Detection of Minimal Residual Disease by Immunostaining of Bone Marrow Biopsies After 2-Chlorodeoxyadenosine for Hairy Cell Leukemia

By David Hakimian, Martin S. Tallman, Carol Kiley, and LoAnn Peterson

2-Chlorodeoxyadenosine (2-CdA) yields high complete remission (CR) rates in patients with hairy cell leukemia (HCL). In an effort to detect minimal residual disease, we studied two B-lineage antibodies, L26 and MB2, and a T-lineage antibody, UCHL-1, in fixed marrow core biopsies from 34 patients with HCL before and after 2-CdA. Before therapy, hairy cells exhibited intense cytoplasmic membrane reactivity with L26 and strong intracytoplasmic reactivity with MB2. UCHL-1 did not react with hairy cells. Thirty-one patients were assessable 3 months after therapy. Five of 24 (21%) patients in CR by routine evaluation had residual HCL detected by immunostaining. Four of these 5 patients have been reevaluated at 1 year. One patient relapsed by routine evaluation, 2 remained positive by immunostaining alone, and 1 patient became negative by immunostaining. A total of 19 patients have been evaluated at 1 year. Only 1 additional patient has become positive by immunostaining alone. Immunostaining using the B-lineage antibodies highlighted the presence of hairy cells with preservation of morphology. This assisted in quantifying the extent of disease, particularly when hairy cells were interstitial and blended with surrounding hematopoietic tissue, when hairy cells were present in hypocellular marrows, when hairy cells were spindle-shaped, and when marrows were markedly fibrotic. Because immunostaining can be easily performed on routinely processed marrows, it is an attractive method to detect minimal residual disease. Our data suggest that some patients in apparent CR after 2-CdA may have minimal residual disease. Patients will need to be observed prospectively to determine if residual disease will be predictive of relapse.

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Materials and Methods

Patients. All patients with HCL treated with 2-CdA (cladribine, leustatin; Ortho Biotech, Raritan, NJ) at The Robert H. Lurie Cancer Center of Northwestern University between February 1991 and August 1992 were eligible. The initial diagnosis was established by standard morphologic criteria and the clinical characteristics of these patients recently have been described. Bone marrow aspirates and core biopsies were obtained from all patients before therapy, then again at 3 months and 1 year after therapy.

Immunohistochemistry. Bone marrow core biopsies were obtained and processed either at Northwestern University or at referral institutions. The biopsies obtained at Northwestern University were fixed in B5 for 90 minutes, decalcified in RDO (Apex Engineering Product Corp, Plainfield, IL), and embedded in paraffin. Blocks of core biopsies and particle clots from referral institutions were sent to Northwestern University, where the immunostaining on all cases was performed. Immunohistochemical studies were performed on 4-μm sections that were hydrated in a graded series of alcohols. The sections were immersed in 0.03% hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase activity. Specimens were incubated with 2% nonfat milk/phosphate-buffered saline (PBS) (pH 9.0) for 30 minutes to block nonspecific Ig binding. The MoAbs UCHL-1, L26 (Dako Corp, Carpenteria, CA), and MB2 (Biotest Diagnostics Corp, Fairfield, NJ) were diluted to titers of 1/500, 1/800, and 1/75, respectively. Slides were incubated with the MoAb overnight at 4°C. Prediluted mouse negative control sera (BioGenex, San Ramon, CA) was used as a control for residual nonspecific Ig binding. After a brief wash in 2% nonfat milk/PBS (pH 8.0), biotinylated antimouse Ig (Vector Laboratories, Burlingame, CA) was applied and incubated for 30 minutes. This application was followed by a brief wash in PBS and incubation with Avidin DH-horseradish peroxidase complex (Vector Laboratories) for 30 minutes. Slides were then washed in PBS (pH 7.4) and diaminobenzidine was applied as a chromogen, followed by a light hematoxylin counterstain.

The slides were examined by light microscopy and the number, pattern of distribution, and morphologic characteristics of the cells exhibiting reactivity were evaluated.

Remission Criteria

Criteria for CR in the previous study included all of the following: (1) absence of hairy cells in the peripheral blood and bone

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IMMUNOSTAINING OF MARROW BIOPSIES AFTER 2-CdA

Fig 1. Bone marrow core biopsy sections from a patient with HCL before therapy with 2-CdA. The hairy cells exhibit intense cytoplasmic membrane reactivity with L26 (CD20). Immunostain with antibodies to L26 (CD20). (Original magnification X 630.)

Fig 2. Bone marrow core biopsy section from a patient with HCL before therapy with 2-CdA. The hairy cells show strong intracytoplasmic positivity with MB2. Immunostain with antibodies to MB2. (Original magnification X 630.)

Fig 3. Bone marrow core biopsy section from a patient with spindle-shaped hairy cells highlighted by reactivity with MB2. Immunostain with antibodies to MB2. (Original magnification X 630.)

Fig 4. Bone marrow core biopsy sections from a patient 3 months after therapy with 2-CdA. The H&E section (A) was interpreted as remission. Hairy cells were identified after immunostaining (B). (A) H&E (original magnification x 400). (B) Immunostain with antibodies to L26 (CD20). (Original magnification X 630.)

marrow; (2) normalization of peripheral blood counts (hemoglobin, $\geq 12$ g/dL; white blood cell count, $>3,000$/μL; neutrophils, $>1,500$/μL; and platelet count, $>100,000$/μL); (3) absence of all constitutional symptoms; (4) absence of all palpable adenopathy and hepatosplenomegaly; and (5) disappearance of all abnormal adenopathy and hepatosplenomegaly by computed tomography (CT) scans. Partial remission (PR) required all of the following: (1) greater than 50% reduction of hairy cells in the bone marrow core biopsy; (2) greater than 50% increase of all abnormally low peripheral blood counts; and (3) greater than 50% reduction in all abnormal adenopathy or hepatosplenomegaly. Patients who did not fulfill the criteria for CR or PR were classified as nonresponders.

In the present study, posttherapy bone marrow sections were interpreted as positive for residual hairy cells only if the following two
criteria were met: (1) the L26-positive cells had the morphologic appearance of hairy cells; and (2) the cells reacting with L26 were more numerous than those reacting with UCHL-1.

RESULTS

Pretherapy. Core biopsies from 34 patients were evaluated before therapy. Hairy cells were the predominant cell in all biopsies, representing 60% to 95% of the nucleated cells. Hairy cells exhibited intense cytoplasmic membrane reactivity with L26, and strong intracytoplasmic reactivity with MB2 (Figs 1 and 2). L26 had no apparent reactivity with other cells, whereas MB2 cross-reacted with myeloid cells. UCHL-1 did not react with hairy cells, but scattered lymphocytes were positive.

Immunostaining using these B-lineage antibodies highlighted the presence of hairy cells with preservation of morphology. This assisted in quantifying the extent of bone marrow involvement and was particularly useful in the following settings: (1) when hairy cells were interstitial and blended with surrounding hematopoietic tissue; (2) when hairy cells infiltrated between fat cells in hypocellular areas of the marrow; (3) when hairy cells were spindle-shaped (Fig 3); and (4) when marrows were markedly fibrotic.

At 3 months. Thirty-three patients were evaluated 3 months after a single cycle of 2-CdA. Immunostaining was inadequate in 2 cases (1 fixed in Bouins, the other in formalin). Therefore, 31 cases were assessable. Twenty-four of the 31 (77%) patients achieved CR and 7 (23%) achieved PR (Table 1). Five of these 24 patients in CR (21%) met the immunostaining criteria for residual HCL. In 3 cases, the marrows were markedly hypocellular and hairy cells were present between fat cells. In the other 2 cases, the marrows were normocellular and the hairy cells infiltrated in an interstitial pattern, blending with surrounding hematopoietic cells (Fig 4). Occasionally, hairy cells were focally concentrated, but no large well-defined aggregates were present.

Table 1. Comparison of Conventional Evaluation and Immunostaining 3 Months After 2-CdA (N = 31)

<table>
<thead>
<tr>
<th>Remission Status by Conventional Evaluation</th>
<th>Residual Disease (%) by Immunostaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>5 (21)</td>
</tr>
<tr>
<td>PR</td>
<td>3 (42)</td>
</tr>
<tr>
<td>Marrow disease</td>
<td>3 (100)</td>
</tr>
<tr>
<td>Enlarged spleen or lymph nodes only</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. Comparison of Conventional Evaluation and Immunostaining 1 Year After 2-CdA (N = 19)

<table>
<thead>
<tr>
<th>Remission Status by Conventional Evaluation</th>
<th>No. of Patients</th>
<th>Residual Disease (%) by Immunostaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>17</td>
<td>3 (18)</td>
</tr>
<tr>
<td>Relapse</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

These 5 cases were reexamined and none had hairy cells identified by morphology alone.

Three of the 7 patients in PR had residual HCL recognized on hematoxylin and eosin (H&E)-stained bone marrow core biopsies. In all 3 cases, residual disease was confirmed by immunostaining. The other 4 patients were classified as PR because of either residual splenomegaly or adenopathy, but no hairy cells were apparent in the bone marrow. Immunostaining in each of these cases was negative.

Lymphoid aggregates were present in 5 of the 31 cases. The aggregates differed from residual HCL by either the predominance of UCHL-1-positive lymphocytes or the presence of approximately equal numbers of UCHL-1- and L26-positive cells.

At 1 year. Nineteen patients achieving CR were evaluated 1 year after 2-CdA therapy and 17 patients remain in CR (Table 2). Three of these 17 are positive by immunostaining, 2 of whom had been positive at 3 months as well. Thus, only 1 additional patient became positive by immunostaining at 1 year. Two of the 17 patients had relapsed by morphologic criteria and each case was confirmed by immunostaining. Only 1 of these 2 had been positive by immunostaining at 3 months.

When analyzing the 5 patients who were positive by immunostaining alone at 3 months, 4 are evaluable at 1 year (1 patient is too early; Table 3). One patient has relapsed by routine evaluation, 2 remain positive by immunostaining alone, and 1 patient with a very hypocellular marrow is now negative by immunostaining.

DISCUSSION

The treatment of HCL has rapidly evolved over the past decade. Splenectomy was the therapy of choice for many years; however, remissions were short and persistent disease was always present in the bone marrow. Interferon yields remissions in many patients, but toxicity is significant, CRs are uncommon, and patients often relapse after discontinuation of therapy. More recently, the two purine analogs, 2-deoxycoformycin (DCF; pentostatin) and 2-CdA have been shown to yield high rates of CR, with few relapses. However, given the indolent nature of this disease, it is unclear whether these patients have been cured.

Immunophenotypic characteristics of hairy cells are well recognized. The pan-B-cell antigens CD19 (Leu12),

Table 3. One-Year Follow-Up of the 5 Patients With Residual Disease by Immunostaining Alone at 3 Months After 2-CdA

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Remission Status by Conventional Evaluation</th>
<th>Residual Disease by Immunostaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CR</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Relapse</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>CR</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>CR</td>
<td>−</td>
</tr>
<tr>
<td>5</td>
<td>TE</td>
<td>TE</td>
</tr>
</tbody>
</table>

Abbreviation: TE, too early.
CD20 (Leu16), and CD22 (Leu14 and HCL-1) are expressed as well as surface Ig and the monocyte-associated antigen CD11c (LeuM5 and HCL3). T-cell antigens are generally not expressed. The majority of the antigens require cell suspensions or frozen tissue for detection, but are destroyed when tissues are fixed and embedded in paraffin. Therefore, immunophenotypic evaluation of patients with HCL is often difficult because circulating hairy cells may be rare and bone marrows are often inaspirable.

MoAbs are now available that detect antigens in fixed, decalcified, and paraffin-embedded bone marrow core biopsies. These antibodies allow assessment of neoplastic processes in bone core biopsies, with preservation of cell morphology and tissue architecture.

The present study confirms that hairy cells react with the B-lineage MoAbs, L26 and MB2 in fixed and paraffin-embedded bone marrow core biopsies. Although these antibodies are not specific for HCL, hairy cells with characteristic morphology are highlighted, aiding in the evaluation of both the presence and extent of disease. In patients with HCL, bone marrows are occasionally markedly hypocellular and hairy cells infiltrate between fat spaces. In some marrows, hairy cells are present in an interstitial pattern rather than in focal aggregates. In other instances, hairy cells are spindle-shaped or are present in marrows with marked fibrosis. In these settings, immunostaining was particularly useful. Differentiation of benign lymphocytic aggregates from hairy cell infiltrates was also facilitated because benign aggregates consisted primarily of UCHL-1-positive cells or approximately equal numbers of UCHL-1- and L26-positive cells. It is important to emphasize that all biopsies at our institution and most submitted from outside institutions were fixed in B5 and these biopsies all stained similarly. A few marrows showed no reactivity with L26 and were therefore unevaluable. These marrows were fixed in either Bouin’s or Formalin. Whether or not the lack of reactivity with L26 is attributable to the specific fixative is not clear. However, previous reports describe absence of reactivity with L26 in Zenker’s fixed tissue.

Our data suggest that some patients in apparent CR after 2-CdA may have minimal residual disease. Twenty-one percent of patients in remission by conventional bone marrow morphology at 3 months were interpreted as having residual disease. The determination of residual disease is difficult because there are no standard criteria; thus, our own criteria were established for this study. Because most patients were in CR and numerous myeloid cells were present, cross-reactivity with MB2 precluded its usefulness in most cases. As a result, our criteria were based only on immunostaining with L26 and UCHL-1. L26 reacted with scattered lymphoid cells in all cases. Although these cells occasionally had the morphologic appearance of hairy cells, we did not feel that their presence alone was sufficient to establish the existence of residual disease. Furthermore, in normal bone marrows, UCHL-1-positive cells usually are more numerous than L26-positive cells. Therefore, we required that L26-positive cells be more numerous than UCHL-1-positive cells as well as have the morphologic appearance of hairy cells. Our immunostaining criteria may be considered strict; however, they were established to avoid an overestimation of residual disease.

The present study is the first to detect residual disease by immunostaining in HCL patients treated with 2-CdA. Our findings are not surprising given that residual disease has been identified in patients in CR after interferon or pentostatin. For example, Falini et al detected residual disease in routinely processed core biopsies in 2 patients achieving CR with interferon using antibodies to L26 (CD20) and 4KB5 (CD45RA). Thaler et al evaluated cryostat sections of bone marrow core biopsies. In five patients attaining complete morphologic remission after treatment with either interferon or pentostatin, residual hairy cells were identified with antibodies against CD22, CD25, and CD11c antigens. Finally, Hunnieu et al has evaluated a new MoAb DBA.44 that recognizes a fixation-resistant B-cell antigen present in centroblastic, immunoblastic, and monocytoid B-cell lymphomas. Forty-one bone marrow biopsies in patients with HCL assessed as uninvolved or equivocal after treatment with interferon were all reclassified as having minimal or moderate infiltration by hairy cells after staining of routinely processed core biopsies by DBA.44. This finding is not unexpected because the CR rate with interferon is low and remissions are not sustained. In contrast, our data are encouraging because residual disease by immunostaining was not present in the majority of our patients. This finding is consistent with the observation that 2-CdA results in sustained CRs in most patients.

Other sensitive methods for detecting minimal residual disease include flow cytometry and Ig gene rearrangement studies. Such studies have been performed in our patients; however, these were seldom useful. Although an occasional patient had evidence of a persistent clonal abnormality, the vast majority of patients were not evaluable because there were insufficient circulating cells and marrows were frequently inaspirable. Because immunostaining can be easily performed on routinely processed bone marrows, it is an attractive alternative for detection of minimal residual disease. Patients will need to be observed prospectively to determine if detection of minimal residual disease by immunostaining will be predictive of relapse.

REFERENCES

of neoplasms in bone marrow biopsies using monoclonal antibodies reactive in paraffin-embedded tissue. Mod Pathol 2:618, 1989


Detection of minimal residual disease by immunostaining of bone marrow biopsies after 2-chlorodeoxyadenosine for hairy cell leukemia

D Hakimian, MS Tallman, C Kiley and L Peterson