Hairy cell leukemia (HCL) is a low-grade B-cell lymphoproliferative disorder with distinctive morphologic, cytochemical, and immunologic characteristics that permit accurate diagnosis and distinction from other disorders in nearly all cases, often with a limited diagnostic evaluation. In a few patients, atypical features, either clinical or morphologic, require a more complete battery of testing to establish a diagnosis of HCL. The diagnostic criteria of this disorder, as well as other low-grade B-cell lymphoproliferations, have been refined over the past several decades, bringing into focus another far less common lymphoproliferative process that has been termed the variant form of HCL (HCL-V). The pathology of these two disorders, with particular emphasis on a practical approach to the diagnostic hematopathology of HCL, is the topic of this article.

THE HAIRY CELL
Cytology
“Accurate diagnosis of this disease rests upon the recognition of the...cells in blood, bone marrow, or spleen. Morphologic observation of these pathognomonic cells is more art than science...” [1]. This trenchant observation of Yam and colleagues is as relevant today as when it was written in 1972 and emphasizes that, despite the ever-growing battery of ancillary studies to assist the hematopathologist, the identification of cytologically characteristic cells remains the diagnostic sine qua non of HCL [2–5]. As such, it is critical to obtain air-dried cytologic material for Wright’s and cytochemical stains. Suitable preparations include smears of the peripheral blood or bone marrow and touch preparations of biopsy samples. The routine preparation of bone marrow core biopsy touch preparations should be a standard component of all bone marrow biopsies, but especially so in patients with inaspirable marrow.

In Wright-stained preparations, the hairy cell is 1.5 to 2 times the size of a mature lymphocyte and the nucleus occupies one half to two thirds of the cell’s

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HCL tends to be a disease of monotonous cells with respect to cytologic characteristics and size. Hence, although there may be a moderate degree of cell size variation between patients, an individual patient usually displays a remarkably homogeneous population of hairy cells (Fig. 1).

The nuclei can have several configurations, including round, oval, spindled, reniform, horseshoe-shaped, and bilobed. Although the hairy cells in an individual vary in nuclear contours, most patients have a preponderant nuclear shape, most commonly oval. Despite variation in nuclear contour, there are several consistent nuclear characteristics of HCL that assist greatly in recognition. Most important, the nuclear membrane is nearly always smooth, imparting a distinct demarcation from the surrounding cytoplasm and lacking the fine surface irregularities that typify many other lymphoproliferative disorders. As well, the nuclear membrane usually appears thickened. The chromatin of HCL

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**Fig. 1.** (A) Classic hairy cell with a smoothly contoured, round nucleus and a rim of textured cytoplasm with circumferential hairlike projections. Peripheral blood (Wright’s stain, original magnification ×100). (B) Hairy cells often have an oval nuclear contour and a single small nucleolus. Peripheral blood (Wright’s stain, original magnification ×100). (C) The nuclei of hairy cells frequently are reniform to lobated. The cytoplasm has a textured, flocculent appearance. Peripheral blood (Wright’s stain, original magnification ×100). (D) Hairy cell with a ribosome-lamella complex (arrow). Peripheral blood (Wright’s stain, original magnification ×100).
has a partially condensed appearance that is intermediate between a mature lymphocyte and a blast. Additionally, the chromatin has a uniform granular appearance in contrast to the irregularly clumped chromatin of other disorders, particularly B-cell chronic lymphocytic leukemia (B-CLL) and splenic marginal zone lymphoma (SMZL). Hairy cells have no evident nucleoli or a single nucleolus. Infrequently, cells with two nucleoli are present. Generally, patients demonstrate a predominance of nucleolated or nonnucleolated cells. The nucleoli are nearly always small, round, and without irregularities in contour.

The cytoplasm of the hairy cell is distinctive. Although the term “hairy cell” derives from supravital preparations that optimally display the unique topology of these cells, air-dried preparations usually reveal occasional cells with a characteristic corona of cytoplasmic hairlike projections. More commonly, the hairy cell has an indistinct cytoplasmic border that is described as serrated, frayed, or wind-blown. In bone marrow aspirate or touch preparations, there often are many bare nuclei, devoid of cytoplasm. The character of the cytoplasm also is useful in identification. The cytoplasm stains pale gray-blue and because of the frayed margins and pale staining it often merges imperceptibly with the unstained background of the blood smear. The cytoplasm has a textured, flocculent appearance imparted by the presence of irregularly distributed areas of more intense staining. In occasional cases, gray-blue circular and rod-shaped structures, which represent ribosome-lamella complexes, are visible in the cytoplasm (see “Ultrastructural findings” below). These structures have an appearance that is similar in shape and tincture to Dohle bodies that are present in neutrophils of reactive processes and May-Hegglin anomaly [6], while lacking the azurophilic staining and crystalline appearance of Auer rods. The central pale zone of the ribosome-lamella complex is observed occasionally (see Fig. 1D).

Within the spectrum of HCL are occasional patients who manifest unusual cytologic features, most commonly a predominance of hairy cells with horsehoe, spindle, or lobated nuclear contours (Fig. 2) [7,8]. As well, there are rare examples of HCL that are characterized by cells that are comparable in size to large cell lymphoma [9]. Despite the variation in nuclear contour or cell size, the hairy cells demonstrate otherwise typical cytologic features, particularly in the character of chromatin and cytoplasm. HCL patients who have these unusual cytologic features demonstrate clinical, morphologic, and immunophenotypic characteristics that are otherwise typical. Hence, it is important to distinguish HCL with atypical cytologic features, from the variant form of HCL (HCL-V), a separate entity with distinctive clinical and morphologic characteristics.

HCL has an extremely low proliferative rate that manifests cytologically as a virtual absence of transformed cells. Hence, although a variable number of prolymphocytes and paraimmunoblasts typify the cytologic findings of B-CLL and a small percentage of immunoblasts or centroblast-like cells are present in SMZL, transformed cells with prominent nucleoli and increased cytoplasmic basophilia are present rarely in HCL. This characteristic is useful in the cytologic
differential diagnosis of HCL from other indolent B-cell lymphoproliferative processes.

Ultrastructural Findings
A plethora of publications during the 1970s was devoted to the ultrastructural characteristics of the hairy cell [10–13]. The distinctive hairlike cytoplasmic projections are demonstrable readily by transmission electron microscopy as are nuclear characteristics of peripherally arrayed heterochromatin and the inconsistent presence of a small nucleolus. The most distinctive ultrastructural finding is the ribosome-lamella complex, which is composed of a tubular concentric array of alternating lamellae and free-lying ribosomes with a central zone devoid of either of these components. These structures, which are present in about half of patients who have HCL, often exceed 1 μm in diameter and 3 μm in length. Hence, they can be resolved easily with light microscopy as Dohle body–like cytoplasmic structures (see Fig. 1D) [6]. Ribosome-lamella complexes are not unique to HCL, but are present only infrequently in other low-grade lymphoproliferative disorders. Hence, the ongoing diagnostic usefulness of the light microscopic identification of this structure is the predominant enduring legacy to the pathology of HCL from the heyday of diagnostic electron microscopy.

Tartrate-Resistant Acid Phosphatase
A critical cytochemical feature of the hairy cell is the expression of isoenzyme 5 of acid phosphatase that is uniquely resistant to treatment with tartaric acid. This characteristic was exploited in the 1970s by the development of cytochemical stains that permitted the identification of tartrate-resistant acid phosphatase (TRAP) activity of cells in cytologic preparations [14]. Virtually all cases of
HCL show expression of TRAP. In contrast, TRAP positivity is demonstrable in only a small percentage of patients with other lymphoproliferative disorders including B-CLL, Sezary syndrome, T-cell prolymphocytic leukemia, and Human T-cell lymphotrophic virus-1 (HTLV-1)–related T-cell leukemia/lymphoma, diseases that are included rarely in the differential diagnosis of HCL [15,16]. Moreover, the bright expression of TRAP is almost exclusive to HCL. TRAP was the principal study that was used during the last decades of the twentieth century to buttress a morphologic diagnosis of HCL. Although immunophenotypic analysis has supplanted the TRAP stain in many centers over the past decade, the procedure retains significant usefulness in the diagnosis of HCL, and seems to have superior specificity to immunohistochemical TRAP stains (see later discussion). Acid phosphatase stains are particularly useful when fresh tissue is not available for phenotypic analysis or in the evaluation of borderline processes with ambiguous morphologic or immunophenotypic features.

A long discussion of TRAP staining is precluded by its fading usefulness under siege by the ongoing introduction of useful flow cytometric and immunohistochemical markers; however, a few comments regarding the interpretation of these stains are relevant to this discussion.

Acid phosphatase stains are technically challenging, and it is critical to confirm their validity, particularly if the stains are used infrequently. Staining for acid phosphatase activity without tartrate treatment always should be performed and reviewed to ensure that there is appropriate staining of normal cells, particularly monocytes and lymphocytes. In the tartrate-treated slides, it is critical to confirm that acid phosphatase activity has been extinguished from these normal cells. Conversely, when TRAP stains are negative, it is generally impractical to exclude a false negative study, as positive control slides rarely are available because of the lability of TRAP activity in air-dried preparations.

A second critical consideration is the definition of a positive TRAP stain. Hairy cells often are heterogeneous in the expression of TRAP with many negative cells. Interpretation of a stain as positive is based predominantly on the intensity of the reaction in individual cells, rather than the number of positive cells. Only a few brightly positive mononuclear cells with cytologic features of HCL are required for a positive study (Fig. 3).

**Antigen Expression**

**General comments**

Despite the nostalgic attractiveness of electron microscopy and cytochemical stains, reality beckons recognition that these methodologies have been supplanted by antibody-mediated identification of expressed antigens as the ancillary method of choice in the confirmation of a morphologic diagnosis of HCL. Immunophenotypic characterization by flow cytometry and immunohistochemical staining of tissue sections are the predominant ancillary methods that are employed in the diagnosis of HCL in the twenty-first century. These
Methodologies are complementary, and access to both is warranted for the optimal diagnosis and assessment of HCL.

The advantages of flow cytometry include:

- Simultaneous multi-parameter assessment for the expression of multiple antigens as well as light scattering characteristics. The capacity to evaluate cells for a profile of three to six (and more) antigens provides a high degree of specificity and sensitivity.
- Superior sensitivity relative to immunohistochemistry for detecting dim expression of surface antigens.
- Larger library of antigens that can be assessed.
- Use of fresh, unfixed tissue, which reduces the number of preanalytical variables that adversely impact tissue immunoreactivity.

Unfortunately, the requirement for fresh tissue is also the chief disadvantage of flow cytometry, because HCL often presents with a dearth of circulating hairy cells and “dry tap” bone marrow aspiration that preclude this analysis unless an unfixed portion of the bone marrow core biopsy is available. Another liability is the inability to correlate cytologic findings and antigen expression patterns directly. Finally, assessment for expression of cytoplasmic antigens by flow cytometry often is technically challenging.

The chief strengths of immunohistochemical stains are:

- Direct visualization of positive cells.
- Use of readily available fixed and embedded tissue.
- Meaningful quantification of marrow involvement by HCL.
- Superior assessment of antigens that are expressed only in the cytoplasm or nucleus.

The inability to assess for expression of multiple antigens on a single slide and insensitivity relative to flow cytometry in the detection of dim surface antigen expression are the chief drawbacks of immunohistochemistry. As well, the deleterious effects of preanalytical specimen processing, including fixation...
and decalcification, must be considered, particularly when assessing the significance of negative studies.

**Flow cytometry**

Immunophenotypic analysis by flow cytometry is addressed extensively elsewhere in this issue and is summarized briefly here. Hairy cells have a distinctive antigen expression profile that can confirm a morphologic impression of HCL. Nevertheless, flow cytometry was used infrequently in the clinical diagnosis of HCL until the 1990s. Since that time, the development of multi-parameter flow cytometry and antibodies of high specificity have permitted the reliable identification of hairy cells, even when they compose less than 0.5% of analyzed cells [17]. Because small populations of circulating hairy cells are present in nearly all untreated patients who have HCL, these developments have established a primary role for immunophenotypic analysis in the diagnosis of HCL.

When CD45 expression and side scatter characteristics are used in the gating of analyzed cells, the distinctive surface irregularities of hairy cells impart side scatter characteristics that frequently locate them within the monocyte gate [18]. At the same time, the monocyte gate in HCL usually is conspicuously devoid of actual monocytes. The characteristic finding of monoclonal B lymphocytes within the monocyte gate is used in many laboratories as a trigger for assessment of HCL-specific antigens.

The typical phenotypic profile of HCL is characterized by the expression of B-cell markers with bright expression of CD20 and CD22. Hairy cells commonly express bright monotypic surface immunoglobulin although occasional cases are surface immunoglobulin negative. Hairy cells almost never express CD5, whereas about 10% of cases show expression of CD10 [19].

Three markers are of particular usefulness in the phenotypic characterization of HCL. Hairy cells show consistent bright expression of CD11c and are almost always positive for CD103 and CD25 [20]. Expression of these markers is not exclusive to HCL. CD11c is expressed commonly in B-CLL and SMZL; however, the intensity of expression of CD11c in HCL is 30-fold greater than in B-CLL and SMZL. Hence, the bright expression of this antigen has a high degree of specificity for HCL. Similarly, CD25 is expressed in other types of B-cell lymphoproliferative disorders, including nearly half of B-CLL. The expression of CD25 tends to be brighter in HCL than in other lymphoproliferative diseases, but with significant overlap in intensity that limits the specificity of this marker. Nevertheless, CD25 is expressed consistently in hairy cells, and the absence of expression of this antigen in a lymphoproliferative process can assist in the exclusion of HCL. Lastly, although CD103 is the most specific of hairy cell antigens, the usefulness of this marker can be overstated because expression has been described in some cases of SMZL, which can mimic HCL clinically and cytologically.

The Royal Marsden group has developed several useful immunophenotypic scoring systems for differentiating B-cell lymphoproliferative disorders [21,22]. A four-point system for HCL uses CD103, CD11c, CD25, and another
antibody with high specificity for HCL, HC2. Although HC2 is not used widely in the United States, a modified three-point system retains usefulness because SMZL and HCL-variant almost never coexpress CD103, CD11c, and CD25, whereas at least 90% of cases of HCL are positive for all three antigens [21].

**Immunohistochemical studies**

The wide accessibility to an ever-expanding library of antibodies and nearly universal use of trephine biopsy in bone marrow pathology have made immunohistochemical stains the primary ancillary tool in the diagnosis of HCL. Additionally, because a remarkable degree of marrow involvement by HCL can be inapparent in routinely stained histologic sections, immunohistochemical studies are requisite for adequate posttreatment evaluation for the efficacy of treatment.

Optimal immunohistochemical evaluation includes generic B-cell antibodies (CD20, CD79a, or PAX5) and markers with specificity for HCL, including TRAP, DBA.44, and cyclin D1. Generic B-cell markers, although lacking specificity for HCL, are highly sensitive and are useful for quantification of marrow involvement. CD20 is preferable because the thick granular membrane pattern is somewhat distinctive for HCL, and the lack of cytoplasmic or nuclear staining permits cytologic assessment of positive cells (Fig. 4A).

DBA.44, an antibody that originally was raised against a centroblastic cell line [23], was found incidentally to be a sensitive marker of HCL, and is expressed in 99% of cases [24]. The antibody displays a granular cytoplasmic pattern of expression and usually stains fewer cells than are demonstrated with

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**Fig. 4.** (A) CD20 immunostain marks HCL with a characteristic thick and granular membrane pattern that permits cytologic assessment of the positive cells. Bone marrow (CD20 immunostain, original magnification ×40). (B) DBA.44 immunostain shows a granular cytoplasmic pattern of positivity and usually stains only a subset of the CD20 positive hairy cells. Bone marrow (CD20 immunostain, original magnification ×40).
CD20 (Fig. 4B). This antibody, although positive in less than 20% of other low-grade B-cell lymphoproliferative diseases, unfortunately is expressed in up to 80% of cases of SMZL, which often is in the morphologic differential diagnosis of HCL \[25\]. DBA.44 also is expressed in a significant minority (20%–40%) of follicular lymphoma, mantle cell lymphoma (MCL), and diffuse large B-cell lymphoma \[24\].

Cytochemical staining for TRAP requires air-dried cytologic preparations and cannot be performed on paraffin-embedded tissue; however, antibodies that are specific for isoenzyme 5 of acid phosphatase have been developed in the last decade \[26–28\]. TRAP antibodies mark greater than 90% of cases of HCL and have sensitivity that is essentially equivalent to the cytochemical stain. Similar to DBA.44, this antibody has a granular cytoplasmic pattern and marks only a subset of the hairy cells, and, thus, is inferior to CD20 for quantification of marrow involvement. The immunohistochemical TRAP stain has a specificity that is significantly inferior to cytochemical TRAP and is reported to be positive in other lymphoproliferative disorders, including B-CLL/SLL, SMZL, and MCL \[28,29\]. As well, immunohistochemical TRAP is positive in several nonneoplastic cells, including osteoclasts, Gaucher cells, mast cells, and activated macrophages \[27\].

Cyclin D1 is overexpressed consistently in MCL, and has great usefulness in differentiating this intermediate-grade lymphoma from other low-grade mimics. Antibodies to this protein also mark nearly all cases of HCL with a dim nuclear pattern of staining in a subset of the hairy cells \[30\].

Immunohistochemical stains serve two primary purposes in the evaluation of HCL. First, a combination of DBA.44, TRAP, and cyclin D1 can be used to confirm a morphologic impression of HCL either in the setting of initial diagnosis or following chemotherapy in assessing a minor population of CD20-positive B cells. A DBA.44+/TRAP+ profile had a 97% specificity for HCL in a recent study \[29\]. Although not documented, it is likely that the addition of cyclin D1 to this panel would enhance specificity. Second, a CD20 immunostain is an excellent tool for quantification of an HCL infiltrate. This stain nearly always reveals more extensive disease than is evident from routinely stained sections and commonly shows persistent disease when hematoxylin-eosin–stained sections show no discernable infiltrate.

This discussion has focused on the use of immunohistochemical stains in bone marrow evaluation, because these specimens constitute most tissue biopsies that are performed on patients who have HCL. They are, of course, similarly useful in supporting a morphologic diagnosis of HCL in spleen, liver, lymph node, and other tissue samples.

**TISSUE-SPECIFIC PATHOLOGY OF HAIRY CELL LEUKEMIA**

**Peripheral Blood**
Abnormalities of the peripheral blood are identified at presentation in nearly all patients who have HCL. Pancytopenia is common, with neutropenia out of proportion to the degree of anemia and thrombocytopenia. Most distinctive
is a dearth of circulating monocytes, a finding so consistent that the diagnosis of HCL is unlikely in the absence of monocytopenia [31]. A note of caution in this regard: many automated hemogram analyzers categorize circulating hairy cells as monocytes. If present in sufficient number, they will mask monocytopenia unless a peripheral blood film is reviewed. The peripheral blood film often displays an increase in circulating large granular lymphocytes. These cells, with abundant gray-blue cytoplasm, can mimic the cytologic appearance of hairy cells. However, hairy cells rarely contain the sparse azurophilic granulation characteristic of large granular lymphocytes. Despite the near constant presence of reticulin myelofibrosis in HCL, leukoerythroblastosis usually is not prominent. Circulating hairy cells are present in variable, usually low, numbers. In most cases a diligent search for cytologically typical hairy cells is required. This search should focus on the thin portions of the blood film. Although thicker portions of the smear accentuate the hairiness of the leukemic cells, this artifact-rich region commonly induces hairy projections of normal lymphocytes while obscuring most of the other characteristic cytologic features of HCL. When rare cells that are suggestive of HCL are identified in leukopenic peripheral blood films, buffy coat preparations often are useful in demonstrating additional hairy cells. As well, these concentrated smears greatly facilitate the interpretation of cytochemical acid phosphatase stains for evidence of TRAP positivity. Although the neoplastic cells in HCL are monotonous in appearance, most of them do not have classic “textbook” cytologic features. Hence, the reviewer commonly encounters several suspicious lymphocytes before encountering a cytologically classic hairy cell. Although leukopenia is the rule in HCL, a leukemic presentation, which is more characteristic of the variant form of HCL and SMZL, is encountered occasionally in patients who have otherwise typical HCL [32]. The peripheral blood film shows abnormal lymphocytosis with many classic hairy cells.

Bone Marrow

Infiltration of the bone marrow by HCL usually is accompanied by reticulin fibrosis, and the marrow often is deemed inaspirable [2]. The incidence of “dry tap” bone marrow aspiration likely is overstated, and reflects the common definition of “dry tap” as an inability to draw liquid bone marrow into the syringe; however, the bone marrow aspirate needle in “dry tap” aspirations often contains a few drops of blood that are rich in bone marrow elements. Expressing even minimal material from the needle directly onto glass slides often results in excellent cytologic preparations. A second alternative to standard bone marrow aspirate smears is the creation of touch preparations of the core biopsy, which should be prepared routinely with all bone marrow biopsies as a backup should the aspirate smears prove to be lacking in marrow particles [33]. If these two techniques are used, adequate cytologic preparations that permit identification of cytologically typical hairy cells and cytochemical staining for TRAP are available in most patients who have HCL. The mechanical
stress of bone marrow aspiration or smear preparation, coupled with the fragility of the neoplastic cells in HCL, results in the presence of numerous nuclei that are stripped of cytoplasm and often outnumber intact hairy cells. In addition to infiltration with HCL, cytologic preparations of bone marrow specimens usually show erythroid-predominant hematopoiesis because of selective suppression of granulocytopenesis [5].

Routinely stained histologic sections of bone marrow in HCL demonstrate a highly distinctive patchy or diffuse mononuclear infiltrate [3]. The aggregates are ill-defined and blend imperceptibly with surrounding residual hematopoiesis that usually is erythroid predominant. Unlike most other lymphoproliferative disorders, HCL demonstrates an infiltrative rather than “pushing” growth pattern that results in at least the focal preservation of marrow adipose tissue, despite extensive HCL surrounding fat lobules. Disruption of bone marrow microvasculature by hairy cell infiltrates results in the presence of extravasated erythrocytes. At low power, the infiltrates of HCL are paucicellular relative to other lymphoproliferative disorders, particularly B-CLL, SMZL, follicular lymphoma, and MCL. The well-spaced appearance is due to the abundant cytoplasm of hairy cells and to the pericellular deposition of fibronectin. Often, the infiltrates are paratrabeicular and intramedullary in distribution and have an extremely monotonous appearance at medium power. The cells have round, oval, reniform, and lobated nuclei and the nuclear contours are not as smooth as in cytologic preparations. Chromatin is condensed partially and granular. The cells are without nucleoli or contain a single small nucleolus. Nuclei with features of transformed cells, including dispersed chromatin and prominent nucleoli, are virtually absent, as are mitotic figures. Cytoplasm is abundant and pale staining. The nuclei are placed centrally within the cytoplasmic domain, which imparts the well-spaced and monotonous appearance at medium power. The abundant and pale-staining cytoplasm surrounding smoothly contoured and round nuclei have inspired many observers to compare hairy cells with “fried eggs” (Fig. 5A). Infrequently, vague cytoplasmic condensations may be identified that represent large ribosome-lamella complexes.

When arrayed as sheets of monotonous cells, hairy cells are identified readily; however, in the context of surrounding hematopoietic elements, individual hairy cells in hematoxylin-eosin–stained sections closely mimic monocytes and myelocytes. Hence, when HCL infiltrates the marrow in an interstitial pattern it can be completely inapparent until immunostains highlight the subtle population of hairy cells.

The myelofibrosis that is associated with HCL represents deposition of fibronectin, which is synthesized and excreted by the hairy cells [34]. This likely explains the uniform pericellular increase in marrow reticulin that is demonstrated with silver stains. Occasional patients who have otherwise typical HCL have no evidence of reticulin deposition and easily aspirable bone marrow. Because the ground substance that is deposited in HCL is fibronectin, trichrome staining typically does not show deposition of mature collagen [35]. Occasional cases show prominent collagen deposition, however [36]. In these
biopsies, fibroblasts are admixed with hairy cells that often take on a spindled fibroblastoid appearance (Fig. 5B). This morphologic variant of HCL must be differentiated from other fibrosing spindle cell proliferations in the bone marrow, and it can be mimicked closely by systemic mast cell disease (see “Differential diagnosis” below).

Occasionally, HCL simulates aplastic anemia [37] (Fig. 6). In this variant of the disease, patients present with pancytopenia and bone marrow biopsy demonstrates a markedly hypocellular medullary space. The infiltrate of hairy cells can be subtle and masked by admixed residual hematopoietic precursors. The

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**Fig. 5.** (A) This is an image of the classic bone marrow infiltrate of HCL. The infiltrate is monotonous and the abundant cytoplasm imparts a well-spaced appearance to the centrally placed nuclei. Bone marrow (hematoxylin-eosin, original magnification ×40). (B) Marrow fibrosis can give a spindle-shaped appearance to the HCL infiltrate. Bone marrow (hematoxylin-eosin, original magnification ×40).

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**Fig. 6.** HCL can present with pancytopenia and a markedly hypoplastic bone marrow that simulates aplastic anemia. Immunostain for CD20 (inset) shows a subtle infiltrate of hairy cells. Bone marrow (hematoxylin-eosin, original magnification ×4). Inset: Bone marrow (CD20 immunostain, original magnification ×40).
possibility of HCL should be considered in all adult patients who have apparent aplastic anemia. A CD20 immunostain is an easy and cost-effective way to evaluate for this neoplastic, but treatable, cause of marrow aplasia (Fig. 6, inset) [38].

There are several nonspecific changes associated with marrow infiltration by HCL. There often is an increase in polyclonal plasma cells [3]. Although a definite association between HCL and multiple myeloma has not been proven, multiple myeloma is a common disease among the elderly patient population that typically is afflicted with HCL, and occasional patients who have both diseases have been reported [39–41]. Hence, a significant degree of plasmacytosis, formation of plasma cell nodules, or atypical cytologic features should prompt immunostaining for evidence of immunoglobulin light-chain restriction to distinguish reactive plasmacytosis and multiple myeloma. An increase in marrow mast cells also is common in HCL [42]. The mast cells are mature, interstitial in distribution, and unlikely to suggest mast cell disease, although a single case of concomitant HCL and systemic mast cell disease has been reported [43]. Lastly, abnormal bone remodeling and lytic bone lesions have been described in rare cases of HCL [44].

**Spleen**

The pathology of the spleen in HCL is well described [2,45–47], reflecting the utility of therapeutic splenectomy before the development of effective chemotherapeutic strategies. However, splenectomy in HCL is now performed only infrequently.

HCL is a disease of the splenic red pulp that results in moderate to marked splenomegaly in nearly all patients. The expanded red pulp shows a variable degree of architectural effacement although sinuses and cords of Billroth—while infiltrated with hairy cells—usually are preserved. Hairy cell infiltrates generally are most prominent in the cords. The histologic appearance of the infiltrate is similar to that described in the bone marrow with a monotonous population of small- to intermediate-sized mononuclear cells with well-spaced nuclei and an absence of transformed cells and mitotic figures (Fig. 7A). Hairy cells infiltrate and replace sinus endothelium and result in degeneration of the sinus basement membrane, which requires endothelial support. Loss of the basement membrane results in dilation of the erythrocyte-filled vascular space, now lined by hairy cells, which creates the characteristic “blood lake” that typifies splenic involvement by HCL (Fig. 7B) [45]. In contrast to the marked red pulp expansion, the white pulp of the spleen frequently is hypoplastic. Although marrow fibrosis is common in HCL, splenic extramedullary hematopoiesis is encountered only infrequently and is not prominent.

**Liver**

Like the spleen, the hepatic pathology of HCL was well described in the era of therapeutic splenectomy when wedge biopsies of the liver were part of the standard surgical staging laparotomy for lymphoma [45,48,49]. Since that time,
liver biopsies involved by HCL have become a curiosity. Nevertheless, the liver is nearly always involved at presentation. Typically, hairy cells infiltrate the hepatic sinuses and portal tracts (Fig. 8, A and B). Sinus involvement often is subtle and is overlooked easily if HCL is not suspected. If a mononuclear infiltrate is recognized, immunostain for CD20 typically demonstrates that the hairy cell infiltrate is out of proportion to that suspected in routinely stained sections. When the portal tracts are involved, there is expansion of this compartment with a histologically characteristic infiltrate that permits ready recognition. A distinctive hepatic lesion in HCL is the “angiomatoid focus,” which is composed of a congeries of apparent vascular spaces that mimic the appearance of a small vascular tumor but actually is composed of a collection of blood-filled spaces that are lined by hairy cells (Fig. 8C) [45]. The formation of these peculiar lesions is believed to be similar to the mechanism of blood lake formation in the spleen.

Lymph Node
Peripheral lymph node enlargement is rare in HCL; however, involvement of splenic hilar lymph nodes is common, and significant abdominal and retroperitoneal node involvement occurs in occasional patients. Such lymphadenopathy may be associated with atypical cytology and a more aggressive clinical course [50]. HCL infiltrates lymph node in a marginal zone or interfollicular pattern. Even when diffuse paracortical involvement is present, nodal sinuses often are preserved. The infiltrate has an appearance similar to that described in other organs. Although blood lake formation is rare, extravasated erythrocytes are common.
Other Tissues

Except for autopsy series that were reported before the advent of effective chemotherapy [51,52], involvement of tissues other than those discussed above is rare. Such oddities include reports of cutaneous involvement [53] and involvement of a hernia sac [54].

ASSESSMENT FOR RESIDUAL DISEASE

The pathologic assessment of patients who have HCL following 2-Chlorodeoxyadenosine (2-CdA) or other effective therapy has revealed that 15% to 50% of patients have demonstrable residual disease using moderately sensitive techniques, including immunohistochemistry and flow cytometry. Residual HCL that is detected by these methods and not evident with routine morphologic examination is termed minimal residual disease (MRD). Although the efficacy of minimal residual HCL for predicting clinical relapse has been suggested by several studies [55,56], it is evident that many patients who have MRD do not progress, or do so only over many years [57]. Hence, the predictive value of MRD detection in HCL is low and this finding has limited clinical usefulness at this time.
The limited usefulness of MRD detection does not obviate the need for ancillary techniques in the evaluation of follow-up bone marrows in patients who have HCL. The morphologic assessment of posttreatment bone marrow using routine histologic and cytologic stains is challenging. The interstitial pattern of disease and histologic resemblance of hairy cells to myelocytes and monocytes can mask residual HCL that composes as much as 10% of marrow cellularity. Hence, immunostains are a critical component of the posttherapy evaluation of HCL to ferret out and reproducibly quantify residual disease. Although many studies have defined relapse or persistent disease as the identification of HCL in routine stains [55,57], the ability to recognize residual HCL is dependent on the experience of the reviewing pathologist. This standard may be suitable in institutions that care for a large number of patients who have HCL, but it is unrealistic for those that rarely encounter HCL. Much as CD138 immunostains have become the method of choice for quantification of multiple myeloma, CD20 or DBA.44 staining serves as an accessible and reproducible method for identifying and quantifying hairy cells; the routine use of these immunostains, although not essential, is justifiable. If immunohistochemical or flow cytometry studies are not performed, the pathologic interpretation should specify that a small amount of residual disease cannot be excluded.

An important caveat in the routine use of these immunostains is the recognition that a small number of CD20- and DBA.44-positive cells, some resembling hairy cells, are present in normal bone marrow [58]. Hence, the findings generally should be considered nondiagnostic when the population of immunohistochemically identified mononuclear cells composes less than 5% of marrow cellularity, and there is no additional supportive evidence of residual disease, such as cytochemical TRAP-positive cells or flow cytometric evidence of monoclonal B cells with a characteristic phenotype. Because the literature lacks clarity regarding morphologic criteria for relapse and the clinical significance of a minor population of clinically inapparent HCL, a cautious approach is to deem 5% to 10% populations as suspicious for HCL and to reserve definitive diagnosis of HCL for when cytologically appropriate B cells exceed 10% of marrow cellularity.

THE DIFFERENTIAL DIAGNOSIS OF HAIRY CELL LEUKEMIA

The initial diagnostic specimens in patients who have HCL rarely are submitted to the pathology department with a clinical history of “Rule out hairy cell leukemia.” Hence, it is important to discuss the broad range of diagnoses that must be considered and excluded in the evaluation of this often clinically obscure disease.

Cytopathologic Differential Diagnosis

The classic cytologic appearance of the hairy cell is distinctive and the presence of numerous characteristic cells is diagnostic of HCL independent of any supportive studies. Unfortunately, HCL often presents with a dearth of evaluable cells, suboptimal cytologic preparations, or a predominance of cells lacking diagnostic features. These settings raise alternative diagnostic considerations.
HCL-V, discussed below, is characterized by cells that can resemble typical HCL (Fig. 9). Shared features include cell size, nuclear contours, and a moderate to abundant rim of cytoplasm with cytoplasmic projections. In contrast to HCL, the cells of HCL-V typically have a single prominent nucleolus, more chromatin condensation, and cells with basophilic cytoplasm. As well, the neoplastic cells of HCL-V tend to be polymorphous relative to the consistent monotony of HCL.

SMZL, also known as splenic lymphoma with villous lymphocytes can resemble HCL; they share an abundant rim of cytoplasm and cytoplasmic projections (Fig. 10A). The nuclear chromatin in SMZL usually is more condensed and irregularly distributed than in HCL. The cytoplasm lacks the characteristic flocculent appearance of the classic hairy cell and often is more basophilic. Villous lymphocytes typically have well-defined cytoplasmic margins without the frayed appearance that is seen in HCL. Lastly, when projections are present, they tend to be polar and blunt, in contrast to the thin and circumferential “hairs” that typify HCL.

**Fig. 9.** (A) HCL-V commonly presents with leukocytosis in contrast to the dearth of hairy cells that typifies the peripheral blood of typical HCL. Peripheral blood (Wright’s stain, original magnification ×100). (B) This is a characteristic cell of HCL-V. Note the prominent nucleolus, smudgy chromatin, and lack of the textured, flocculent cytoplasm of typical HCL. Peripheral blood (Wright’s stain, original magnification ×100). (C) Unusual nuclear configurations, such as this binucleate form, often are present in HCL-V. Peripheral blood (Wright’s stain, original magnification ×100).
Cells with an unusually generous rim of cytoplasm that bear some resemblance to HCL can characterize B-CLL and B-cell prolymphocytic leukemia (Fig. 10B). Nuclear features, especially the distinctive “ginger snap” chromatin pattern of B-CLL and the presence of a minor population of prolymphocytes, usually permit ready distinction from HCL.

From the relatively informed perspective of the twenty-first century, it is important to recognize that the hairy cell has cytologic features that are more akin to a monocyte than to a mature lymphocyte. The descriptive nature of the moniker “hairy cell leukemia” supplanted the earlier term “leukemic reticuloendotheliosis” specifically to reflect the ambiguous lineage of the neoplastic cells that remained unresolved until the 1980s [5,59]. Acute monocytic...
leukemia (AML-M5b in the French-American-British [FAB] system) occasionally shows differentiation toward a distinctive cell type that is referred to as a plasmacytoid monocyte. These cells have round or oval nuclei without the characteristic folding of typical monocytes. The abundant rim of cytoplasm generally is agranular and lacks vacuolization. Moreover, the cells are fragile with numerous ruptured cells in cytologic preparations. Hence, these cells can resemble HCL reflected in the conflation of monocytic leukemia and HCL in early publications [2,60]. The immature chromatin of these atypical promonocytes and occasional forms with sparse azurophilic granulation assist in the cytologic discrimination from hairy cells.

The neoplastic cells in systemic mast cell disease can be devoid of the metachromatic granules that crowd the cytoplasm of normal mast cells. These agranular forms display nuclear features that are remarkably similar to HCL with round, oval, reniform, and dumbbell-shaped nuclei and partially condensed, evenly dispersed chromatin (Fig. 10C). The abundant cytoplasm also is similar to hairy cells; however, mast cells have well-defined cytoplasmic borders and lack hairy projections. As well, careful review usually reveals the presence of some cells with at least sparse granulation.

Finally, rare cases of multiple myeloma display a subset of neoplastic cells that is virtually indistinguishable from HCL (Fig. 10D) [61]. Although the clinical context and admixed typical myeloma cells seem to permit ready distinction from true hairy cells, the two diseases occasionally occur synchronously, an intriguing, but likely coincidental association. Careful review for cells with intermediate features of myeloma and HCL favors an interpretation of multiple myeloma with hairy cell–like features, but flow cytometry may be required to evaluate for concomitant HCL.

**Histopathologic Differential Diagnosis**

The diffuse monotonous infiltrate of small- to intermediate-sized mononuclear cells with well-spaced nuclei that is characteristic of HCL is mimicked by several other malignant infiltrates. Marginal zone lymphoma (splenic, extranodal, or nodal) with monocytoid B cells can be indistinguishable from HCL in routinely stained sections. HCL-V has a similar low- and medium-power appearance to typical HCL, although the cytologic features of this disease usually permit distinction. Mast cell disease can bear a close resemblance to HCL, although mast cell infiltrates tend to be more discrete than HCL. Admixed eosinophils are an important clue to the diagnosis of systemic mast cell disease. Two forms of acute myeloid leukemia, acute monocytic leukemia (AML-M5b) and acute promyelocytic leukemia (AML-M3), are characterized by monotonous sheets of cells with a well-spaced appearance. Like HCL, the nuclear contours frequently are reniform to bilobed; however, the immature chromatin pattern and numerous mitotic figures of these disorders usually permit ready distinction from HCL. Lastly, several types of metastatic disease enter the “monotonous well-spaced nuclei” differential diagnosis, particularly lobular carcinoma of the breast and malignant melanoma.
Tissue-Specific Considerations

Bone marrow
In the evaluation of bone marrow infiltrates, there are several features that assist in the morphologic differential diagnosis. Although SMZL can have a monocytoid appearance that mimics HCL, there often is a small lymphocytic component with lymphoid aggregates of tightly packed small lymphocytes similar to those of B-CLL that are not a feature of HCL. As well, SMZL often displays a sinusoidal growth pattern and the occasional presence of benign germinal centers, two features that help in distinction from HCL. Mast cell infiltrates tend to be circumscribed relative to the ill-defined hairy cell aggregates, often are paratrabecular, and have associated eosinophils.

Spleen
The exclusive red pulp pattern of splenic HCL is a useful feature in differential diagnosis. SMZL, MCL, and follicular lymphoma principally involve the white pulp; red pulp involvement, if present, usually has a micronodular appearance that reflects expansion of the periarteriolar lymphoid sheaths. Hence, these disorders are distinguished readily from HCL. Mast cell disease involves the spleen in a patchy manner with most aggregates at the red/white pulp interface; it is not a significant consideration, despite the similarities discussed above. B-CLL typically involves red and white pulp, which results in blurring of the margins between these two compartments. Although this pattern of involvement can resemble HCL, the expanded white pulp and tightly packed small lymphocytic infiltrate of B-CLL/SLL usually permits ready distinction.

Lymphoproliferative disorders that are characterized by a pure red pulp pattern include HCL, HCL-V, hepatosplenic T-cell lymphoma, and rare cases of SMZL. HCL-V can mimic the classic histologic pattern of HCL, including diffuse red pulp involvement with the formation of blood lakes that are lined by neoplastic cells. Recognition of the atypical cytologic features is the only morphologic means of distinguishing these disorders. SMZL with a red pulp pattern [62] lacks blood lake formation and usually contains a variable number of transformed cells that are absent in HCL. Parenthetically, the distinction between HCL-V and the red pulp variant of SMZL is a greater challenge than is distinguishing either of these disorders from HCL; it requires characterization of the neoplastic cells with flow cytometry. Hepatosplenic T-cell lymphoma is an aggressive process with an atypical histologic appearance and high proliferative rate that does not mimic the cytologic appearance of HCL. Lastly, acute leukemia is characterized by involvement of the splenic red pulp. Hence, acute monocytic leukemia (AML-M5b) can mimic HCL in the spleen; however, monocytic leukemia lacks the blood lakes of HCL and usually has immature nuclear features and mitotic figures.

Resolution of Morphologic Differential Diagnoses
The above discussion of differential diagnosis is directed toward the initial morphologic evaluation of tissue samples from patients who do not have a pre-existing diagnosis of HCL. Resolution of these considerations is based on
the application of a panel of cytochemistry, flow cytometry, or immunohistochemistry studies to demonstrate the diagnostic characteristics of HCL that were described in detail above. Additionally, a focused battery of tests to exclude other diagnostic possibilities should be performed, although the specifics of this evaluation vary depending on the specific pathologic findings and are beyond the scope of this discussion.

**CATEGORIZATION OF HAIRY CELL LEUKEMIA AND RELATED DISORDERS**

The following categorization is suggested in the evaluation of low-grade B-cell lymphoproliferative disorders with features that are characteristic of, or closely related to, HCL:

- **HCL**
  - Typical
  - With unusual features:
    - Clinical
    - Cytologic
    - Immunophenotypic
- **HCL-V**
- **SMZL**
- Low-grade B-cell lymphoproliferative disorder, HCL-V versus SMZL

**Hairy Cell Leukemia**

“Typical HCL” is applied to lymphoproliferative disorders with cytologic and histologic features that are characteristic of HCL. It is first and foremost, a morphologic diagnosis. Some degree of ancillary testing is desirable to confirm a morphologic diagnosis and may include cytochemical staining for TRAP, immunophenotypic analysis by flow cytometry, and immunohistochemical stains. In a morphologically typical case, only one or two of these modalities is necessary to confirm a diagnosis of HCL.

As illustrated in the list above, the subcategory of “HCL with unusual features” is used to highlight cases of HCL that have some atypical characteristic. The presence of an unusual feature should prompt a more extensive panel of ancillary studies than for typical HCL. This panel must generate findings that are consonant with a diagnosis of HCL. Atypical clinical characteristics include isolated skeletal lesions [63,64], leukemia cutis [65], retroperitoneal mass [50], or significant leukocytosis [32]. Cytologically unusual cases generally include otherwise typical hairy cells of large size [9] or cells that demonstrate exaggerated nuclear configurations, including numerous horseshoe-shaped, spindled, or lobated forms [7,8]. Immunophenotypically unusual cases show some phenotypic characteristics that stray from the expected, including expression of CD10 or lack of one of the signature hairy cell markers (bright CD11c, CD25, CD103). Despite these clinical, cytologic, or immunophenotypic deviations, these cases can be categorized unequivocally as “HCL.” The atypical features can be elaborated in the interpretive comment.
Variant Form of Hairy Cell Leukemia

In stark contrast to the clearly defined criteria and unique characteristics of HCL, HCL-V lacks such well-limned contours. This rare disease, at least 10-fold less common than HCL [66,67], has features that suggest to varying degrees HCL, SMZL, and prolymphocytic leukemia. The perplexity of this borderline lesion is heightened by the relationship to the similarly ill-defined prolymphocytic leukemia and an irresolvable overlap in some patients who have SMZL. As well, from a terminology perspective, “HCL-V” invites confusion with cases of HCL with atypical features as described above. This distinction is not trivial because patients who have HCL-V have significantly inferior response to 2-CdA and other effective HCL treatment strategies [66–68]. Add to this the existence of a Japanese variant form of HCL and there is a “perfect storm” of confusion that swirls around HCL-V. In such a morass, it is useful to ask, “Is there a set of clinical and pathologic criteria that permits diagnostic certitude on par with that of other lymphoproliferative disorders?”. This discussion attempts to demonstrate an affirmative answer to that query and briefly explores the approach to borderline lesions.

Clinical characteristics of the variant form of hairy cell leukemia

HCL-V presents at an older age than does HCL (eighth versus sixth decades), and shows only a modest male predominance (<2:1) that contrasts with the 5:1 male/female distribution of HCL. Patients present with splenomegaly, lymphocytosis, and cytopenias, particularly anemia and thrombocytopenia. In contrast to HCL, monocytopenia and neutropenia are not features of HCL-V. Hence, the clinical presentation of HCL-V is unlike HCL and resembles SMZL [68].

Pathologic characteristics of the variant form of hairy cell leukemia

Cytopathology. The neoplastic cells of HCL-V are intermediate in size with nuclei that generally are round and have partially condensed chromatin that lacks the regular distribution of HCL and tends to have a smudged appearance (see Fig. 9). The cells often have a single medium to large nucleolus, although it recently was suggested that this cytologic feature may have been overemphasized and is not always present [69]. The cytoplasm is abundant with an appearance that is more basophilic and uniform than that of HCL with cytoplasmic margins that are well defined. Some cells have villous or hairlike projections. The cells lack the monotony of HCL and there are cell-to-cell variations in cytoplasmic basophilia, chromatin condensation, and nucleoli. As well, occasional transformed-appearing large cells may be present [68].

Ancillary studies. TRAP staining in HCL-V is variable and may be negative, brightly positive as in HCL, or show uniform dim positivity. Flow cytometry generally shows monoclonal B-cells that lack expression of CD5 and CD10. The cells predictably express CD11c, whereas about 60% express CD103. HCL-V is almost always negative for CD25. Immunohistochemical studies typically show expression of generic B-cell antigens. DBA.44 usually is positive [68,69]. A rare report [70] and a single evaluated case at Scripps Clinic
(personal observation) indicate that the cells can express cyclin D1 in a manner similar to typical HCL.

**Histopathology.** The histopathology of HCL-V essentially mimics that of HCL, except for the differing cytologic characteristics. Specifically, the marrow infiltrates have the ill-defined and well-spaced appearance that typifies HCL, although fibrosis generally is mild or absent. There may be a sinusoidal growth pattern of infiltration [69]. As well, the selective granulocytic hypoplasia that characterizes HCL is not typical of HCL-V. In the spleen, HCL-V shows a red pulp pattern and some cases have “blood lake” formation. Hepatic involvement in HCL-V is likewise similar to HCL with sinusoidal and portal tract involvement [68].

**Natural history**

HCL-V tends to follow a more aggressive clinical course than does HCL and generally is less responsive to chemotherapy. The median survival is about a decade from diagnosis. As well, and in contrast to typical HCL, about 5% to 10% of cases of HCL-V undergo morphologic transformation to a large cell process that otherwise retains immunophenotypic characteristics of HCL-V. This transformation is associated with marked leukocytosis, B symptoms, and short survival [66,68].

**Categorization of Cases in the Variant Form of Hairy Cell Leukemia/Splenic Marginal Zone Lymphoma Spectrum**

A survey of the literature of HCL-V makes evident that there is no uniformity of diagnostic criteria, particularly in the distinction between HCL-V and SMZL. In this murky context, a conservative approach to the diagnosis of HCL-V is recommended. Definitive categorization of a lymphoproliferative process as HCL-V is warranted when a population of cytologically characteristic cells is present, including the uniform presence of prominent nucleoli. The lymphocytes must display an appropriate immunophenotypic profile characterized by expression of CD11c and a lack CD25. If the cytologic features are ambiguous with an admixture of cells that suggest both HCL-V and SMZL, a diagnosis of HCL-V should be reserved for patients with a characteristic phenotypic profile as well as bone marrow and splenic findings (including a red pulp pattern of splenic involvement) that resemble typical HCL. Alternatively, when only bone marrow biopsy is available, immunohistochemical evidence of cyclin D1 overexpression can be used to distinguish HCL-V from SMZL, as the latter should always be cyclin D1 negative. In the setting of a lymphoproliferative disorder that meets neither the above criteria nor those for SMZL, it is prudent to classify the process as “low-grade B-cell lymphoproliferative disorder, SMZL, or HCL-V.”

**SUMMARY**

The pathology of HCL has been reviewed with a focus on the diagnostic hemopathology of this rare, but fascinating, disease. The discrimination of HCL
from other B-cell lymphoproliferations, particularly HCL-V and SMZL, has been emphasized. The unique responsiveness of HCL to 2-CdA and other chemotherapeutic agents makes this distinction critical. Fortunately, HCL has consistent cytologic, histologic, cytochemical, and immunologic features that make classification reliable and reproducible. Less straightforward is the differential diagnosis of SMZL and HCL-V, problematic because of the rarity of both disorders, lack of discriminating evidence-based criteria, and perhaps a biologic kinship between these two disorders that share many clinical and pathologic features. Fortunately, this is not a clinically critical distinction.

References


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