Shared Idiotype Expression by Chronic Lymphocytic Leukemia and B-Cell Lymphoma

By Malaya Chatterjee, Maurice Barcos, Tin Han, Xilin Liu, Zale Bernstein, and Kenneth A. Foon

Antidiotype (Id) antibodies identify unique determinants within the surface immunoglobulin (Ig) that are present on B-cell tumors. Anti-Ids have been used for diagnosis and therapy of B-cell lymphoma and leukemia. A panel of 29 anti-id monoclonal antibodies (MoAbs) that recognize shared idiotypes (Slds) on B-cell lymphomas was tested for reactivity with both B-cell leukemias and lymphomas. Ten of 40 (25%) cases of chronic lymphocytic leukemia (CLL) reacted with at least one of the 29 anti-Sld MoAbs. Three cases reacted with more than one anti-Sld MoAb, but there was no repetitive pattern of a single anti-Sld MoAb reacting with a large proportion of CLL cases. In contrast, for B-cell lymphomas, in which 11 of 31 (36%) cases reacted, one anti-Sld (B4-1) reacted with five of the positive cases; all were diffuse histology. Restricted anti-Sld reactivity may lead to important insights into the etiology of certain B-cell lymphomas. In addition, these anti-Slds may obviate the need to develop “tailor-made” antibodies for individual patients.

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Table 1. Anti-Sld Reactivity With CLL

<table>
<thead>
<tr>
<th>Patient</th>
<th>Isotype</th>
<th>Anti-Sld</th>
</tr>
</thead>
<tbody>
<tr>
<td>RN</td>
<td>γ, κ</td>
<td>W15-26, W15-82</td>
</tr>
<tr>
<td>HH</td>
<td>γ, κ</td>
<td>W15-26, H48-3</td>
</tr>
<tr>
<td>MS</td>
<td>μ, δ, κ</td>
<td>H48-3</td>
</tr>
<tr>
<td>VS</td>
<td>γ, κ</td>
<td>S2-33</td>
</tr>
<tr>
<td>EM</td>
<td>μ, δ, κ</td>
<td>S2-33, H27-17</td>
</tr>
<tr>
<td>WK</td>
<td>γ, κ</td>
<td>ND (pool 6)</td>
</tr>
<tr>
<td>JL</td>
<td>μ, λ</td>
<td>S66-76</td>
</tr>
<tr>
<td>DG</td>
<td>γ, λ</td>
<td>ND (pool 1)</td>
</tr>
<tr>
<td>AB</td>
<td>μ, δ, λ</td>
<td>S37-48</td>
</tr>
</tbody>
</table>

Ten of 40 (25%) positive.

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determined first by indirect immunofluorescent staining using the nine pools of anti-SId MoAbs and fluorescein-conjugated goat anti-mouse Ig (TAGO) second-step reagent. If a positive pool was identified, the individual components of that pool were tested to determine which of the MoAbs was reactive. In some cases, Id expression was determined by a very sensitive immunoperoxidase staining technique using biotin-streptavidin reagents (ICN Universal Kit, ICN Immunobiologicals, Lisle, IL).

Measurement of serum Id levels. Specific anti-SId MoAbs were diluted to 5 μg/mL in 0.05 mol/L sodium bicarbonate (pH 9.5), and 100 μL MoAb was added per well and incubated overnight at 4°C. The plates were washed five times with buffer. Then 100 μL diluted

Table 2. Anti-SId Reactivity With Lymphoma

<table>
<thead>
<tr>
<th>Patient</th>
<th>Isotype</th>
<th>Anti-SId</th>
<th>Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>MY</td>
<td>κ</td>
<td>B4-1</td>
<td>MLDLg</td>
</tr>
<tr>
<td>MW</td>
<td>λ</td>
<td>B4-1</td>
<td>MLDLg</td>
</tr>
<tr>
<td>MH</td>
<td>γ, κ</td>
<td>B4-1</td>
<td>MLDM</td>
</tr>
<tr>
<td>HW</td>
<td>γ, μ, κ</td>
<td>B4-1</td>
<td>MLSmLy</td>
</tr>
<tr>
<td>DG</td>
<td>=</td>
<td>B4-1</td>
<td>MLSmLy</td>
</tr>
<tr>
<td>ER</td>
<td>γ, κ</td>
<td>S2-33</td>
<td>MLSmCl</td>
</tr>
<tr>
<td>HL</td>
<td>=</td>
<td>S2-33</td>
<td>MLDM</td>
</tr>
<tr>
<td>FW</td>
<td>λ</td>
<td>W15-82</td>
<td>MLDLg</td>
</tr>
<tr>
<td>JB</td>
<td>μ, κ</td>
<td>H27-17</td>
<td>MLSmCl</td>
</tr>
<tr>
<td>LM</td>
<td>γ, λ</td>
<td>S26-16</td>
<td>MLFSmCl</td>
</tr>
<tr>
<td>CB</td>
<td>γ, λ</td>
<td>C52-22</td>
<td>MLFSmCl</td>
</tr>
</tbody>
</table>

MLDLg, malignant lymphoma diffuse large cell; MLDM, malignant lymphoma diffuse mixed cell; MLSmLy, malignant lymphoma, small lymphocytic; MLSmCl, malignant lymphoma, diffuse small cleaved; MLFSmCl, malignant lymphoma follicular small cleaved.

Eleven of 31 (36%) positive.

*(=) Not tested or unable to determine.
buffer [5% Carnation Instant Milk in phosphate-buffered saline (PBS)] was added per well and incubated for 30 minutes at room temperature. The plates were washed five times with buffer, and 100 μL dilution buffer was added per well. Then 100 μL Id standard [previously diluted to 10 μg/mL in 1% PBS-bovine serum albumin (BSA)] was added to one well and diluted serially. Similarly, 100 μL of the patients' serum was added to one well in the next row and was also diluted serially. The plates were then incubated for 1 hour at room temperature and washed five times with buffer. Biotin-labeled anti-Sld was then added per well (100 μL) and incubated for 1 hour at room temperature and washed five times in buffer. Avidin-horseradish peroxidase in dilution buffer was added (100 μL per well) and incubated for 1 hour at room temperature. Finally, the ABTS substrate (100 μL per well) was added and read at 405 nm.

RESULTS

Reactivity of anti-Slds with CLL cells. Forty cases of surface Ig-positive CLL were studied by flow cytometry. Ten cases reacted with the pooled anti-Slds, and in eight cases the specific anti-Sld(s) involved were identified (Table I). Cells were not available to determine the specific anti-Sld in the other two cases. Flow cytometry demonstrating identical patterns of reaction with a goat anti-lg reagent and the

Fig 3. Immunoperoxidase staining of specimens from a patient (M.W., isotype λ) with diffuse lymphoma with the anti-Sld B4-1 (isotype IgM-λ). B4-1 reacted intensely with nearly 100% of the tumor cells in A. No staining with a nonreactive anti-Sld was evident in B.
specific anti-SId suggests that the anti-SId MoAbs bound to all available surface Ig on the positive cells (Fig 1a). In three cases, two different anti-SIds reacted with the same tumor (Fig 2).

Reactivity of anti-SIds with B-cell lymphoma. A variety of histologic subtypes of B-cell lymphomas were studied by immunoperoxidase staining and flow cytometry against the panel of 47 anti-SId antibodies. Eleven of 31 cases reacted with at least one of these anti-SId MoAbs. Table 2 summarizes the positive cases.

Five of the 11 positive cases reacted with the same anti-SId MoAb, B4-1; all five cases were diffuse lymphoma with three morphologic subtypes. Immunoperoxidase staining pattern from one of these five cases is shown in Fig 3. The anti-SId B4-1 reacted intensely with almost 100% of the tumor cells, whereas there was no staining with other anti-SIds tested. The reactivity pattern of B4-1 with tumor cells from four other patients was very similar.

Id serum levels. Twelve SId-positive patients (nine CLL, three non-Hodgkin’s lymphoma) were tested for circulating Id levels in their serum (Fig 4). Serum levels ranged from 0.2 to 2,900 µg/mL with a median level of 0.6 µg/mL. The patient with 2,900 µg/mL also had a monoclonal spike on serum protein electrophoresis. Only two patients had levels greater than 50 µg/mL. For the remaining patients, the serum Id levels were almost all within a few micrograms of the level of normal controls in the primary screening assay.21

DISCUSSION

Miller et al21 devised a screening assay to select anti-SId MoAbs that reacted with a small fraction of normal serum Ig. They screened 199 anti-Ids cross-reacted with some of these anti-SIds. Table 2 summarizes the positive cases.

Fifteen of the 11 positive cases reacted with the same anti-SId MoAb, B4-1; all five cases were diffuse lymphoma with three morphologic subtypes. Immunoperoxidase staining pattern from one of these five cases is shown in Fig 3. The anti-SId B4-1 reacted intensely with almost 100% of the tumor cells, whereas there was no staining with other anti-SIds tested. The reactivity pattern of B4-1 with tumor cells from four other patients was very similar.

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Fig 4. Serum levels of Id in SId-positive patients.
SHARED IDIOTYPES IN CLL AND B-CELL LYMPHOMA

would have reacted if the anti-Ids were raised against the Ig from CLL cells. Nonetheless, the results of present study together with the data obtained by Miller et al. demonstrate the feasibility of these anti-SId panels in possible diagnosis and therapy of CLL and B-cell lymphomas. At least one third of the B-cell lymphoma patients and one fourth of CLL patients potentially could be treated with anti-SId MoAbs.

To be a candidate for treatment with anti-SId MoAb, a patient must have less than 50 μg/mL circulating Id in the serum or the murine anti-SId will be bound in the "blood" and may not be available to penetrate the tumor. Less than 50 μg/mL may even be too high for successful targeting to tumor cells. Determining the critical level of circulating Id will be one of the objectives of this clinical trial. We determined the serum Id level in 12 SId-positive CLL and diffuse lymphoma patients. Only two patients had serum levels greater than 50 μg/ml, making them unsuitable for initiating anti-SId therapy.

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REFERENCES

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