 positivity in 7 of 77 cases of CLL, all of which also had atypical morphologic features, strong CD11c expression, and loss of CD25 expression, raising the diagnostic possibility of HCLv. This is in contrast with the typical morphologic features, CD25 positivity, and partial dim CD11c expression in CLL cases in the present study. CD123 positivity was also demonstrated in 1 of 12 MCLs and 1 of 5 FLs. However, CD103 was not examined in this study, and, furthermore, this study was performed using a limited 3-color FC method, precluding effective assessment of coexpression of CD123 and other relevant antigens on the neoplastic cells.

In a subsequent study tnat focused primarily on tne expression of CD123 in B-cell disorders with villous lymphocytes, Del Giudice and coworkers[3] examined cases of HCL, HCLv, splenic lymphoma with villous lymphocytes, and CLL. Notably, in concordance with our findings, the study by Del Giudice et al[3] corroborated the moderatestrong expression of CD123 in most HCLs (24/25 cases), all of which were CD103+. In addition, only 1 of their 11 HCLv cases showed expression of CD 123, whereas CD103 was partially expressed in 4 of 11 HCLv cases. Only 3% of their cases of splenic lymphoma with villous lymphocytes expressed CD123. Although all CLL cases in this study were CD103− and CD123−, only 12 cases of CLL were examined,[3] FL and MCL were not examined, and furthermore, this study was done using a limited 3-color strategy, precluding effective assessment of CD103 and CD123 on appropriate disease-specific gated B-cell subpopulations. In a more recent study, CD123 expression was investigated in classical Hodgkin lymphoma and a limited number of non-Hodgkin lymphoma cases.[13] Although the majority of Hodgkin and Reed-Sternberg cells and all 3 HCL cases studied showed CD123 expression, only 3 of the 29 CLLs showed CD123 expression, and cases of HCLv and SMZL were not included in the non-Hodgkin lymphoma group.[13]

The use or CD103 and CD123 in a standard panel is valuable for determining the diagnosis of HCL and HCLv. The present study identified that CD103 is specific for HCL and HCLv and is not observed in SMZL, CLL, MCL, and FL. Of note, we identified uniform bright CD123 expression as a specific feature of HCL that distinguishes it from HCLv. Although dim CD123 expression may be observed in HCLv, SMZL, MCL, CLL, and FL, the intensity of CD123 expression fully facilitates differentiation of these entities from HCL. In view of these findings, the inclusion of CD123 and CD103 in panels for BC-LPDs may further aid in the diagnosis of these diseases.

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Upon completion of this activity you will be able to:

- list the disease entities in which CD123 expression may be observed.
- discuss the expression patterns of CD123 in a variety of B-cell lymphoproliterative disorders.
- formulate a comprehensive flow cytometry panel that distinguishes between hairy cell leukemia, hairy cell leukemia variant, and related B-cell lymphoproliterative disorders.

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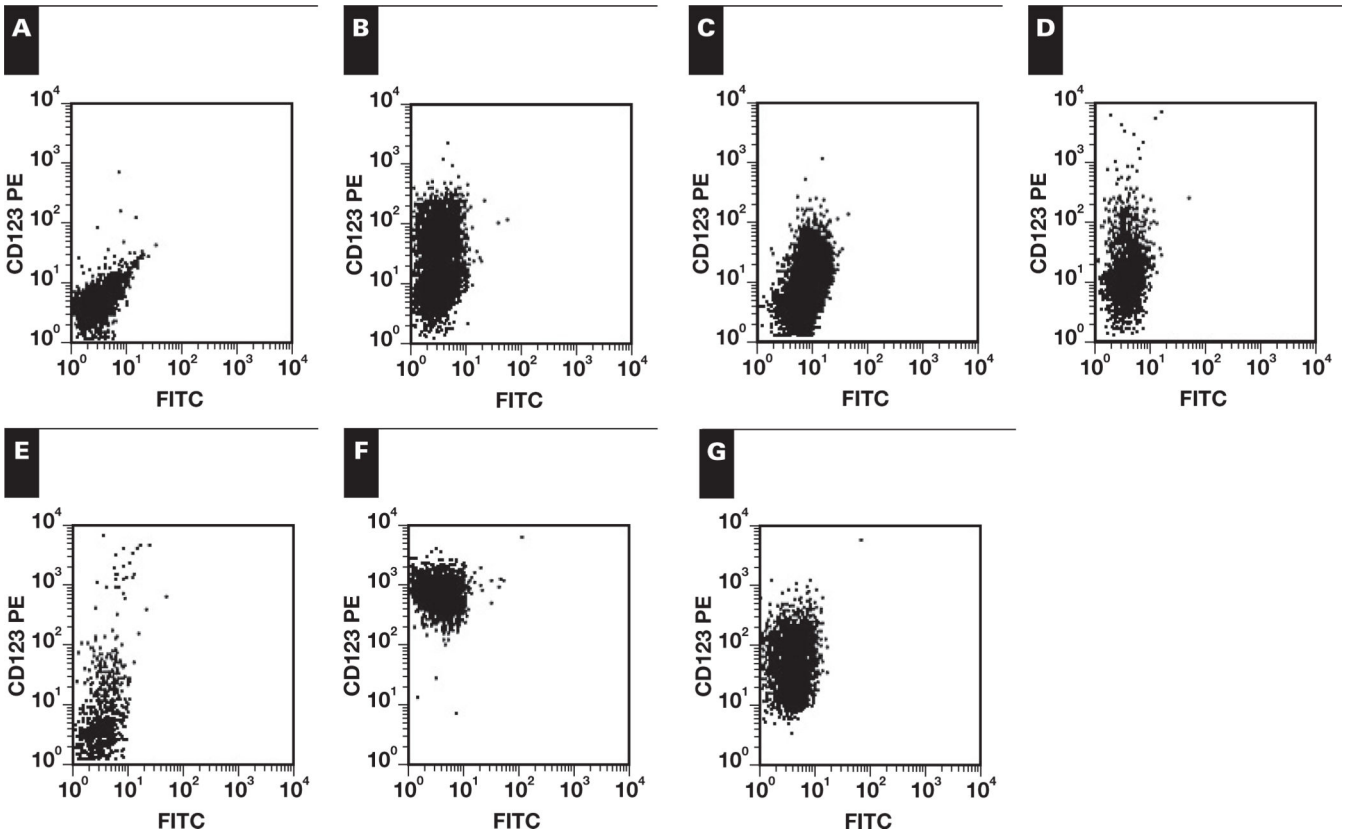

Image 1.

Flow cytometric immunophenotypic detection of CD123 expression. **A**, Chronic lymphocytic leukemia (CLL) case negative for CD123. Analysis gate was drawn around the CD19+ and CD5+ CLL cells. **B**, CLL case with partial moderate CD123. Analysis gate was drawn around the CD19+ and CD5+ CLL cells. **C**, Mantle cell lymphoma (MCL) case with partial dim CD123. Analysis gate was drawn around the CD19+ and CD5+ MCL cells. **D**, Follicular lymphoma (FL) case with partial dim CD123. Analysis gate was drawn around the CD19+ and CD10+ FL cells. **E**, Splenic marginal zone lymphoma (SMZL) case with partial dim CD123. Analysis gate was drawn around the CD19+ SMZL cells (all B cells monoclonal). **F**, Hairy cell leukemia (HCL) case with uniform bright CD123. Analysis gate was drawn around the bright CD20+ and CD11c+ HCL cells. **G**, HCL-variant (HCLv) case with dim CD123. Analysis gate was drawn around the bright CD20+ and CD11c+ HCLv cells. FITC, fluorescein isothiocyanate; PE, phycoerythrin.

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Table 1

Antibody Combinations for Characterization of B Cells *

FITC	PE	PerCP/Cy5.5	PC7	APC	AH7	V500	V450
CD103	CD25	CD20	—	CD11c	—	—	—
—	CD123	CD20	—	CD-X	—	—	—
—	CD123	CD19	—	CD-X	—	—	—
κ	λ	CD19	—	CD-X	CD-X	—	—
κ	CD22	CD20	—	CD-X	CD-X	—	—
λ	CD22	CD20	—	CD-X	CD-X	—	—
κ	CD22	CD5	CD19	CD10	CD20	CD45	CD11c
λ	CD22	CD5	CD19	CD10	CD20	CD45	CD11c
CD103	CD25	CD123	CD19	CD23	CD20	CD45	CD11c

AH7, allophycocyanin/cyanin tandem conjugate; APC, allophycocyanin; Cy, cyanine; FITC, fluorescein isothiocyanate; PC7, phycoerythrin/cyanin dye 7 tandem conjúgate; PE,phycoerythrin; PerCP, peridinin chlorophyll protein.

* For hairy cell leukemia and hairy cell leukemia-variant specimens, X indicates CD11c; for mantle cell lymphoma and chronic lymphocytic leukemia, X indicates CD5; forfollicular lymphoma, X indicates CD10; for splenic marginal zone lymphoma cases, X indicates CD5, CD10, or CD11c, depending on the submitted patient history. The panelof antibodies for initial screening also included CD2, CD3, CD4, CD5, CD7, CD8, CD14, CD16, CD56, CD57, T-cell receptor (TCR)ap, and TCRyó.

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Table 2

Immunophenotypic Profile of Non–Hairy Cell Leukemia B-Cell Lymphoproliferative Disorders That Express CD123

	CD19	CD20	CD22	CD5	CD10	CD11c	CD23	CD25	CD38
MCL	+	+	+	+	−	−	−	+	+
MCL	+	+	+	+	−	−	−	+	NA
MCL	+	+	+	+	NA	−	NA	+	+
MCL	+	+	+	+	−	−	−	+	+
MCL	+	+	+	+	−	−	−	+	+
MCL	+	+	+	+	NA	−	−	+	+
MCL	+	+	+	+	−	−	−	+	+
CLL	+	+ Dim	+ Dim	+	−	+ Partial dim	+	+	+
CLL	+	+ Dim	+ Dim	+	−	+ Partial dim	+	+	+ Partial
CLL	+	+ Dim	+ Dim	+	−	+ Partial dim	+	−	+ Partial
CLL	+	+ Dim	+ Dim	+	−	+ Partial dim	+	+	+
CLL	+	+ Dim	+ Dim	+	−	+ Partial dim	+	+	+
FL	+ Dim	+	+	−	+	−	+	−	NA
SMZL	+	+	+	−	−	−	+ Partial dim	+ Dim	NA

CLL, chronic lymphocytic leukemia; FL, follicular lymphoma; MCL, mantle cell lymphoma; NA, not available; SMZL, splenic marginal zone lymphoma; +, positive; −, negative.

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Table 3

CD103 and CD123 Expression in B-Cell Lymphoproliferative Disorders

Diagnosis	CD103+/CD123−	CD123+/CD103−	CD103+/CD123+	CD103−/CD123−	Total
HCL	0	0	114	0	114
HCLv	12	0	8	0	20
SMZL *	0	1	0	3	4
MCL	0	7	0	14	21
CLL	0	5	0	128	133
FL	0	1	0	2	3

CLL, chronic lymphocytic leukemia; FL, follicular lymphoma; HCL, hairy celi leukemia; HCLv, HCL variant; MCL, mantle celi lymphoma; SMZL, splenic marginal zone lymphoma.

* 5 additional SMZL cases were evaluated for CD103 only; all 5 were negative.