CD20 (Pan-B Cell Antigen) Expression on Bone Marrow-Derived T Cells

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Antibodies directed against CD20 (L26, Leu 16, and Bl) are frequently used to determine the presence of B lymphocytes. However, recent publications describe the unexpected presence of CD20-positive T cells in the peripheral blood of normal subjects and occasional T-cell neoplasms that express CD20. To determine the presence of CD20-positive T cells in bone marrow, flow cytometric analysis was performed on 34 aspirate specimens (14 normal, 5 acute lymphoblastic lymphoma [ALL], 5 acute myelogenous leukemia [AML], 4 HIV positive, 2 myelodysplastic/myeloproliferative, 2 chronic myelogenous leukemia [CML], 1 chronic lymphocytic lymphoma [CLL], 1 multiple myeloma). A small population of cells coexpressing CD3 (Leu 4) and CD20dim (Leu 16) was identified in 94% of the specimens, representing 0% to 11% of marrow mononuclear cells and 0% to 22.2% (mean 6.54%) of marrow lymphoid cells. There was no correlation between the percentage of CD20-positive T cells and the CD4:CD8 ratio, patient age, gender, or diagnosis. CD20dim positive cells included immature B cells and CD20-positive T cells. Although evaluation of CD20 expression is useful in delineating B-cell processes, caution should be exercised in interpreting its expression on bone marrow T-lymphoid cells. CD20 expression on T cells may be seen in either normal, reactive, or neoplastic processes. (Key words: Lymphocytes; Immunophenotyping; Hematopoietic differentiation antigens; Hematologic diseases) Am J Clin Pathol 1996;106:78-81.

Phenotyping of human lymphoid proliferations is of value in determining cell lineage. Immunophenotyping uses monoclonal antibodies directed against B- or T-cell specific antigens. B lymphocytes are identified by the presence of the antigens CD19, CD20, CD21, and CD22. CD20 is a 33-kDa membrane phosphoprotein with three hydrophobic regions that traverse the cell membrane, creating a structure similar to an ion channel. This protein is expressed on the majority of B cells, appearing early in B-cell development (following CD19 and CD10, but preceding CD21, cell surface expression of CD22, and surface immunoglobulin). CD20 is retained on mature B cells until plasma cell development. The CD20 antigen can be identified by immunohistochemistry or flow cytometry using several commercially available monoclonal antibodies: Leu 16, B1, and L26 (Leu 16 from Becton Dickinson Immunocytometry Systems, [San Jose, CA]; B1 from Coulter Corporation [Hialeah, FL]; and L26 from Dako [Santa Barbara, CA]). Therefore, this antigen is frequently used to designate B-cell lineage.

Recent studies question the diagnostic utility of the CD20 antigen. Benign and neoplastic populations of T cells have been identified that also mark with the CD20 antigen. During a routine flow cytometric analysis of bone marrow specimens, several cases were identified in which the number of cells expressing CD20 exceeded the number of CD19-positive cells. Therefore, a prospective evaluation of 34 bone marrow specimens was undertaken using multi-parameter flow cytometric analysis to identify T cells that coexpressed CD20.

MATERIALS AND METHODS

Consecutive, routinely submitted posterior iliac bone marrow aspirate specimens were collected and anticoagulated with EDTA. The specimens were processed within 24 hours of collection and analyzed by flow cytometry using direct immunofluorescence.

The bone marrow cells were separated by Ficoll gradient, red blood cells were lysed with ammonium chloride, and cells were resuspended in phosphate buffered saline containing fetal calf serum (Irvine Scientific, Santa Ana, CA) to inhibit nonspecific binding. The specimens were then incubated with monoclonal antibodies for 30 minutes at 4 °C, washed with phosphate buffered saline, and...
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<table>
<thead>
<tr>
<th>Study</th>
<th>No. of Patients</th>
<th>% of Peripheral Blood Lymphocytes</th>
<th>% of CD3/CD20 Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quintanilla-Martinez et al. 7</td>
<td>11</td>
<td>14.4</td>
<td>3.5</td>
</tr>
<tr>
<td>Hultin et al. 6</td>
<td>50</td>
<td>11.6</td>
<td>2.4</td>
</tr>
</tbody>
</table>

* Smaller study of 7 to 18 patients.

RESULTS

Two-color flow cytometric analysis of bone marrow aspirates from 34 consecutive routinely submitted specimens stained with anti-CD20 (Leu16) and anti-CD3 (Leu4) revealed a small subset of T lymphocytes co-expressing CD20 antigen. This represented a mean percentage of 1.77 ± 2.36% (range 0.0%-11.0%) of the total marrow mononuclear cells and 6.54 ± 5.15% (range 0.0%-22.2%) of marrow lymphoid cells. CD20 expression on T cells was less intense than that seen on B cells. However, CD20positive cells in the bone marrow also included CD3-negative immature B cells.

The samples containing CD20-positive T cells were obtained from 21 men and 13 women having a mean age of 52.2 ± 19.4 years (range 20-84 years). Diagnoses included acute lymphoblastic lymphoma (5), acute myelogenous leukemia (5), HIV infection (4), myelodysplastic/myeloproliferative disorder (2), chronic myelogenous leukemia (2), chronic lymphocytic lymphoma (1), multiple myeloma (1), and several cases studied for staging purposes which exhibited a normal cell composition (14). Analysis revealed no correlation between the percentage of CD20-positive T lymphocytes and the CD4:CD8 ratio, patient age, gender, or diagnosis.

DISCUSSION

Analysis of 34 bone marrow specimens revealed 32 (94%) containing a subset of T lymphocytes expressing the CD20 antigen (Fig. 1). CD20 expression on T cells was of similar, dim intensity to that seen on immature B

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**TABLE 1. PERIPHERAL BLOOD LYMPHOCYTES COEXPRESSING CD3/CD20**

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of Patients</th>
<th>% of Peripheral Blood Lymphocytes</th>
<th>% of CD3/CD20 Cells</th>
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<td>50</td>
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</tr>
</tbody>
</table>

* Smaller study of 7 to 18 patients.

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**TABLE 2. T-CELL MALIGNANCIES EXPRESSING CD20 REACTIVITY**

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of Cases Studied</th>
<th>No. Expressing CD20</th>
<th>% Expressing CD20</th>
<th>CD20 Antibody*</th>
<th>Method to Determine Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norton and Isaacson 14</td>
<td>18</td>
<td>1</td>
<td>5.56</td>
<td>L26</td>
<td>TCR, IH-P</td>
</tr>
<tr>
<td>Linder et al. 12</td>
<td>42</td>
<td>1</td>
<td>4.76</td>
<td>L26</td>
<td>IH-F</td>
</tr>
<tr>
<td>Pallesen 13</td>
<td>4</td>
<td>1</td>
<td>25.00</td>
<td>B1</td>
<td>IH-P</td>
</tr>
<tr>
<td>Hamilton-Dutoit and Pallesen 9</td>
<td>50</td>
<td>4</td>
<td>8.00</td>
<td>L26</td>
<td>IH-F</td>
</tr>
<tr>
<td>Warzynski et al. 11</td>
<td>15</td>
<td>4</td>
<td>26.67</td>
<td>L26</td>
<td>M, FC</td>
</tr>
<tr>
<td>Quintanilla-Martinez et al. 7</td>
<td>NA</td>
<td>1</td>
<td>NA</td>
<td>L26</td>
<td>IH-P</td>
</tr>
<tr>
<td>Total</td>
<td>129</td>
<td>13</td>
<td>9.30</td>
<td></td>
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</tbody>
</table>

FC = flow cytometry; IH-F = immunohistochemistry on frozen sections; IH-P = immunohistochemistry on paraffin sections; M = morphology; NA = not applicable; TCR = T-cell receptor gene rearrangement. * Antibody L26 from Dako/Dakopatts, except Linder et al. 12 received antibody L26 from Yoshifumi Ishii (Sapporo, Japan); antibody B1 from Coulter Electronics. † Single case report, data not included in totals.
antilla-Martinez and colleagues confirmed the presence of CD20+, CD38-, CD4-, CD45RA-. In T-cell receptor analysis of the CD20-positive circulating T cells revealed additional study identified a high percentage of T-cell lymphocytes. The T-cell nature of the CD20 coexpressing cells was confirmed by induction of calcium influx after cross-linking the cell surface CD3 antigen. Further three-color analysis of the CD20-positive circulating T cells revealed a resting mature T-cell phenotype: CD8+, CD45RO+, T-cell receptor γ/δ+, CD38–, CD4–, CD45RA–. In addition, the T cells expressing CD20 expressed the pan-T-cell antigens CD2 and CD5. Subsequently, Quin-antilla-Martinez and colleagues confirmed the presence of reactive circulating CD20-positive T cells (Table 1.) Review of the literature also reveals several reports of neoplastic T cells expressing CD20 as determined by immunohistochemistry. These reports include cases classified as pleomorphic medium and large cell lymphoma, immunoblastic lymphoma, anaplastic large cell lymphoma, acute lymphoblastic leukemia, and T-cell/natural killer cell lymphoma (Table 2). Although an additional study identified a high percentage of T-cell lymphomas exhibiting reactivity to the antibody L26, these cases exhibited reactivity of the endothelial cells, macrophages, and epithelial cells. Subsequent review indicates there may have been nonspecific binding of the CD20 antibody. Three cases of T-acute lymphoblastic leukemia and an unusual lymphoma exhibiting natural killer/T lymphocytic immunophenotype are the only cases reported to date on which CD20 expression was detected by flow cytometric analysis. Studies also revealed the neoplastic populations exhibited several T-cell antigens including CD2, CD3, CD4, CD5 and CD7.

The identification of CD20 antigen expression on T cells is reminiscent of the well-recognized normal B-cell subset expressing CD5 antigen. Initially, the CD5 expression on B cells was shown to identify and classify a subset of lymphoproliferative disorders (ie, chronic lymphocytic leukemia/small lymphocytic lymphoma [CLL/SLL]). Subsequent studies revealed a normal B-cell population with the same phenotype. CD5-positive B cells constitute a major B-cell subpopulation in fetal spleen and umbilical cord blood and a smaller subpopulation in adult peripheral blood, spleen, and lymph nodes. However, this population is generally not detectable in the bone marrow. Circulating normal CD5 B cells are increased some disease processes, most notably in autoimmune disease. CD20-positive T cells may represent an analogous population of lymphoid cells and may be identified in a variety of clinical situations. The presence of CD20 on reactive T cells may represent retention of an immature/fetal antigen on mature post-thymic T cells, gain of CD20 by mature post-thymic T cells, or a discrete, small population of T cells with a unique phenotype and possibly a distinct function. CD20-positive T cell malignancies may represent either expansion of the population of normal CD20-positive T cells, re-expression of a fetal antigen, or aberrant expression of CD20 by the neoplastic cells. The purpose of CD20 expression on T cells is unknown. However, recognition of this distinct subset of T cells is essential when interpreting immunophenotypic analysis of peripheral blood and bone marrow specimens. This normal subset of T cells generally comprises a small subpopulation, whereas rare larger populations have been shown to represent neoplastic proliferations. However, it is important to recognize that not all CD20dim cells present in the bone marrow are immature B cells, and that the presence of CD20 expression on T-cell proliferations does not necessarily indicate the presence of malignancy. Certain immunophenotypic panels and patterns may be helpful in delineating this T-cell population. Expression of CD19 on B-cells is highly conserved and is first identified before the expression of CD20. A T-cell population exhibiting CD20 should be suspected if the CD20 population is greater than the CD19 population. Diagnostic difficulties may be resolved by including CD3/CD20 in an analytic panel. High conservation of CD3 on T cells will help enumerate the CD20dim T cells in the specimen. A T-cell population expressing CD20 may be further defined with either two- or three-color
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analysis. CD20-positive T cells typically co-express CD2, CD5, CD7, CD8, CD45RO, and T-cell receptor γ/δ, and lack CD38, CD4, and CD45RA.6-8 The combination of CD5 and CD20 is not recommended for identification of this unusual subset of T cells, because of confusion with CD5 expressing B cells. Therefore, the combination of CD5 and CD19 is recommended for identification of CLL, SLL, or mantle cell lymphoma, and CD3 and CD20 for identification of CD20 expressing T cells (Fig. 1).

In summary, although antibodies against the CD20 antigen are still of value in immunophenotyping studies, care should be used selecting antibody combinations and interpreting the results.

REFERENCES

6. Hultin LE, Hausner MA, Hultin PM, Giogi JV. CD20 (pan-B cell) antigen is expressed at a low level on a subpopulation of human T lymphocytes. Cytometry 1993; 14:196-204.