CME/SAM

Immunohistochemical Detection of Hairy Cell Leukemia in Paraffin Sections Using a Highly Effective CD103 Rabbit Monoclonal Antibody

Elizabeth A. Morgan, MD,[1] Hongbo Yu, MD, PhD,[2] Jack L. Pinkus, PhD,[1] and Geraldine S. Pinkus, MD[1]

Key Words: Hairy cell leukemia; CD103; Paraffin sections; Monoclonal antibody; Immunohistochemistry

DOI: 10.1309/AJCPHW7RULIZT2GB

Upon completion of this activity you will be able to:
- describe the reactivity pattern of CD103 in nonneoplastic tissues.
- discuss the utility of the CD103 antibody in the diagnosis of hairy cell leukemia in paraffin-embedded tissues, particularly in the setting of various fixation mediums.
- define the staining pattern of CD103 in neoplastic B cells.

The ASCP is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians. The ASCP designates this journal-based CME activity for a maximum of 1 *AMA PRA Category 1 Credit*™ per article. Physicians should claim only the credit commensurate with the extent of their participation in the activity. This activity qualifies as an American Board of Pathology Maintenance of Certification Part II Self-Assessment Module.

The authors of this article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.

Questions appear on p 256. Exam is located at www.ascp.org/ajcpcme.

Abstract

Detection of the integrin subunit CD103 is a useful diagnostic tool in the diagnosis of hairy cell leukemia (HCL). Currently, flow cytometric analysis (FC) and frozen section immunohistochemistry (IHC) represent the only available methods of detection. This study is the first to describe the successful use of a CD103 antibody to identify HCL and HCL-variant in paraffin sections of formalin- or Bouin solution–fixed specimens (n = 68) using an immunoperoxidase technique. In other B-cell lymphoproliferative disorders that morphologically may resemble HCL, including chronic lymphocytic leukemia/small lymphocytic lymphoma (n = 32), mantle cell lymphoma (n = 23), lymphoplasmacytic lymphoma (n = 27), follicular lymphoma (n = 7), and marginal zone lymphoma (n = 13), lymphoid cells are nonreactive for CD103. In HCL, the CD103 staining pattern is predominantly membranous with delineation of delicate cytoplasmic projections. This CD103 antibody is an extremely valuable addition to the IHC panel for the diagnosis of HCL, especially in cases lacking FC analysis.

CD103 is an integrin subunit, αE, encoded by the *ITGAE* gene on chromosome 17.[1,2] αE binds with the β7 integrin subunit to form the heterodimer integrin αEβ7 (also referred to as the human mucosal lymphocyte-1 [HML-1] antigen). The existence of the human protein was first described in 1987 following development of a monoclonal antibody, HML-1, which was raised against human intestinal intraepithelial lymphocytes (IELs). The antibody reacted with all intestinal IELs as well as IELs at other mucosal sites, but only showed reactivity with rare lymphocytes in the peripheral blood (less than 2%), bone marrow, and other lymphoid sites.[3] A monoclonal antibody reactive to hairy cell leukemia (HCL) cells called B-ly7[4] was found to have similar specificity to HML-1, as did the monoclonal antibodies Ber-ACT8 and LF61.[5-7] These antibodies, along with 3 others, were clustered to CD103 in 1993.[2] None revealed immunoreactivity in paraffin sections.

Since those initial studies, expression of CD103 has been detected using flow cytometric (FC) analysis or frozen section immunohistochemistry (IHC) on nearly all reported cases of HCL as well as a significant proportion of cases classified as HCL-variant (HCL-v). The vast majority of other B-cell neoplasms, however, do not show CD103 expression.[4,7-14] CD103 expression has only rarely been reported in splenic marginal zone lymphoma[15,16] and infrequently in the 2008 World Health Organization (WHO) provisional entity splenic diffuse red pulp small B-cell lymphoma.[17,18] Based on this highly selective staining pattern, CD103 is routinely used in FC panels for identifying HCL in peripheral blood, bone marrow aspirate,

and tissue samples. The role of CD103 in the mechanism of HCL is largely unknown.

Because of the associated reticulin fibrosis that can occur in HCL, aspirate samples may yield little to no diagnostic material for FC. Thus, morphologic and IHC analysis of the bone marrow core biopsy specimen is critical for diagnosis of this entity. At present, markers available for detection of HCL in paraffin-embedded tissue sections (depending on fixation and decalcification protocols) include TRAP, DBA.44, and annexin A1 as well as CD123, cyclin D1, T-bet, and CD200. No single marker is diagnostic of HCL. Several markers, including CD123, cyclin D1, T-bet, and CD200, are positive in other lymphoproliferative disorders.[11,19,20] Although antibodies against annexin A1 are highly specific for HCL compared with other B-cell neoplasms,[21] they also detect myeloid cells and some T cells, thus creating difficulties in interpretation, particularly if involvement by HCL is limited. Also, scattered cells demonstrating TRAP and DBA.44 reactivity have been reported in normal bone marrow,[22] further confounding evaluation for minimal tumor involvement. Thus, the ability to detect CD103-positive cells in paraffin sections would be extremely helpful for the diagnosis of HCL.

To our knowledge, the successful use of a CD103 antibody for IHC detection of HCL in paraffin-embedded sections has not been described in the literature. The purpose of our study was to examine the reactivity pattern of a CD103 rabbit monoclonal antibody in paraffin-embedded sections of a large series of HCL and other B-cell lymphoproliferative disorders, particularly those considered in the differential diagnosis of HCL; we also wished to profile its staining pattern in nonneoplastic hematopoietic tissues. In addition, variations in immunoreactivity for CD103 as related to different fixatives used in bone marrow biopsies, the main tissue type used in the diagnosis of HCL, were evaluated.

Materials and Methods

Case Selection

Cases were obtained from Department of Pathology files with the approval of the institutional review boards of Brigham and Women's Hospital (Boston, MA) and UMass Memorial Medical Center (Worcester, MA). Classification of disease using morphologic and immunophenotypic features was performed according to the 2008 *WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues*.[18] Diagnosis was confirmed by means of slide review, FC reports, and clinical findings. Cases included 62 HCL (59 bone marrow, 3 spleen); 6 HCL-v (4 bone marrow, 2 spleen); 32 chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) (26 bone marrow, 4 spleen, 2 lymph nodes); 23 mantle cell lymphoma (MCL) (18 bone marrow, 3 spleen, 2 lymph nodes); 27 lymphoplasmacytic lymphoma (LPL) (26 bone marrow, 1 spleen); 7 follicular lymphoma (FL) (4 bone marrow, 3 lymph nodes); 2 nodal marginal zone lymphoma (NMZL; lymph nodes); and 11 splenic marginal zone lymphoma (SMZL) (7 bone marrow, 4 spleen). Fixatives included 10% neutral-buffered formalin, Bouin solution (StatLab Medical Products, McKinney, TX), Zenker fixative, B-plus fixative (BBC Biochemical, Mount Vernon, WA), or B-5 fixative (Table 1) and (Table 2). Nonneoplastic tissue specimens included 8 bone marrows, 4 lymph nodes, 7 spleens, 6 tonsils, 1 thymus, 1 stomach biopsy, and 5 intestinal biopsies. Bone marrow biopsy specimens were fixed in Bouin solution; other

Table 1
CD103 Immunoreactivity in Bone Marrow Biopsy Specimens and Spleens Involved by HCL and HCL-v

Diagnosis	Specimen Type	Fixative	No./Total (%) of CD103-Positive Cases
HCL	Bone marrow (n = 59)	Bouin solution	13/13 (100)
		Formalin	28/28 (100)
		Zenker solution	6/13 (46)[a]
		Unknown	4/5 (80)
	Spleen (n = 3)	B-5	2/2 (100)
		Formalin	1/1 (100)
HCL-v	Bone marrow (n = 4)	Bouin solution	2/2 (100)
		Formalin	1/2 (50)
	Spleen (n = 2)	B-plus fixative	2/2 (100)

HCL, hairy cell leukemia; HCL-v, hairy cell leukemia–variant.
[a] One CD103-negative case was also negative for CD103 on flow cytometry but was clinically consistent with HCL.

Table 2
CD103 Immunoreactivity in 102 Specimens Involved by Various B-Cell Lymphoproliferative Disorders

Diagnosis, Specimen Type, and Fixative	No./Total (%) of CD103-Positive Cases
CLL/SLL (n = 32)	
Bone marrow (16 B, 10 F)	0/26 (0)
Spleen (3 B-P, 1 F)	0/4 (0)
Lymph node (1 B-P, 1 F)	0/2 (0)
MCL (n = 23)	
Bone marrow (8 B, 10 F)	0/18 (0)
Spleen (1 B-P, 2 F)	0/3 (0)
Lymph node (2 F)	0/2 (0)
LPL (n = 27)	
Bone marrow (16 B, 10 F)	0/26 (0)
Spleen (1 B-P)	0/1 (0)
FL (n = 7)	
Bone marrow (4 B)	0/4 (0
Lymph node (3 F)	0/3 (0)
NMZL (n = 2)	
Lymph node (1 B-P, 1 F)	0/2 (0)
SMZL (n = 11)	
Bone marrow (5 B, 2 F)	0/7 (0)
Spleen (4 B-P)	0/4 (0)

B, Bouin solution; B-P, B-plus fixative; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; F, 10% neutral-buffered formalin; FL, follicular lymphoma; LPL, lymphoplasmacytic lymphoma; MCL, mantle cell lymphoma; NMZL, nodal marginal zone lymphoma; SMZL, splenic marginal zone lymphoma.

DOI: 10.1309/AJCPHW7RULIZT2GB

tissue specimens were fixed in 10% neutral-buffered formalin and B-plus fixative. A multitissue microarray of formalin-fixed normal tissue specimens was evaluated (NormalGrid multi-tissue control slides, Biomeda, Foster City, CA).

Immunohistochemical Analysis

Immunohistochemical studies were performed on paraffin sections using a rabbit monoclonal antibody to CD103 (integrin αE; clone EPR4166[2] Epitomics, Burlingame, CA). A synthetic peptide corresponding to residues in the extracellular domain of human CD103/integrin αE was used as immunogen. Briefly, following deparaffinization, slides were treated for 5 minutes with 3% hydrogen peroxide to inactivate endogenous peroxidase and then washed. Picrates were removed from Bouin solution–fixed tissue using a saturated alcoholic solution of lithium carbonate. Heat-induced epitope retrieval was performed using EDTA (0.001 mol/L), pH 8.0 (Invitrogen, San Francisco, CA), for 30 minutes in a steamer (model HS80, Black & Decker, Shelton, CT), then allowed to remain in the hot EDTA solution for an additional 10 minutes at room temperature. Slides were washed and placed in Tris buffer (Covance, Dedham, MA), then incubated with CD103 antibody (1:3,000 dilution was found to be optimal; 1:1,000 dilution was also used for some bone marrow specimens) for 50 minutes at room temperature. Slides were then washed and incubated for 30 minutes at room temperature with a horseradish peroxidase–labeled polymer conjugated to goat antirabbit immunoglobulin antibodies (PowerVision, Leica Microsystems, Buffalo Grove, IL). For antibody localization, a peroxidase reaction was performed using the DAB+ reagent (Dako, Carpinteria, CA). Reaction product was enhanced by dipping slides into a solution of copper sulfate. Slides were counterstained with hematoxylin, dehydrated, and mounted. Positive control slides (HCL involving bone marrow or spleen) were included in all runs. Negative control studies were performed using matched isotype control rabbit IgG (Epitomics).

Results

Nonneoplastic Tissues

Nonneoplastic tissue specimens evaluated for CD103 immunoreactivity included bone marrow biopsy specimens with maturing trilineage hematopoiesis (n = 8) as well as specimens from the lymph nodes (4), spleen (7), tonsil (6), thymus (1), stomach (1), and intestine (5). The bone marrow specimens (all fixed in Bouin solution) demonstrated a few CD103-positive mononuclear cells (<1% of cellularity) that were scattered in the interstitium or located in a paratrabecular distribution **Image 1A** and exhibited cytoplasmic reactivity of variable intensity (Image 1A, inset). In lymph nodes, small mononuclear cells with strong CD103 immunoreactivity were detected predominantly in a scattered distribution in the interfollicular area and were also present in fewer numbers in the mantle zones and in germinal centers **Image 1B**. Larger cells resembling dendritic cells in the interfollicular area demonstrated reactivity for CD103 (Image 1B, inset). Splenic sections showed scattered cells predominantly in the red pulp as well as fewer cells distributed in the white pulp **Image 1C**. Within the tonsil, the CD103 antibody

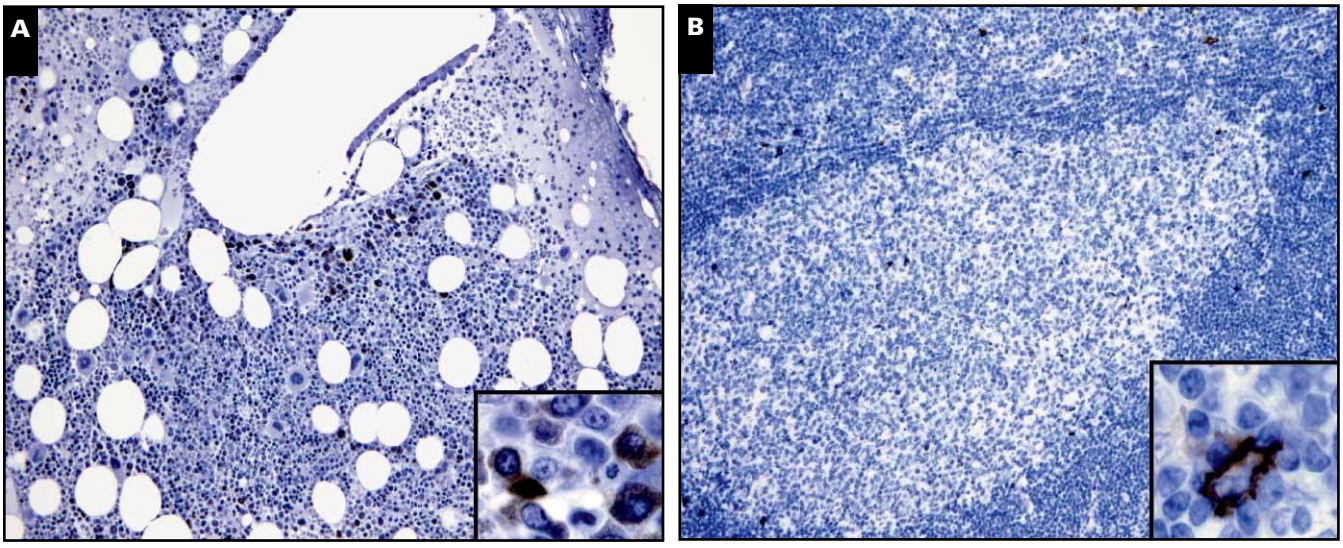
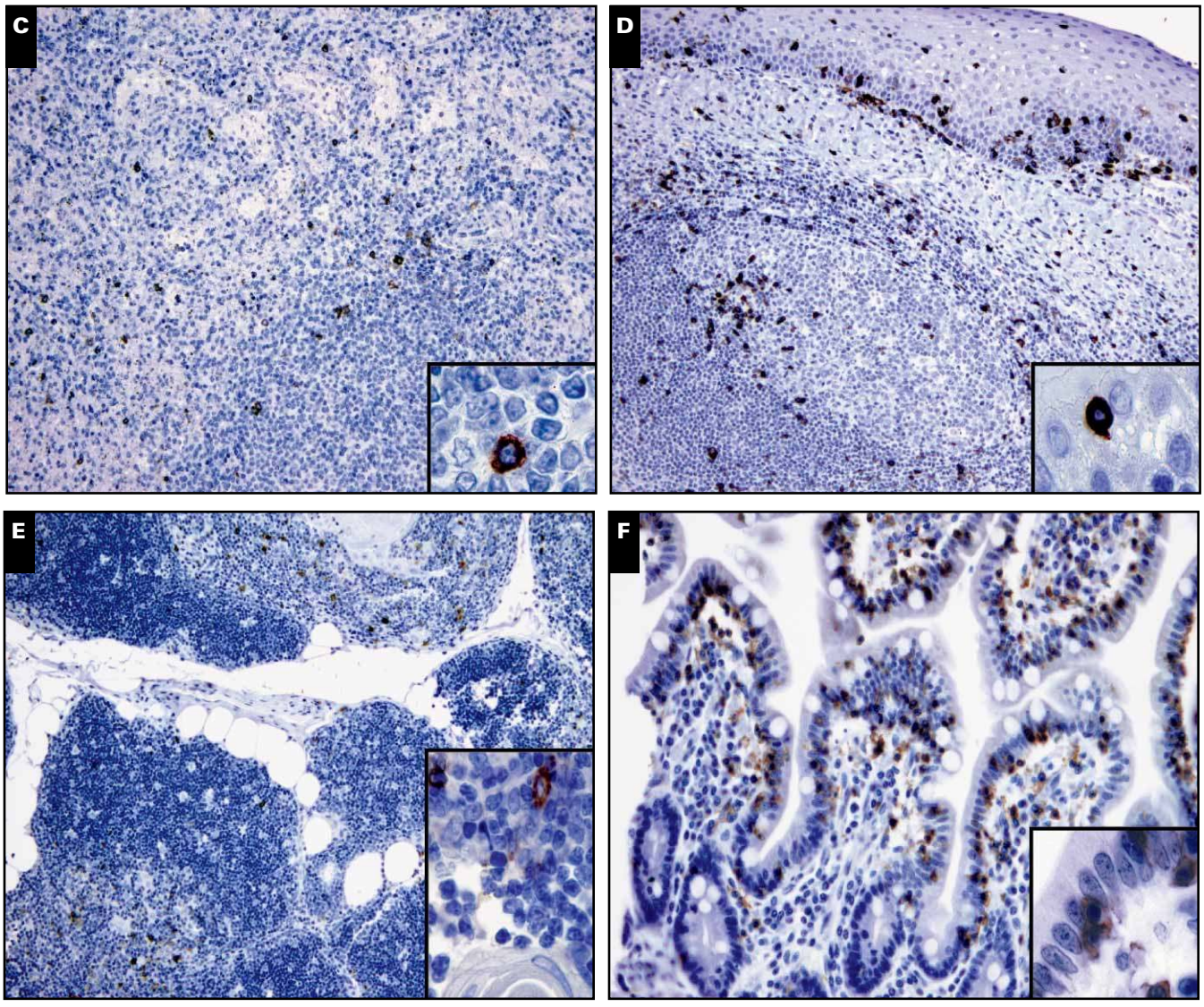


Image 1 CD103 expression in nonneoplastic tissues. **A**, Bone marrow biopsy specimen, Bouin solution fixed. Scattered cells with cytoplasmic CD103 positivity are present, particularly in a paratrabecular distribution. **B**, Lymph node, B-plus fixed. A few scattered CD103-positive cells with cytoplasmic reactivity are seen predominantly in the interfollicular space, some with morphologic features of dendritic cells as noted in the inset image.

DOI: 10.1309/AJCPHW7RULIZT2GB

highlighted intraepithelial cells in the overlying mucosa, located primarily toward the basal layer, as well as scattered cells in the underlying tonsillar lymphoid tissue (**Image 1D**). Within the thymus, CD103-positive cells localized primarily to the thymic medulla (**Image 1E**) (inset). As expected, the majority of gastric and small and large intestinal IELs as well as lymphocytes in the lamina propria were CD103-positive (**Image 1F**) (inset) (small intestine) (stomach and large intestine, data not shown). Sections of other tested organs (brain, pituitary, adrenal gland, breast, heart, liver, skin, kidney, pancreas, lung, testis, ovary, uterus, placenta, prostate, and thyroid) did not demonstrate any organ-specific staining patterns (data not shown).

As described before, a subset of CD103-positive cells was present in the paratrabecular areas of nonneoplastic bone marrow biopsy specimens, reminiscent of the localization of immature myeloid elements. To explore this further, we examined pretreatment bone marrow specimens obtained from 4 patients with chronic myelogenous leukemia (CML) and 5 patients with acute myeloid leukemia (AML), including 2 acute promyelocytic leukemias (APML), as well as 5 splenectomy specimens showing extramedullary hematopoiesis secondary to myelofibrosis (data not shown). In 3 of 4 CML cases, the expanded paratrabecular cuffs as well as the interstitial space contained an increased number of CD103-positive cells compared with the nonneoplastic



C, Spleen, B-plus fixed. Scattered CD103-positive cells are present in both the red and white pulp. **D**, Tonsil, formalin fixed. CD103-positive cells are identified in intraepithelial and lymphoid areas. **E**, Thymus, formalin fixed. Most of the scattered CD103-positive cells are present in the medulla and exhibit cytoplasmic staining. **F**, Small intestine, formalin fixed. Most of the intestinal intraepithelial lymphocytes, as well as lymphocytes in the lamina propria, are strongly positive for CD103. (Immunoperoxidase, hematoxylin counterstain; all images, ×200; insets, ×1,000.)

DOI: 10.1309/AJCPHW7RULIZT2GB

bone marrow samples; however, uniform reactivity of all myeloid elements was not apparent. None of the AML cases, including APML, showed an increase in paratrabecular or interstitial CD103-reactive cells compared with the nonneoplastic samples; in fact, most showed little to no reactivity because the intertrabecular space was occupied almost entirely by blast forms. In one of the spleens, many CD103-positive cells were present in clusters associated with megakaryocytes. In the remainder of the splenectomy specimens, the number of scattered CD103-positive cells was no greater than that observed in the nonneoplastic spleen sections described earlier.

HCL, Typical and Variant

In all bone marrow biopsy specimens of HCL fixed in either Bouin solution or formalin (Table 1), the lesional cells exhibited uniform strong CD103 immunoreactivity. In half of the Zenker solution–fixed bone marrow biopsy specimens, the lesional cells showed little to no reactivity with the CD103 antibody. This was despite a burden of disease averaging 60% of the cellularity as determined by CD20 expression, suggesting suboptimal antigen preservation with this fixative (Table 1). Reactivity patterns for Zenker solution–fixed tissue specimens showed little or no improvement with different retrieval solutions including those with high pH. In 5 bone marrow biopsy specimens from our consultation files demonstrating HCL, in which the fixation medium is unknown, 4 (80%) demonstrated CD103 positivity in the lymphoid infiltrate (Table 1). In all HCL bone marrow biopsy specimens, CD103 expression was detected via diagnostic FC when performed (36 of 59 aspirates), with 1 exception. That case, which was morphologically and clinically consistent with HCL, was negative for CD103 on FC as well as IHC (Zenker solution–fixed biopsy specimen).

In general, the cytologic staining pattern for CD103 is membranous; cytoplasmic reactivity ranging from weak to intermediate may also be seen **Image 2A**, **Image 2B**, and **Image 2C**. Irregular cytoplasmic borders, consistent with projections, are detectable at high power **Image 2D**. The vast majority of the biopsy specimens tested had a high burden of involvement by HCL (average extent of involvement by CD20 staining was 60%-70% of the cellularity), but in 5 specimens the extent of neoplastic involvement was 10% or less. In both scenarios, the expression pattern of CD103 mirrored that of CD20 performed on a sequential section, confirming that the observed CD103 reactivity was occurring in neoplastic B cells **Image 3A**, **Image 3C**, **Image 3E**, **Image 3B**, **Image 3D**, and **Image 3F**. Assessment of annexin A1 expression in the same set of cases revealed that the neoplastic infiltrate was obscured by background staining of granulocytes when disease burden was low **Image 3H**, but this background staining was inconsequential in more extensive infiltrates **Image 3G**.

Three HCL splenectomy specimens also demonstrated CD103 positivity in the neoplastic infiltrate, regardless of fixation medium (Table 1) **Image 4A**, **Image 4C**, **Image 4E**, and **Image 4G**.

HCL-v cases were identified based on immunophenotypic features and clinical characteristics. Nearly all samples tested (3 of 4 bone marrow biopsy specimens and 2 of 2 splenectomy specimens) demonstrated immunoreactivity with the CD103 antibody (Table 1) **Image 4B**, **Image 4D**, **Image 4F**, and **Image 4H**. All 6 of these specimens demonstrated CD103 expression on diagnostic FC. Sections processed using the isotype control rabbit antibody were all negative.

Other B-Cell Lymphoproliferative Disorders

Biopsy specimens from 102 other B-cell lymphoproliferative disorders (CLL/SLL [n = 32], MCL [n = 23], LPL [n = 27], FL [n = 7], NMZL [n = 2], and SMZL [n = 11]) were evaluated for CD103 immunoreactivity. Neoplastic cells were not reactive for CD103 in any of the cases (Table 2). In the bone marrow biopsy specimens with CLL/SLL, MCL, and LPL, particularly those with extensive involvement, few cells with cytoplasmic reactivity were noted in the paratrabecular area similar to those observed in nonneoplastic bone marrow biopsy specimens. In cases with patchy neoplastic involvement, scattered CD103-positive cells with variable cytoplasmic intensity were present in the uninvolved interstitial space. However, cells with a membranous staining pattern and cytoplasmic projections were not identified in either the paratrabecular or interstitial locations (data not shown).

Discussion

Cerf-Bensussan and colleagues[3] first identified the CD103 molecule (the αE subunit of the integrin αEβ7) based on its expression by intestinal IELs and lamina propria lymphocytes. Expression of αEβ7 by these lymphocytes, which are predominantly effector/memory CD8-positive T cells, mediates adhesion of the lymphocytes to the ligand E-cadherin, which is expressed on the basolateral surface of enterocytes.[23] CD103 expression has also been identified on lymphocytes (primarily CD8-positive T cells) in the epithelium of the bronchi, oral cavity and tonsil, esophagus, and stomach as well as on fewer cells associated with epithelium of the breast, uterus, conjunctiva, parotid gland, and pancreas, earning the molecule the designation "mucosal lymphocyte antigen" or MLA.[3,6,9,24,25] Typically, less than 5% of epidermal and dermal lymphocytes in benign and inflammatory skin biopsy specimens show reactivity with the HLM-1 clone.[26,27]

DOI: 10.1309/AJCPHW7RULIZT2GB

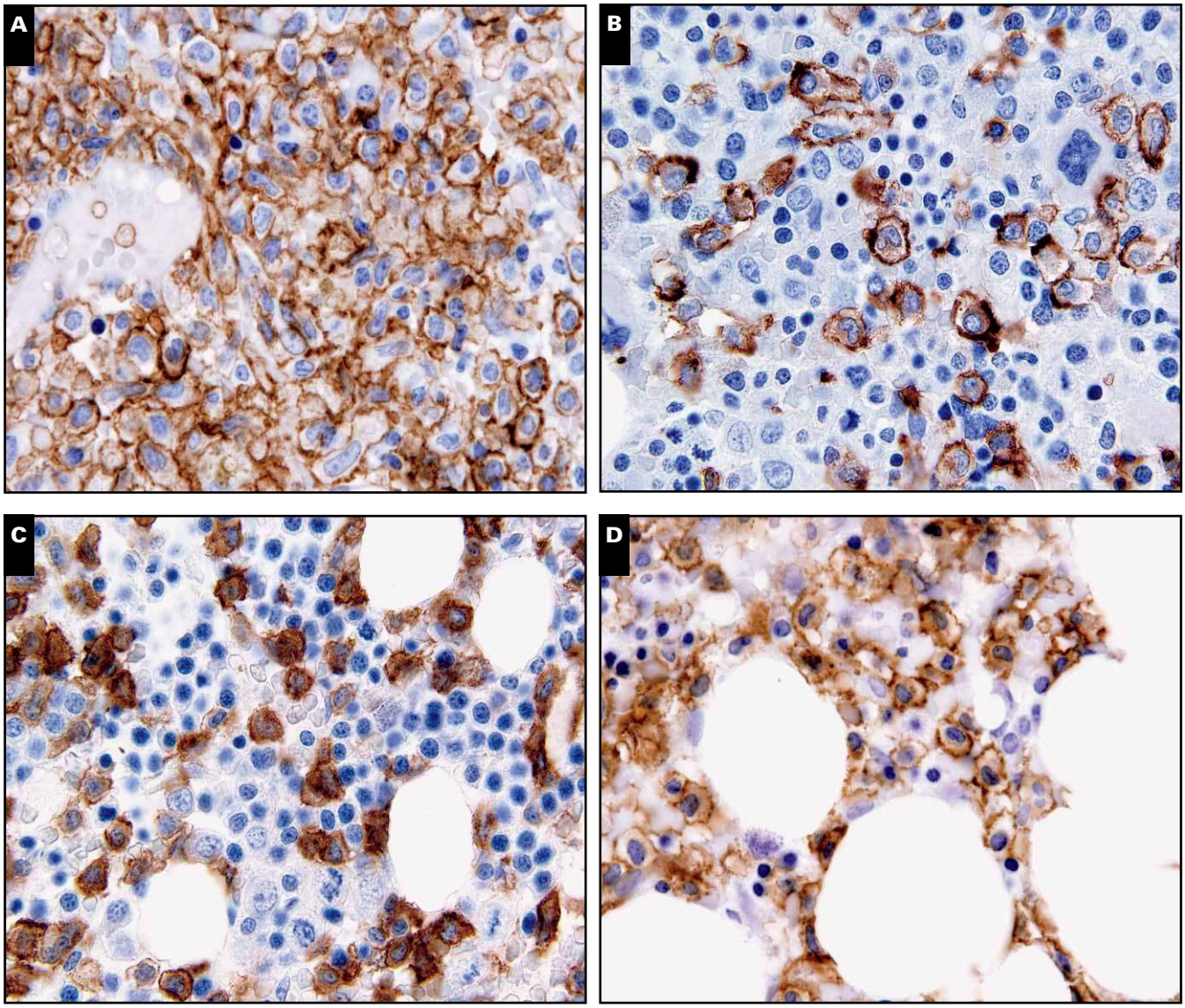


Image 2 CD103 pattern of immunoreactivity in hairy cell leukemia seen in bone marrow biopsy specimens. Immunoreactivity for CD103 is predominantly membranous (**A**) with some cells also showing cytoplasmic reactivity of weak (**B**) to intermediate (**C**) intensity. Delicate cytoplasmic projections are discernible (**D**). (**A-C**, Bouin solution; **D**, formalin; immunoperoxidase, hematoxylin counterstain; all images, ×1,000.)

In the hematopoietic organs, scattered cells with reactivity to 1 or more antibody clones directed against CD103 (LP61, B-ly7, Ber-ACT8, or HML-1) are present in the interfollicular areas of the tonsil, red pulp of the spleen, interfollicular space and mantle zones of lymph nodes, and thymic medulla.[3,6,7,9] Based on double-labeling experiments and/or morphologic features, these cells are generally interpreted to be CD8-positive T cells. Examination of normal bone marrow revealed that less than 1% of cells are positive with antibodies to CD103.[3,10] Less than 2% of peripheral blood cells are positive with the HML-1 antibody.[3] Although early reports suggested that these peripheral blood cells were within the B-cell compartment and represented the physiologic counterpart of HCL cells,[4] subsequent double immunofluorescence studies confirmed that the cells are predominantly CD8-positive memory T cells with a homing receptor phenotype; these cells are thought to be a recirculating component of the mucosa-associated T cells.[7,28] In addition, CD103 expression has been reported in T-cell lymphomas primarily arising from the intestinal tract.[29,30] Subsequent investigations have revealed that the integrin is expressed not only on effector/memory CD8-positive T cells but also on subsets of CD4-positive and CD8-positive regulatory T cells and on dendritic cells in the intestinal mucosal and mesenteric lymph nodes,[31-33] and plays an important role in mucosal immunity.

Expression of CD103 has been detected on FC or frozen section IHC in nearly all reported cases of HCL, a significant

DOI: 10.1309/AJCPHW7RULIZT2GB

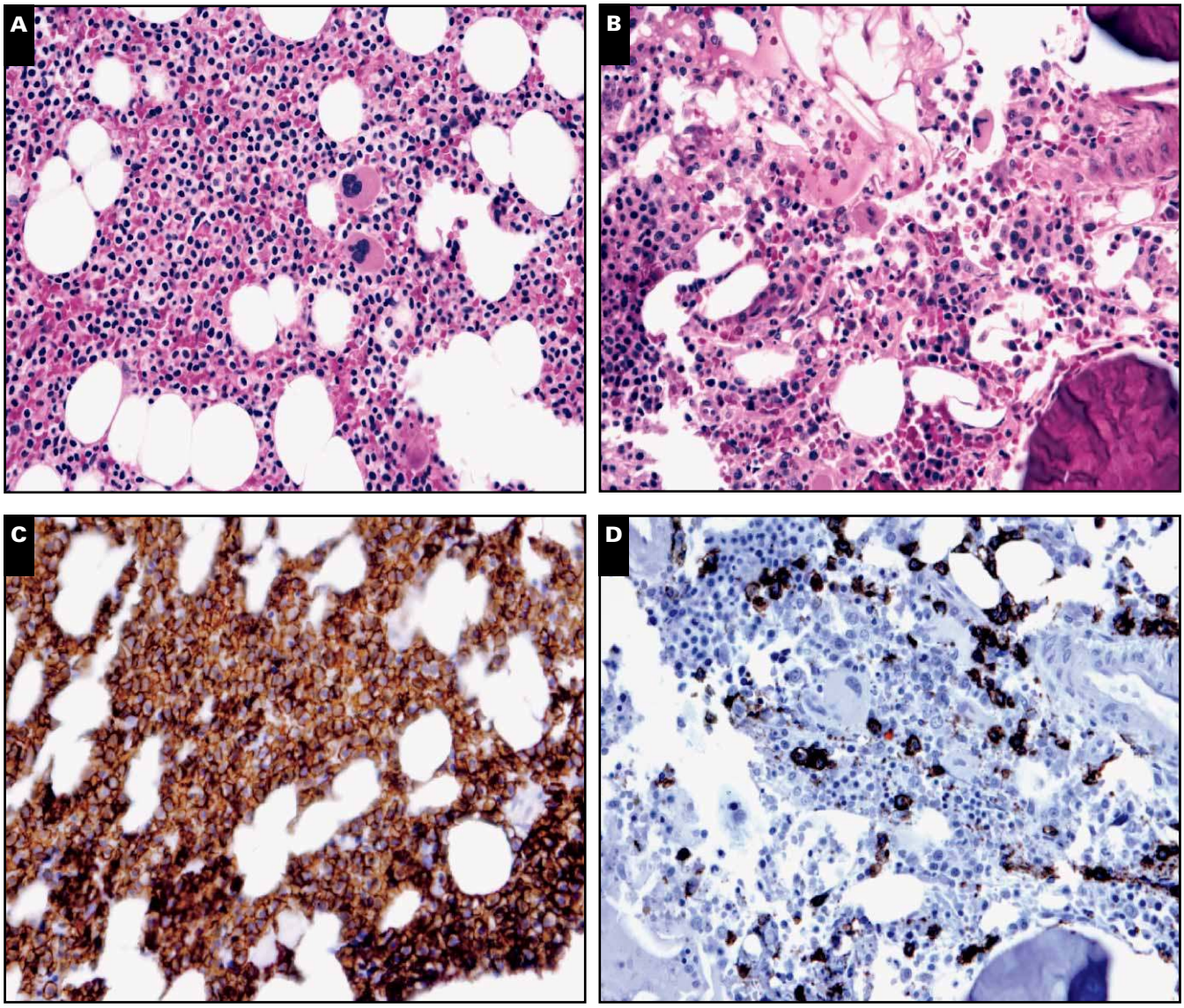
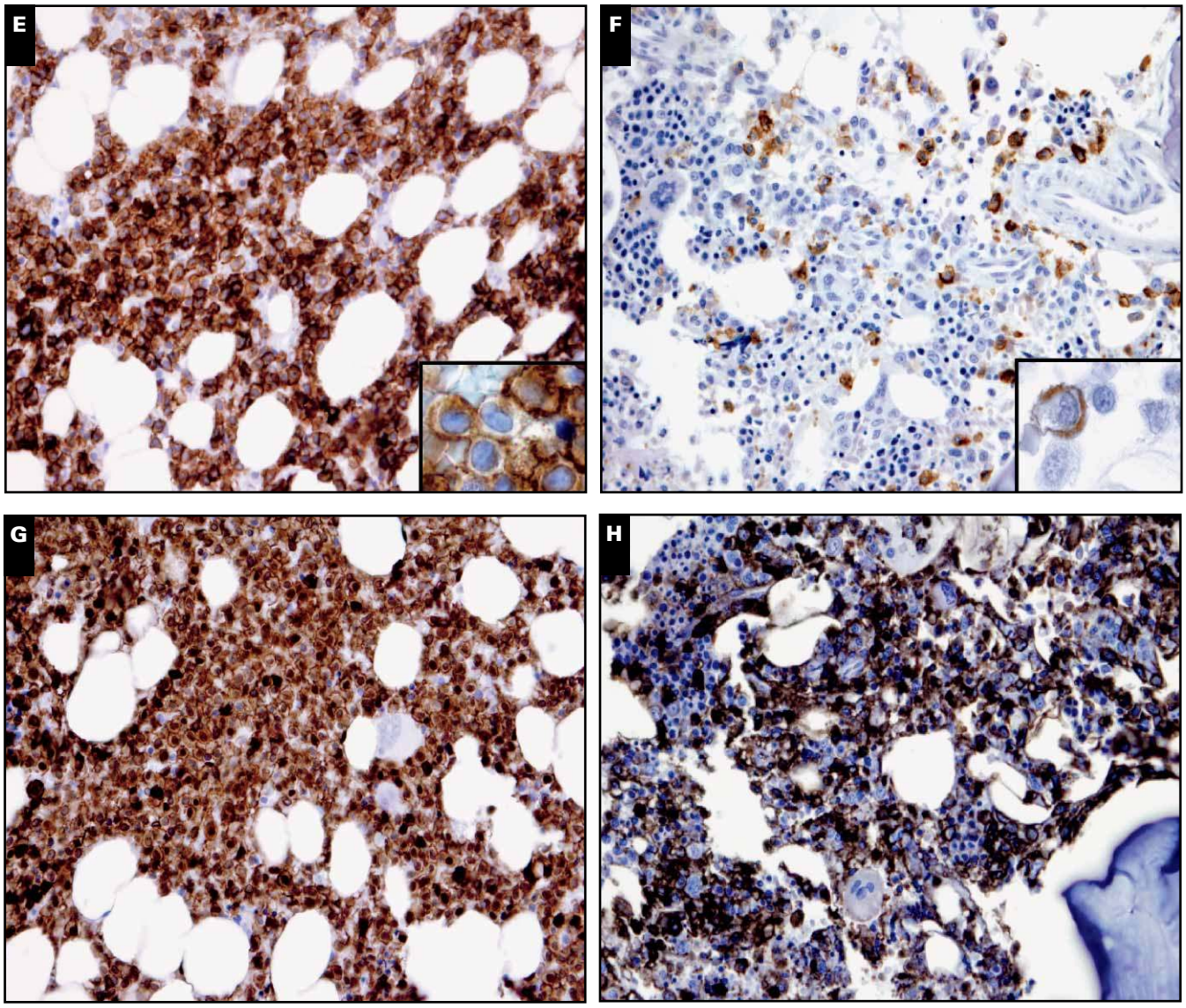


Image 3 CD103 compared with annexin A1 in both high-level (90%, **A**, **C**, **E**, **G**) and low-level (10%, **B**, **D**, **F**, **H**) involvement by hairy cell leukemia (HCL) in formalin- (**A**, **C**, **E**, **G**) and Bouin (**B**, **D**, **F**, **H**) solution–fixed bone marrow biopsy specimens. Expression of CD103 (**E**-**F**) mirrors that of CD20 (**C**-**D**) in both high-level and low-level involvement by HCL.

proportion of HCL-v cases, and infrequently in SMZL and the 2008 WHO provisional entity splenic diffuse red pulp small B-cell lymphoma. The role of CD103 on the neoplastic hairy cells is unknown, but it is a useful aberrant marker for diagnosis of B-cell neoplasms. Our study demonstrates that a rabbit monoclonal antibody to CD103 reacts with neoplastic cells of HCL and HCL-v in paraffin-embedded tissue and is highly effective for detecting these cells. To our knowledge, this is the first report of successful immunodetection of CD103 in paraffin-embedded sections of fixed tissues.

All HCL bone marrow biopsy specimens fixed in Bouin solution or formalin showed CD103 expression in a pattern mirroring CD20 reactivity, whereas no cases of CLL/SLL, MCL, LPL, FL, or NMZL revealed CD103 expression in the neoplastic B-cell populations. Thus this marker provides a helpful diagnostic discriminant in distinguishing HCL from other lymphoproliferative disorders. In 5 cases with an HCL infiltrate occupying 10% or less of the cellularity, CD103 expression by the neoplastic B cells was characterized by a predominantly membranous staining pattern that accentuated cytoplasmic projections. Similar morphologic features were previously described with DBA.44 staining.[34] This is advantageous for the detection of minimal disease involvement compared with annexin A1 studies, which are virtually uninterpretable in this setting because of the extensive reactivity for myeloid elements. However, the presence of a small population (<1%) of CD103-reactive mononuclear cells in nonneoplastic bone marrow interstitium, as demonstrated

DOI: 10.1309/AJCPHW7RULIZT2GB



Note the membranous reactivity highlighting cytoplasmic projections in the case of limited disease (**F**, inset). Annexin A1 is useful in cases with high-level disease burden (**G**) but low-level disease involvement is obscured because of reactivity of abundant myeloid cells (**H**). (**A**-**B**, H&E; **C**-**H**, immunoperoxidase, hematoxylin counterstain; all images, ×400; insets, ×1,000.)

historically and confirmed in this study, diminishes the absolute specificity of CD103 immunoreactivity in minimal disease cases. Importantly, the nonneoplastic cells exhibit cytoplasmic reactivity for CD103 and lack the membranous staining pattern and cytoplasmic projections that characterize true HCL cells. Thus, careful examination of the expression pattern can help to identify HCL cells in cases of minimal residual disease.

Only half of the bone marrow biopsy specimens fixed in Zenker solution showed CD103 expression in the neoplastic population. Therefore, the CD103 antibody is a very effective marker in paraffin-embedded bone marrow biopsy specimens fixed in Bouin solution or formalin but is not reliable in specimens fixed in Zenker solution (which contains mercuric chloride). Interestingly, our studies of lesional tissue fixed in B-5 fixative, which also contains mercuric chloride, showed preservation of CD103 reactivity. This suggests that the potassium dichromate in Zenker solution may be the basis for suboptimal immunoreactivity.

CD103 expression has been documented in a significant proportion of HCL-v cases and a small number of SMZL cases.[18] In this study, 6 HCL-v cases were available for evaluation. All cases were positive for CD103 expression on diagnostic FC. On IHC, 5 of 6 HCL-v specimens demonstrated positivity for CD103. In 1 CD103-negative case, the discrepant IHC/FC pattern may have resulted from fixation conditions. CD103 expression was not detected in any of the SMZL specimens tested.

DOI: 10.1309/AJCPHW7RULIZT2GB

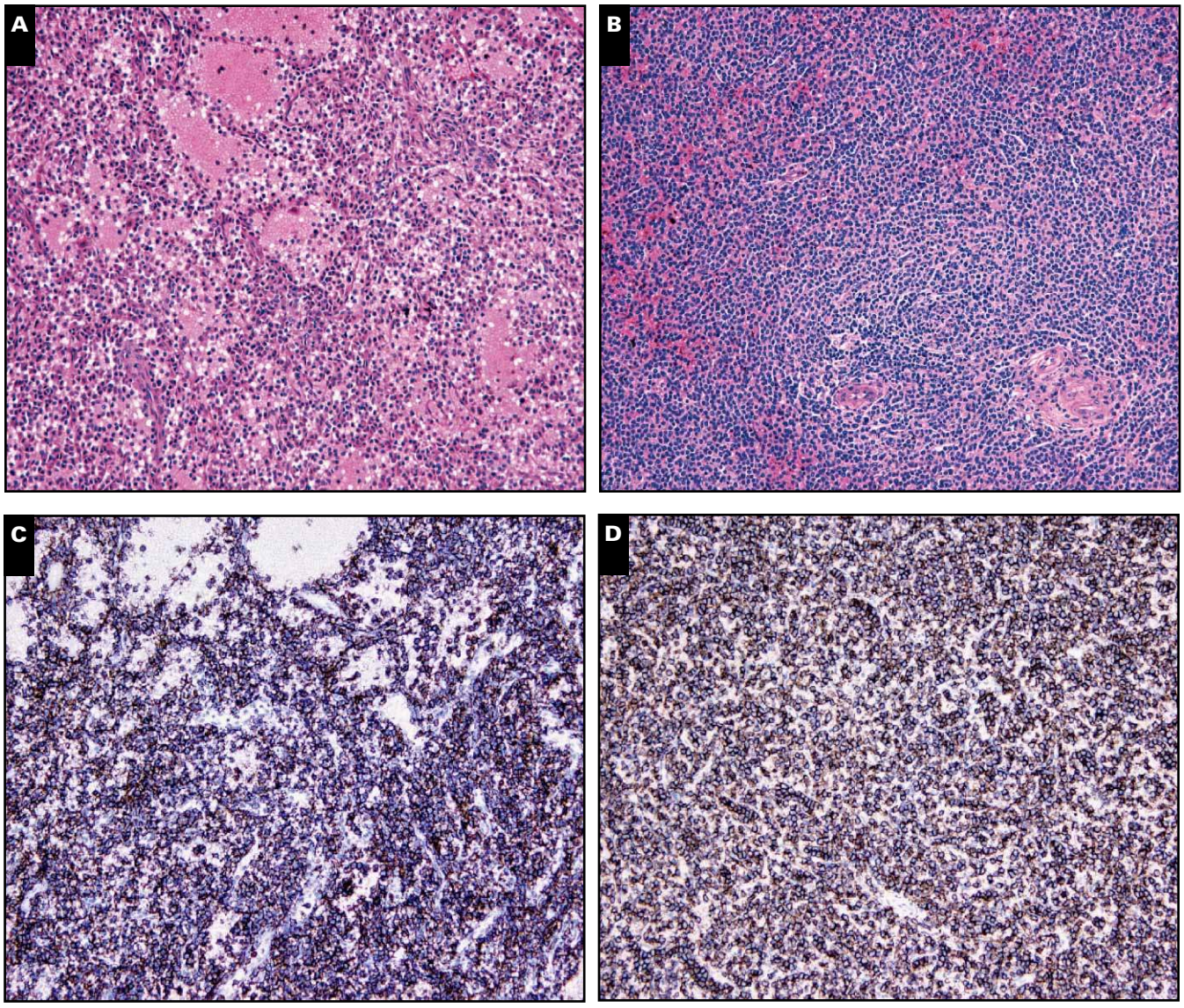
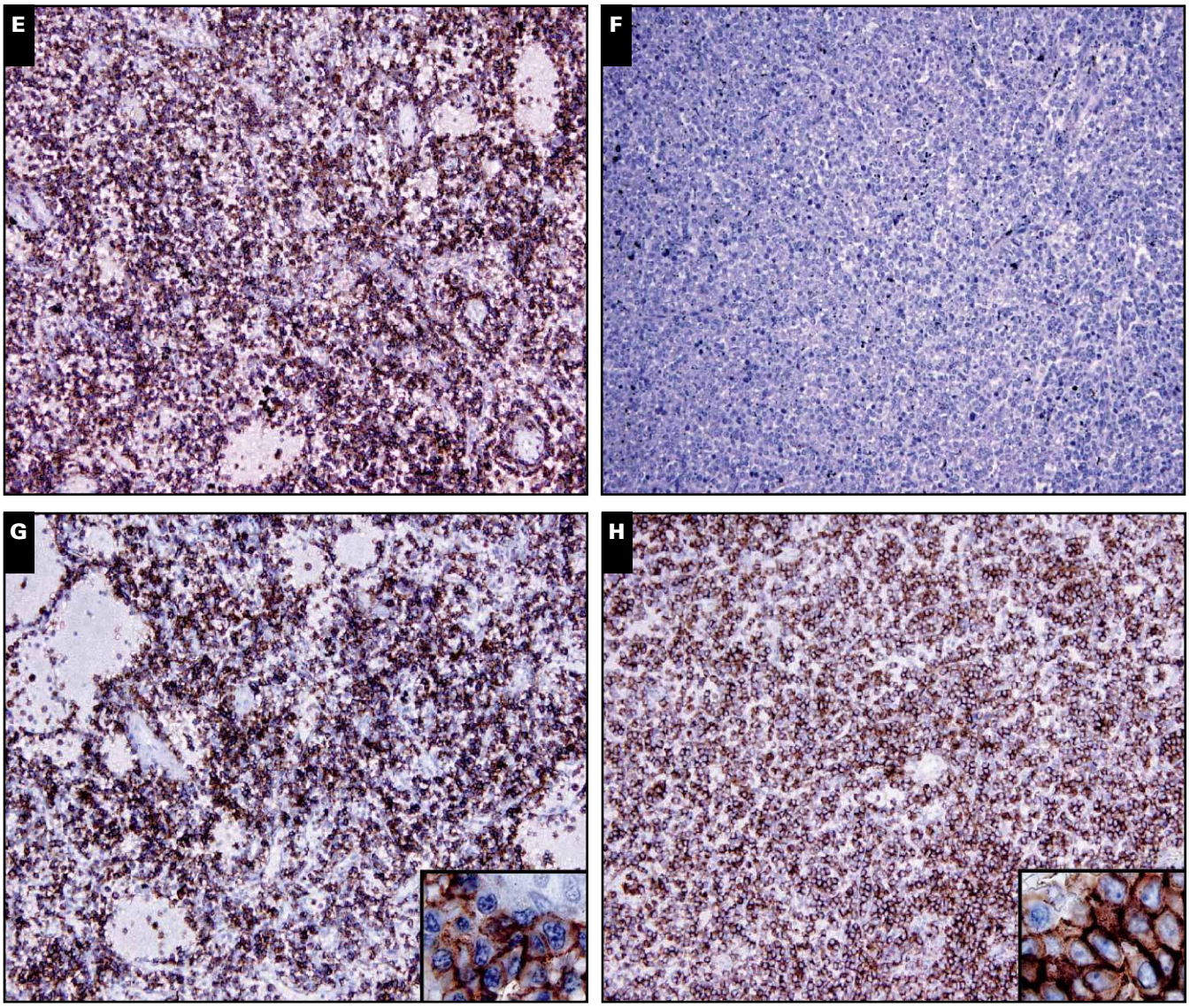


Image 4 CD103 expression in spleen sections involved by hairy cell leukemia (HCL) and HCL-variant (HCL-v) in B-5 (**A**, **C**, **E**, **G**) and B-plus (**B**, **D**, **F**, **H**) fixed sections. HCL (**A**) is characterized by expression of CD20 (**C**), CD25 (**E**), and CD103 (**G**), as well as the presence of blood lakes.

CD103-negative HCL cases are exceedingly rare.[12,35,36] In our files we identified 1 clonal B-cell neoplasm clinically consistent with HCL that expressed CD11c and CD25 on FC and DBA.44 on IHC, but lacked CD103 expression on FC. In our study, CD103 was negative on IHC in this Zenker solution–fixed bone marrow biopsy specimen (repeated over a broad spectrum of antibody titers for confirmation). Interestingly, this specimen was also positive for CD10 on FC and IHC and for CD23(dim) on FC at the time of diagnosis, similar to prior reports.[35,36]

In nonneoplastic hematopoietic tissues examined in this study, the pattern of CD103 reactivity in paraffin sections that we observed is similar to that described in initial reports. In the bone marrow, we also observed cytoplasmic staining in a subset of mononuclear cells in the paratrabecular area, reminiscent of an immature myeloid population. In 3 of 4 cases of CML (ie, a neoplasm characterized by an expanded and left-shifted myeloid population), the CD103 expression pattern was increased, particularly in the expanded paratrabecular cuffs, but was not uniformly present for all myeloid elements. CD103 expression was not seen in 5 cases of AML (including 2 cases of APML). To our knowledge, expression of the integrin αEβ7 has not been described on a myeloid-restricted population. It is unknown whether the cytoplasmic CD103 expression that we have observed represents cross-reactivity with another antigen or is a novel finding. Further investigation is needed to explore these possibilities.

DOI: 10.1309/AJCPHW7RULIZT2GB



HCL-v (**B**) is characterized by expression of CD20 (**D**) and CD103 (**H**), and the absence of CD25 (**F**). (**A**-**E**, H&E; **B**-**H**, immunoperoxidase, hematoxylin counterstain; all images, ×200; insets, ×1,000.)

In summary, we demonstrate that a rabbit monoclonal antibody to CD103 can successfully detect the lesional cells of HCL in paraffin-embedded sections and can effectively distinguish HCL and HCL-v from other B-cell neoplasms. In particular, the antibody exhibits excellent immunoreactivity in bone marrow biopsy specimens fixed in Bouin solution or formalin but is not reliable in Zenker solution–fixed samples. The positivity of CD103 for occasional background cell types diminishes the absolute specificity of the antibody; this includes the expression of integrin αEβ7 on a subset of CD4-positive and CD8-positive T cells as well as dendritic cells and possibly a subset of myeloid cells. However, careful interpretation in cases of minimal disease involvement, including close examination of immunoreactive cells for membranous staining and cytoplasmic projections, should permit distinction between neoplastic and nonneoplastic cells. Overall, this CD103 antibody is an extremely valuable addition to IHC panels in the evaluation of HCL and will greatly facilitate the diagnosis of this disorder, particularly in cases lacking FC analysis.

From the [1]Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, and the [2]Department of Pathology, UMass Memorial Medical Center and University of Massachusetts Medical School, Worcester.

Address reprint requests to Dr Pinkus: Dept of Pathology, Brigham and Women's Hospital, 75 Francis St, Amory 3, Boston, MA 02115; gpinkus@partners.org.

References

1. Shaw SK, Cepek KL, Murphy EA, et al. Molecular cloning of the human mucosal lymphocyte integrin alpha E subunit: unusual structure and restricted RNA distribution. *J Biol Chem*. 1994;269:6016-6025.

DOI: 10.1309/AJCPHW7RULIZT2GB

2. Cepek KL, Wong DA, Brenner MB, et al. AS7/8.4. CD103 (alphaE) cluster report. In: Schlossman SF, Boumsell L, Gilks W, et al, eds. *Leukocyte Typing V: White Cell Differentiation Antigens*. Oxford: Oxford University Press; 1995:1666-1667.
3. Cerf-Bensussan N, Jarry A, Brousse N, et al. A monoclonal antibody (HML-1) defining a novel membrane molecule present on human intestinal lymphocytes. *Eur J Immunol*. 1987;17:1279-1285.
4. Visser L, Shaw A, Slupsky J, et al. Monoclonal antibodies reactive with hairy cell leukemia. *Blood*. 1989;74:320-325.
5. Schwarting R, Dienemann D, Kruschwitz M, et al. Specificities of monoclonal antibodies B-ly7 and HML-1 are identical. *Blood*. 1990;75:320-321.
6. Kruschwitz M, Fritzsche G, Schwarting R, et al. Ber-ACT8: new monoclonal antibody to the mucosa lymphocyte antigen. *J Clin Pathol*. 1991;44:636-645.
7. Flenghi L, Spinozzi F, Stein H, et al. LF61: a new monoclonal antibody directed against a trimeric molecule (150 kDa, 125 kDa, 105 kDa) associated with hairy cell leukaemia. *Br J Haematol*. 1990;76:451-459.
8. Mulligan SP, Travade P, Matutes E, et al. B-ly-7, a monoclonal antibody reactive with hairy cell leukemia, also defines an activation antigen on normal CD8+ T cells. *Blood*. 1990;76:959-964.
9. Moller P, Mielke B, Moldenhauer G. Monoclonal antibody HML-1, a marker for intraepithelial T cells and lymphomas derived thereof, also recognizes hairy cell leukemia and some B-cell lymphomas. *Am J Pathol*. 1990;136:509-512.
10. Thaler J, Dietze O, Faber V, et al. Monoclonal antibody B-ly7: a sensitive marker for detection of minimal residual disease in hairy cell leukemia. *Leukemia*. 1990;4:170-176.
11. Venkataraman G, Aguhar C, Kreitman RJ, et al. Characteristic CD103 and CD123 expression pattern defines hairy cell leukemia: usefulness of CD123 and CD103 in the diagnosis of mature B-cell lymphoproliferative disorders. *Am J Clin Pathol*. 2011;136:625-630.
12. Dong HY, Weisberger J, Liu Z, et al. Immunophenotypic analysis of CD103+ B-lymphoproliferative disorders: hairy cell leukemia and its mimics. *Am J Clin Pathol*. 2009;131:586-595.
13. Carlsson M, Hassan IB, Paul G, et al. Hairy cell leukemia (HCL) associated antigen B-ly7 is an activation/differentiation-associated antigen on HCL and normal B cells but not on B-type chronic lymphocytic leukemia (B-CLL) cells. *Leuk Lymphoma*. 1992;6:133-143.
14. Hassan IB, Hagberg H, Sundstrom C. Immunophenotype of hairy-cell leukemia. *Eur J Haematol*. 1990;45:172-176.
15. Matutes E, Morilla R, Owusu-Ankomah K, et al. The immunophenotype of splenic lymphoma with villous lymphocytes and its relevance to the differential diagnosis with other B-cell disorders. *Blood*. 1994;83:1558-1562.
16. Morice WG, Kurtin PJ, Hodnefield JM, et al. Predictive value of blood and bone marrow flow cytometry in B-cell lymphoma classification: comparative analysis of flow cytometry and tissue biopsy in 252 patients. *Mayo Clin Proc*. 2008;83:776-785.
17. Traverse-Glehen A, Baseggio L, Bauchu EC, et al. Splenic red pulp lymphoma with numerous basophilic villous lymphocytes: a distinct clinicopathologic and molecular entity? *Blood*. 2008;111:2253-2260.
18. Swerdlow S, Campo E, Harris NL, et al, eds. *WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2008.
19. Dorfman DM, Hwang ES, Shahsafaei A, et al. T-bet, a T-cell-associated transcription factor, is expressed in a subset of B-cell lymphoproliferative disorders. *Am J Clin Pathol*. 2004;122:292-297.
20. Dorfman DM, Shahsafaei A. CD200 (OX-2 membrane glycoprotein) expression in b cell-derived neoplasms. *Am J Clin Pathol*. 2010;134:726-733.
21. Falini B, Tiacci E, Liso A, et al. Simple diagnostic assay for hairy cell leukaemia by immunocytochemical detection of annexin A1 (ANXA1). *Lancet*. 2004;363:1869-1870.
22. Matutes E. Immunophenotyping and differential diagnosis of hairy cell leukemia. *Hematol Oncol Clin North Am*. 2006;20:1051-1063.
23. Cepek KL, Shaw SK, Parker CM, et al. Adhesion between epithelial cells and T lymphocytes mediated by E-cadherin and the alpha E beta 7 integrin. *Nature*. 1994;372:190-193.
24. Hingorani M, Metz D, Lightman SL. Characterisation of the normal conjunctival leukocyte population. *Exp Eye Res*. 1997;64:905-912.
25. Woodberry T, Suscovich TJ, Henry LM, et al. Alpha E beta 7 (CD103) expression identifies a highly active, tonsil-resident effector-memory CTL population. *J Immunol*. 2005;175:4355-4362.
26. Sperling M, Kaudewitz P, Braun-Falco O, et al. Reactivity of T cells in mycosis fungoides exhibiting marked epidermotropism with the monoclonal antibody HML-1 that defines a membrane molecule on human mucosal lymphocytes. *Am J Pathol*. 1989;134:955-960.
27. Simonitsch I, Volc-Platzer B, Mosberger I, et al. Expression of monoclonal antibody HML-1-defined alpha E beta 7 integrin in cutaneous T cell lymphoma. *Am J Pathol*. 1994;145:1148-1158.
28. Picker LJ, Martin RJ, Trumble A, et al. Differential expression of lymphocyte homing receptors by human memory/effector T cells in pulmonary versus cutaneous immune effector sites. *Eur J Immunol*. 1994;24:1269-1277.
29. Stein H, Dienemann D, Sperling M, et al. Identification of a T cell lymphoma category derived from intestinal-mucosa-associated T cells. *Lancet*. 1988;2:1053-1054.
30. Spencer J, Cerf-Bensussan N, Jarry A, et al. Enteropathy-associated T cell lymphoma (malignant histiocytosis of the intestine) is recognized by a monoclonal antibody (HML-1) that defines a membrane molecule on human mucosal lymphocytes. *Am J Pathol*. 1988;132:1-5.
31. Lehmann J, Huehn J, de la Rosa M, et al. Expression of the integrin alpha Ebeta 7 identifies unique subsets of CD25+ as well as CD25- regulatory T cells. *Proc Natl Acad Sci U S A*. 2002;99:13031-13036.
32. Uss E, Rowshani AT, Hooibrink B, et al. CD103 is a marker for alloantigen-induced regulatory CD8+ T cells. *J Immunol*. 2006;177:2775-2783.
33. Jaensson E, Uronen-Hansson H, Pabst O, et al. Small intestinal CD103+ dendritic cells display unique functional properties that are conserved between mice and humans. *J Exp Med*. 2008;205:2139-2149.
34. Hasserjian RP, Pinkus GS. DBA.44: an effective marker for detection of hairy cell leukemia in bone marrow biopsies. *Appl Immunohistochem*. 1994;2:197-204.
35. Wu ML, Kwaan HC, Goolsby CL. Atypical hairy cell leukemia. *Arch Pathol Lab Med*. 2000;124:1710-1713.
36. Chen YH, Tallman MS, Goolsby C, et al. Immunophenotypic variations in hairy cell leukemia. *Am J Clin Pathol*. 2006;125:251-259.

DOI: 10.1309/AJCPHW7RULIZT2GB