Anti-CD34 Immunoperoxidase Staining in Paraffin Sections of Acute Leukemia:

Comparison With Flow Cytometric Immunophenotyping

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Anti-CD34 is a monoclonal antibody that reacts with bone marrow progenitor cells and leukemic blasts, and is expressed on 30% to 50% of all acute leukemias. Detection of CD34 has previously been restricted to flow cytometric studies. To expand the utility of CD34, we immunostained 46 paraffin-embedded bone marrow specimens with acute leukemia; results were compared with flow cytometric studies. CD34 reactivity was also evaluated in nine chronic leukemia cases, 27 malignant lymphoma cases (Hodgkin's disease and non-Hodgkin's lymphoma), six normal bone marrow specimens, and three benign, hyperplastic lymph node specimens. All cases that were CD34 positive by flow cytometry (11 of 19 B-cell precursor acute lymphoblastic leukemia cases, one of six T-cell acute lymphoblastic leukemia cases, and seven of 21 acute myeloblastic leukemia cases) were also CD34 positive in paraffin sections. Both cell membrane and cytoplasmic staining was seen. The positivity percentage and fluorescence intensity by flow cytometry correlated with the estimated number of stained cells and the intensity of immunoperoxidase staining in 18 of 19 CD34-positive cases. The remaining bone marrow and lymph node cases studied were CD34 negative; prominent endothelial cell staining, however, was noted. This is the first report of anti-CD34 staining of acute leukemia in paraffin-embedded sections. In contrast to other monoclonal antibodies reactive in bone marrow paraffin sections with leukemia, anti-CD34 immunoperoxidase staining is limited to leukemic blasts and may provide useful diagnostic information when flow cytometric studies are not available. HUM PATHOL 23:26-32. Copyright © 1992 by W.B. Saunders Company

The CD34 antigen is a monomeric, 115-kd protein that is expressed on normal hematopoietic progenitors[1-4] and in 30% to 50% of both acute lymphoblastic and acute myeloblastic leukemia cases[5-9]; mature lymphoid and myeloid proliferations have not been shown to express this protein.[10] Although the gene for CD34 has been localized to the long arm of chromosome 1 in the region of 1q12/1qter and its cDNA partially sequenced,[11,12] the function of this protein has remained an enigma.

The detection of surface and cytoplasmic antigens relevant to diagnostic hematopathology can be accomplished by flow cytometric immunophenotyping of single cell suspensions or by paraffin section immunoperoxidase studies. Most monoclonal antibodies (MoAbs) relevant to diagnostic hematopathology are used in conjunction with flow cytometry and detect surface antigens that are destroyed by routine tissue fixation and processing.[13-15] Monoclonal antibodies that have been used in paraffin sections of bone marrow are not restricted to acute leukemia and usually are not lineage specific.[16-20] Because of these limitations, paraffin section studies of acute leukemia have not been widely implemented.

To expand the diagnostic repertoire of immunoperoxidase studies in acute leukemia, we have compared anti-CD34 staining of paraffin sections of bone marrow with flow cytometric immunophenotyping of bone marrow cell suspensions in 46 cases of acute leukemia and in 36 cases of other hematopoietic and lymphoid malignancies. The results of paraffin section staining with anti-CD34 paralleled those found by flow cytometric surface immunophenotyping. This finding provides the pathologist with an additional MoAb to use in evaluating paraffin sections of acute leukemia.

MATERIALS AND METHODS

The cases of acute leukemia selected for this study were chosen retrospectively on the basis of having available both flow cytometric immunophenotyping (including CD34 staining) and paraffin-embedded bone marrow trephine and/or clot sections. Forty-six acute leukemias that met this criteria were selected; all subtypes of acute lymphoid and myeloid leukemia were represented. To confirm the known distribution of CD34, we also evaluated paraffin sections of nine cases of chronic leukemia and 27 cases of malignant lymphoma. Six normal bone marrow specimens and three benign, hyperplastic lymph nodes were evaluated by paraffin section immunoperoxidase studies; three of the normal bone marrow cases were also immunophenotyped by flow cytometric analysis. Fifteen additional bone marrow trephine biopsies were also examined for CD34 staining; these biopsies represented a variety of nonmalignant bone marrow cases. Table 1 lists the distribution of cases studied by diagnosis. The 46 bone marrow cases with acute leukemia included 24 with decalcified, B-5 fixed trephine biopsy specimens, 12 with both B-5 fixed trephine biopsy specimens and formalin-fixed clot sections, and 10 with clot sections only. Of the 27 lymph node cases, 16 were studied in B5-fixed tissue and 11 in formalin-fixed tissue.

Flow cytometric immunophenotyping methods used in this study have been reported previously.[21] A broad panel of lymphoid- and myeloid-associated MoAbs was used. B-cell–associated MoAbs included CD19 (B4), CD20 (B1), and CD22 (Leu-14). T-cell–associated MoAbs included CD1 (T6), CD2 (T11), CD3 (T3), CD4 (T4), CD5 (Leu-1), CD7 (Leu-9), and CD8 (T8). Myeloid-associated MoAbs included CD11b (Leu-15), CD13 (My7), CD14 (My4), and CD33 (My9). Other MoAbs used included CD10 (J5), CD34 (HPCA-1), and HLA-DR.[14]

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TABLE 1. Immunoperoxidase Staining With Anti-CD34 in Paraffin Sections

Diagnosis	Cases With CD34 Staining/Total No. of Cases
Acute leukemia	
Acute lymphocytic leukemia	12/25
B-cell precursor	11/19
T cell	1/6
Acute myelogenous leukemia	7/21
AML-M1	3/5
AML-M2	3/6
AML-M3	0/3
AML-M4	0/3
AML-M5	1/2
AML-M7	0/2
Chronic leukemia	
Chronic myelogenous leukemia	0/2
Chronic lymphocytic leukemia	0/4
Hairy cell leukemia	0/1
Multiple myeloma	0/2
Malignant lymphoma	
Non-Hodgkin's lymphoma	0/18
Small lymphocytic lymphoma	0/3
Small cleaved cell, follicular center cell type	0/3
Diffuse, mixed cell lymphoma (T cell)	0/2
Diffuse large cell lymphoma (T cell)	0/1
Immunoblastic lymphoma	0/2
Lymphoblastic lymphoma (T cell)	0/4
Burkitt's lymphoma	0/3
Hodgkin's disease	0/9
Lymphocyte predominant, nodular	0/2
Lymphocyte predominant, diffuse	0/1
Nodular sclerosing	0/3
Mixed cellularity	0/3
Normal or nonmalignant bone marrows/ lymph node	
Normal bone marrows	0/6
Infant	0/3
Adult	0/3
Nonmalignant bone marrows	0/15
Megaloblastic anemia	0/2
Granulocytic hyperplasia	0/4
Erythroid hyperplasia	0/3
Regenerating marrow (postchemotherapy)	0/4
Regenerating marrow (posttransplantation)	0/2
Benign lymph node hyperplasia	0/3

Anti-CD34 (HPCA-1; Becton-Dickinson, Mountain View, CA) was used at a dilution of 1:10. The criterion for CD34 positivity was the expression of that antigen by at least 20% of the blast cell population, if the morphologic evaluation of a cytocentrifuge preparation showed a high percentage of leukemia blasts. In addition to determining the positivity percentage of cellular staining with each MoAb, the mean channel of fluorescence intensity was also determined. This mean channel number was used as a direct indicator of the intensity of fluorescent staining.[22]

Immunoperoxidase staining with anti-CD34 was performed using well-established methods.[23] The same antibody source (Becton-Dickinson) and dilution (1:10) for anti-CD34 were used as in flow cytometric immunophenotyping. The interpretation of anti-CD34 immunoperoxidase staining involved both an attempt to quantify the percentage of cells staining as well as an estimate of the intensity of staining. Positivity was roughly approximated as follows: 0 to 25%, 25% to 50%, 50% to 75%, and 75% to 100% positive-staining cells. The intensity of staining was also approximated based on the intensity of immunoperoxidase staining: −, negative; +, weak staining; ++, moderate staining; and +++, intense staining.

RESULTS

Flow Cytometric Immunophenotyping

The distribution of anti-CD34 staining in the acute leukemia cases by flow cytometric immunophenotyping was similar to that reported in previous studies.[5-9] Twelve of 25 (48%) acute lymphocytic leukemia cases and seven of 21 (33%) acute myeloid leukemia cases in this study expressed the CD34 antigen. Only one of six T-cell acute lymphocytic leukemias showed anti-CD34 staining. The percentage of cells expressing CD34, as determined by flow cytometry, ranged from 19.2% to 93.0% in those acute leukemia cases interpreted as being positive for CD34 (median, 78.4%). The mean channel of fluorescence intensity of anti-CD34, which was based on a 1 to 256, three-decade logarithmic scale, ranged from 36 to 115 channels (median, 73.8) in the CD34-positive cases. For purposes of comparison with the immunoperoxidase staining, cases below the median were arbitrarily interpreted as being of dim intensity, while those above the median were arbitrarily designated as being of bright intensity[15,22] (Table 2).

Immunoperoxidase Staining

Results obtained by immunoperoxidase staining with anti-CD34 paralleled those obtained by flow cytometric immunophenotyping (Table 2). The staining was distributed both on the cell surface membrane and in the cytoplasm; the majority of staining appeared to be membrane bound (Fig 1). When the percentage of immunoperoxidase anti-CD34 staining was estimated (Table 2), results closely approximated the percentage of leukemic cells stained by flow cytometry. In addition, the estimate of immunoperoxidase staining intensity of anti-CD34 also correlated with the mean channel of fluorescence intensity as determined by flow cytometry. No significant differences in immunoperoxidase staining were noted in those cases with both B-5 and formalin-fixed tissue. Decalcification of the trephine biopsies had no apparent deleterious effect on the intensity of CD34 staining.

Only one of the 46 acute leukemia cases showed any significant discrepancy of anti-CD34 staining between immunoperoxidase and flow cytometric studies. This case was an acute myelogenous leukemia (case no. 33) having a mean channel number of fluorescence intensity of 66.4, in which flow cytometry showed 27.4% of blast cells staining with anti-CD34. In contrast, immunoperoxidase studies showed that approximately 50% to 75% of the leukemic cells have an intense (+++) staining reaction with anti-CD34 (Fig 2).

To assess the specificity of anti-CD34 staining for leukemic blasts, nine chronic leukemia cases and 27 malignant lymphoma cases were stained with anti-CD34 in paraffin sections. None of the nine chronic leukemia cases, including chronic myelogenous leukemia, chronic lymphocytic leukemia, hairy cell leukemia, and multiple myeloma, showed anti-CD34 immunoperoxidase staining. Of the 27 malignant lymphoma cases evaluated in this study, nine were Hodgkin's disease (including lym-

TABLE 2. CD34 Staining in Acute Leukemia: Flow Cytometry and Paraffin Section Immunoperoxidase Techniques

Case No.	Diagnosis	FC%	FC-MCFI	IP%	IP Intensity
Acute lymphocytic leukemia (ALL)					
1	ALL(B)	60.8	49.8(D)	50-75	+
2	ALL(B)	49.4	66.5(D)	25-50	+
3	ALL(B)	69.4	62.1(D)	25-50	++
4	ALL(B)	93.0	115.0(B)	75-100	+++
5	ALL(B)	68.2	73.8(B)	75-100	++
6	ALL(B)	78.6	131.0(B)	75-100	+++
7	ALL(B)	83.0	65.0(D)	75-100	+
8	ALL(B)	50.8	84.4(B)	25-50	+
9	ALL(B)	89.8	111.5(B)	50-75	+++
10	ALL(B)	19.2	53.1(D)	0-25	+
11	ALL(B)	87.8	94.0(B)	75-100	+++
12	ALL(B)	1.5	22.1	0-25	+
13	ALL(B)	0.0	0.0	0-25	−
14	ALL(B)	14.0	12.3	0-25	−
15	ALL(B)	6.2	30.1	0-25	−
16	ALL(B)	1.4	37.2	0-25	−
17	ALL(B)	0.0	0.0	0-25	−
18	ALL(B)	1.6	41.7	0-25	−
19	ALL(B)	1.3	56.2	0-25	−
20	ALL(T)	78.4	103.3(B)	75-100	++
21	ALL(T)	0.1	24.3	0-25	−
22	ALL(T)	1.7	59.2	0-25	−
23	ALL(T)	8.1	48.9	0-25	−
24	ALL(T)	0.3	15.0	0-25	−
25	ALL(T)	8.0	23.1	0-25	−
Acute myelogenous leukemia (AML)					
26	AML-M1	84.0	58.5(D)	75-100	+
27	AML-M1	85.1	66.2(D)	75-100	++
28	AML-M1	32.1	65.5(D)	50-75	+
29	AML-M1	1.6	70.4	0-25	−
30	AML-M1	1.0	21.0	0-25	−
31	AML-M2	88.7	113.6(B)	75-100	+++
32	AML-M2	89.0	98.1(B)	75-100	++
33	AML-M2	27.4	66.4(D)	50-75	+++
34	AML-M2	6.2	31.0	0-25	−
35	AML-M2	10.2	29.8	0-25	−
36	AML-M2	0.0	0.0	0-25	−
37	AML-M3	0.0	0.0	0-25	−
38	AML-M3	4.7	75.0	0-25	−
39	AML-M3	0.0	0.0	0-25	−
40	AML-M4	0.0	0.0	0-25	−
41	AML-M4	14.0	12.9	0-25	−
42	AML-M4	19.0	56.0	0-25	−
43	AML-M5	39.2	78.2(B)	50-75	++
44	AML-M5	0.0	0.0	0-25	−
45	AML-M7	3.2	24.5	0-25	−
46	AML-M7	1.2	37.5	0-25	−

Abbreviations: FC%, percentage of cells staining with anti-CD34 by flow cytometric methods. FC-MCFI, the flow cytometric mean channel of fluorescence intensity, corresponding to the intensity of staining (D, dim staining; B, bright staining) (see text for definitions). IP%, approximation of the percentage of cells stained by immunoperoxidase: 0 to 25%, 25% to 50%, 50% to 75%, and 75% to 100%. IP intensity, approximation of the immunoperoxidase staining intensity (−, negative; +, weak; ++, moderate; +++, intense). ALL(B), B-precursor ALL; ALL(T), T-ALL.

phocyte predominant, nodular sclerosing, and mixed cellularity types). Eighteen non-Hodgkin's lymphoma cases were also evaluated, including representative subtypes from low-grade, intermediate-grade, and high-grade types; six of these non-Hodgkin's lymphoma cases were of T-cell origin. Flow cytometric immunophenotyping was not performed in these cases. No anti-CD34 immunoperoxidase staining was found in any of the lymphoma cases, including four cases of lymphoblastic lymphoma (Fig 3) and three cases of Burkitt's lymphoma (Fig 3). Anti-CD34 did show prominent vascular endothelial cell staining, which has been described in previous studies (Fig 4).[24] In addition, there was anti-CD34 staining of perivascular and pericapsular spindle-shaped cells (Fig 5). These CD34-positive, dendritic-like cells had elongated, pale-staining nuclei, but no nucleoli. Dendritic cells found in other locations, such as in normal skin,[25,26] have previously been described by Nickoloff as being CD34 positive.[27]

Anti-CD34 immunoperoxidase staining was also performed on six normal bone marrow specimens, 15 nonmalignant bone marrow specimens, and three benign, hyperplastic lymph node specimens. Concurrent flow cytometric studies were done on three of the six normal bone marrow specimens. These three specimens were from children less than 2 years of age who were being evaluated for possible acute leukemia; morphologically, all three specimens had increased numbers of bone marrow hematogones, which probably represent human progenitor B cells.[28,29] Flow cytometric immunophenotyping in these three cases showed increased numbers of B-cell precursors, which were positive for CD10 (CALLA), CD19, TdT, and HLA-DR. No anti-CD34 staining was identified by either flow cytometry or paraffin section immunoperoxidase in these three normal bone marrow specimens. The remaining three normal bone marrow specimens, the 15 nonmalignant bone marrow specimens, and the three benign lymph node specimens were all from adult patients. No anti-CD34 staining of mononuclear cells was identified in these benign marrow specimens or lymph nodes; only endothelial cells showed any immunoperoxidase CD34 staining. Although CD34-positive progenitor cells undoubtedly were present in the bone marrow specimens, the extremely small percentage of such cells probably precluded their detection by immunoperoxidase methods.

DISCUSSION

The CD34 protein is an intriguing molecule that undoubtedly plays a major role in progenitor cell development. In addition to its presence on hematopoietic stem cells[1-4] and endothelial cells,[24] a recent report has described a distinctive CD34-positive population of cells located in the perifollicular "bulge" area on the hair follicle[27] that appears to represent the hair follicle stem cells.[30] The biochemical properties of the CD34 protein along with its expression on both endothelial and bone marrow progenitor cells suggest that it may have an adhesion-like function that could enhance the interaction between progenitor cells and their surrounding stromal cells.[11,12] This interaction would give the progenitor cells a direct source of needed cytokines and interleukins necessary for cellular development and differentiation.

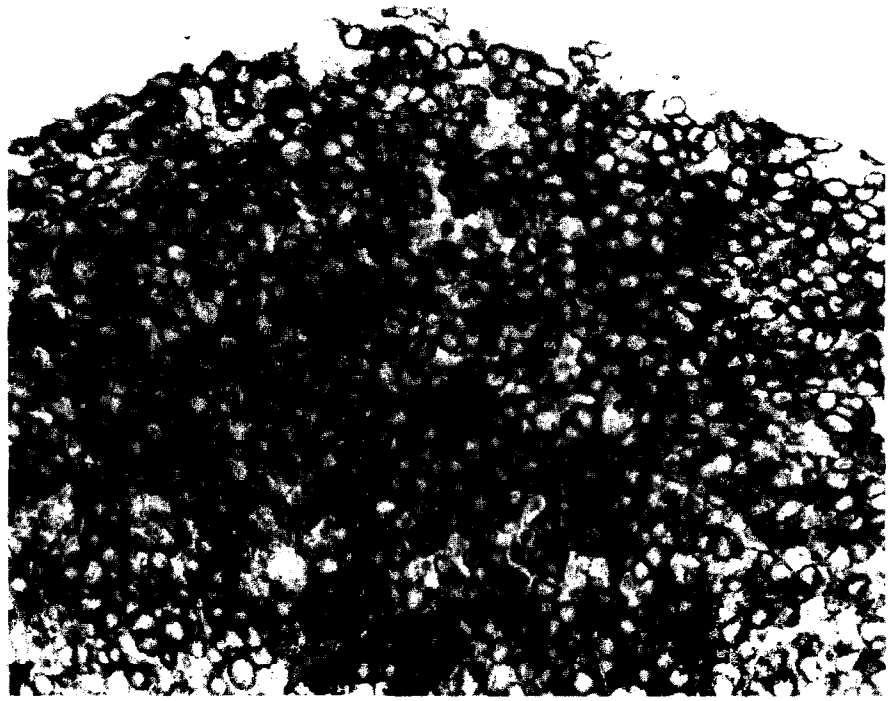

FIGURE 1. Anti-CD34 immunoperoxidase staining of acute lymphoblastic leukemia (case no. 4). Both cell membrane and cytoplasmic staining is seen. (Magnification ×400.)

In addition to its potential importance in hematopoietic cellular development, CD34 expression has been used in diagnostic immunophenotyping as a leukemia-restricted marker[5-9]; various MoAbs (My10, PHCA-1, BI-3C5, ICH3, 12.8, 8G12) have been developed that recognize this antigen. Although not lineage restricted to either B- or T-lymphocyte precursors or myeloblasts, anti-CD34 staining is restricted to normal progenitor cells or leukemic blasts among bone marrow cells. Cooperative group studies of CD34-positive acute lymphocytic leukemia in the pediatric age group have suggested that CD34 expression may define a subgroup of leukemias with a good clinical prognosis.[9] Conversely, CD34 expression in adult patients with acute myelogenous leukemia has been associated with shorter remissions and a poorer clinical outcome.[8] Regardless of its potential clinical relevancy, the expression on leukemic cells has helped define some of the sequential steps of normal hematopoietic and lymphoid development.[15,22]

Paraffin-embedded sections have increasingly been used as an additional source of material for immunophenotyping in diagnostic hematopathology. The use of

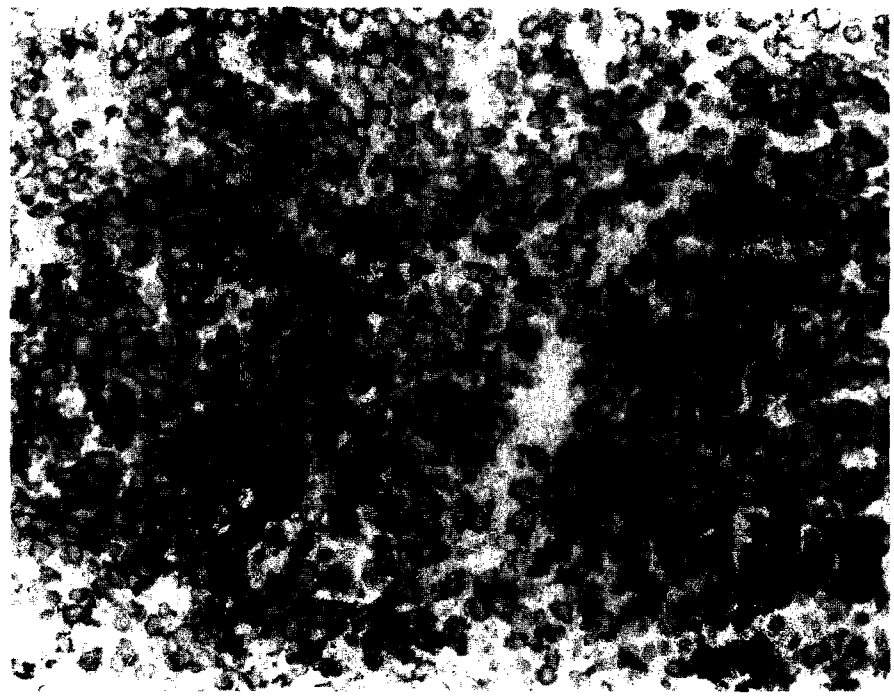

FIGURE 2. Anti-CD34 immunoperoxidase staining of acute myeloblastic leukemia, FAB-M2 (case no. 33). An intense staining reaction is seen in approximately 50% to 75% of the leukemic cells. Both the percentage of positivity and the estimation of staining intensity are much greater than corresponding results obtained by flow cytometry. (Magnification ×400.)

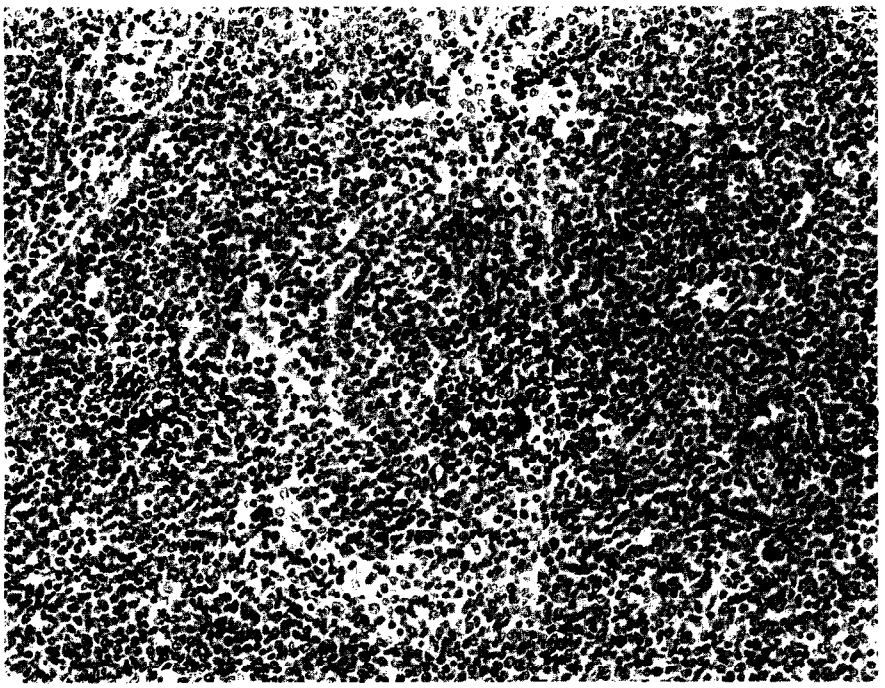

FIGURE 3. Anti-CD34 immunoperoxidase staining in a lymph node section of T-cell lymphoblastic lymphoma. No CD34 staining is identified. (Magnification ×200.)

paraffin material with immunoperoxidase staining has permitted retrospective analysis and has provided diagnostic material in those cases without fresh, single-cell suspensions. Only a limited number of studies have evaluated the utility of MoAbs in paraffin sections of bone marrow.[16-20] In general, the antibodies used for these studies are not lineage restricted and frequently cross-react with normal hematopoietic elements. Furthermore, most of these hematopoietic/lymphoreticular antibodies were developed and initially used as markers of malignant lymphomas (ie, mature lymphoid cells) and uniformly have not been leukemia restricted. Thus, paraffin section antibody studies have had significant limitations in the routine evaluation of acute leukemia.

This is the first report of anti-CD34 staining of acute leukemia in paraffin-embedded sections. In this study, we compared the reactivity of anti-CD34 in paraffin sections to that of routine flow cytometric immunophenotyping. No significant discrepancies were identified between these two methodologies. The results suggest that anti-CD34 staining by immunoperoxidase methods may be diagnostically useful to pathologists evaluating bone marrow specimens for acute leukemia. Current knowledge of the CD34 antigen does not explain why

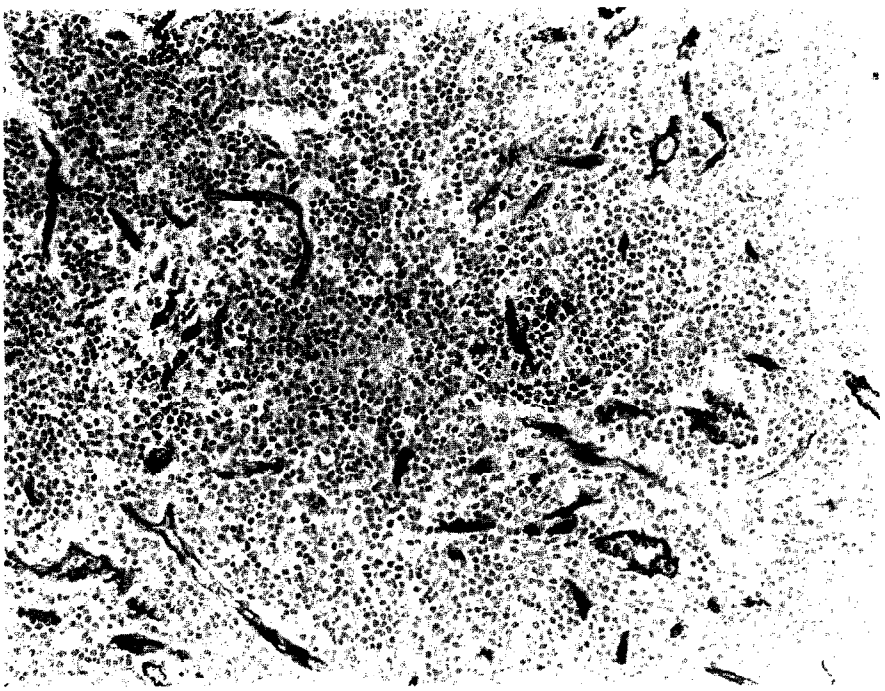

FIGURE 4. Anti-CD34 immunoperoxidase staining in a peripheral T-cell lymphoma. Most of the endothelial cells show prominent staining with this antibody. (Magnification ×200.)

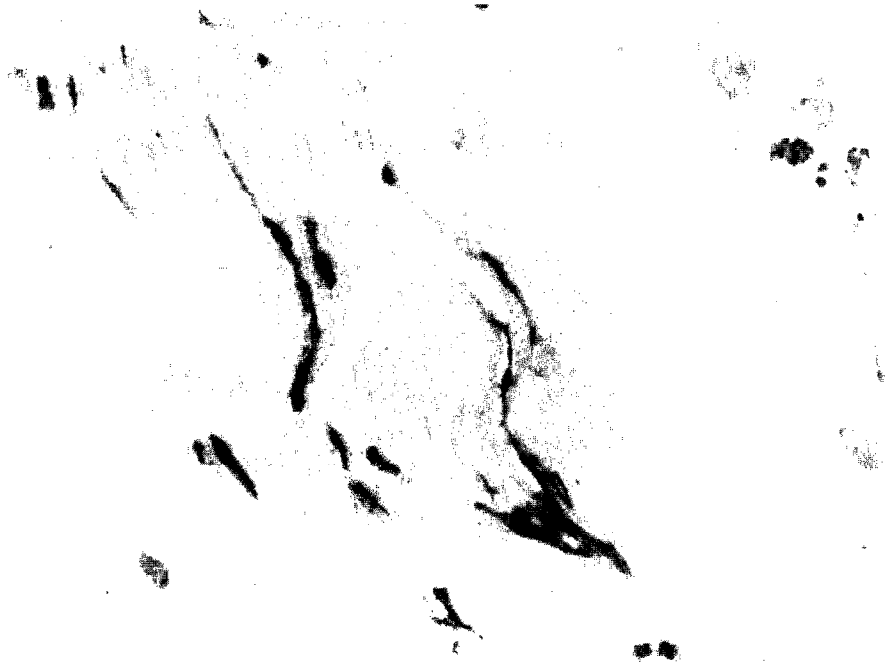

FIGURE 5. Anti-CD34 immunoperoxidase staining of pericapsular, spindle-shaped cells. These cells have a dendritic-like appearance. (Magnification ×400.)

this protein preferentially survives routine fixation and processing as compared with other leukemia-associated surface antigens. Although both surface and cytoplasmic staining was identified in the paraffin sections, the close correlation between flow cytometric surface studies and immunoperoxidase staining suggests that cytoplasmic expression of CD34 cannot be the sole explanation for the paraffin staining. Until this protein and the corresponding gene are more completely characterized, we can only speculate that the basic protein structure of the CD34 antigen allows for its preferential survival after routine fixation.

Although it is not useful for subclassifying an acute leukemia, anti-CD34 staining by immunoperoxidase methods may be useful in confirming the identity of a leukemic process when traditional methods are unavailable or are not diagnostic. Since not all acute leukemias are CD34 positive, lack of staining would not rule out an acute leukemic process. If the leukemic cells are CD34 positive at the time of diagnosis, theoretically, one could use this antibody in paraffin sections to detect residual or early relapsing foci of leukemia that are not morphologically evident. In addition, if CD34 expression continues to be prognostically important in the evaluation of pediatric acute leukemias,[8,9] then the use of paraffin sections for CD34 staining could significantly expand the amount of material available for such clinical studies.

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