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 idiotypic (Sld) 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 lymphoma, 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>
<thead>
<tr>
<th>Table 1. Anti-Sld Reactivity With CLL</th>
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<tr>
<td>Patient</td>
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<tr>
<td>RN</td>
</tr>
<tr>
<td>HH</td>
</tr>
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<td>MS</td>
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<td>VS</td>
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<td>JL</td>
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<tr>
<td>DG</td>
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<td>AB</td>
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</table>

Ten of 40 (25%) positive.

**IMMUNOGLOBULIN (Ig) molecules possess variable regions specific for antigen recognition.** The variable region is encoded by V\(_{\text{H}}\), D, and J\(_{\text{H}}\) genes for the heavy chains and V\(_{\text{L}}\) and J\(_{\text{L}}\) chains for the light chain.¹ The variable regions contain determinants known as idiotypes (Ids) which are themselves immunogenic. Antibodies can be made to many structures in the variable region associated with the light chain, heavy chain, or a combination of both chains.²³ These individual determinants show a continuum of specificity from more or less private to semipublic⁵; e.g., if different antibodies are coded by the same V\(_{\text{H}}\) gene segment, a semipublic Id may be found. Malignant B cells, like their normal counterparts, express Id determinants which may be used for antibody-targeted therapy.⁶⁷

The utility of anti-Id antibodies as therapeutic agents for B-cell tumors has been demonstrated in animal models.⁸¹⁹ In early clinical trials, antitumor effects were reproducibly demonstrated.¹⁵⁻²⁰ Some anti-Id monoclonal antibodies (MoAbs) made against an individual patient's tumor cells were recently shown to cross-react with more than one patient's tumor cells. A panel of 29 anti-shared Id (Sld) MoAbs that reacted with approximately one third of cases of B-cell lymphoma has been identified.¹³ These anti-Sld MoAbs also detected rare normal B cells and minor components of serum Ig. In this study, using this same panel of anti-Sld MoAbs, we extended previous observations to other B-cell malignancies such as chronic lymphocytic leukemia (CLL) and diffuse B-cell lymphomas. Development of such anti-Sld MoAb reagents would facilitate application of anti-Id MoAbs for diagnosis and therapy of B-cell leukemia/lymphoma.

**MATERIALS AND METHODS**

Selection of anti-Id MoAbs reactive with Slds. Anti-Sld MoAbs were provided by IDEC Pharmaceutical (Mountainview, CA). The original anti-Id MoAbs were generated as previously described.¹⁸ The selection of anti-Ids with reactivity with multiple tumors was described in detail previously²¹ and is briefly summarized. A primary screen was used to identify anti-Id MoAbs that showed detectable binding to normal human serum Ig. Anti-Ids were diluted serially and mixed with either undiluted normal human serum pooled from 40 donors or horse serum as a control.²² After 1 hour, samples were added to a microtiter plate previously coated with tumor Id and incubated for an additional hour. Plates were washed, and horseradish peroxidase-conjugated goat anti-mouse IgG (TAGO, Burlingame, CA) was added. Bound anti-Id was measured by adding ABTS-hydrogen peroxide substrate, and the absorbance was measured at 405 nm by an automated enzyme-linked immunosorbent assay (ELISA) plate reader. Binding to normal human serum Ig was considered significant when there was a fourfold or greater inhibition of anti-Id reactivity with the tumor Ig by pooled human serum as compared with control horse serum. From a pool of 199 anti-Id MoAbs, 47 were determined to react with normal human Ig. Twenty-nine of these 47 MoAbs cross-reacted with lymphomas from multiple patients. These antibodies were mixed together in groups to form nine different pools.

Sld expression on B-cell lymphoma and CLL cells. Tumor specimens were obtained from patients with a diagnosis of CLL or B-cell lymphoma. The pools of anti-Sld MoAbs were initially screened by either flow cytometry or immunoperoxidase staining of fresh-frozen cryostat sections or cell suspensions. Surface Ig expression was determined by staining with fluorescein-conjugated goat anti-Ig heavy or light chain reagents (TAGO). Id expression was

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Fluorescence

**Measurement of serum Id levels.** Specific anti-Id 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-Id Reactivity With Lymphoma**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Isotype*</th>
<th>Anti-Id</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>MLDSmCl</td>
</tr>
<tr>
<td>HL</td>
<td>=</td>
<td>S2-33</td>
<td>MLDSmCl</td>
</tr>
<tr>
<td>FW</td>
<td>λ</td>
<td>W15-82</td>
<td>MLDSmLg</td>
</tr>
<tr>
<td>JB</td>
<td>μ, κ</td>
<td>H27-17</td>
<td>MLDSmCl</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; MLDSmCl, 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 Ig 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-Ig 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-κ). 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 normal serum Ig. These 47 anti-Ids were then screened against B-cell tumors. Forty-seven of these 199 anti-Ids cross-reacted with normal serum Ig. These 47 anti-Ids were then screened against B-cell tumors, primarily malignant lymphoma, follicular small cleaved cell. Twenty-nine of the 47 anti-Ids cross-reacted with some of these B-cell lymphomas.21

In this study, we used the same panel of anti-SId MoAbs to study patients with CLL and primarily diffuse B-cell lymphoma. The overall percentage of reactivity (36%) in this group of 31 lymphoma patients was very similar to that reported by Miller et al21 for follicular lymphoma (33%). Twenty-five percent of the CLL patients showed reactivity against at least one of the anti-SId MoAbs in this panel. There appeared to be no consistent pattern of anti-SId reactivity, although three different anti-SIds reacted with cells of two different patients. In contrast, for B-cell lymphoma, there was a repetitive pattern of anti-SId reactivity with the B4-1 MoAb that reacted with 45% of the positive lymphomas. These lymphomas were all diffuse and expressed a variety of phenotypes, suggesting that this reactivity was not isotypic or allotypic. This will require confirmation on a larger panel of diffuse lymphomas. Nevertheless, the data suggest that diffuse lymphomas may have restricted anti-SId reactivity. In a recent study using this same panel of anti-SIds, paraproteins from 42 individuals with multiple myeloma and monoclonal gammapathy of undetermined significance were tested.22 Thirteen individuals were positive and one anti-SId (S30-47) reacted with 60% of the positive myeloma paraproteins.

Miller et al21 did not find a high frequency of individual anti-SId reactivity with follicular lymphomas as we found with the B4-1 MoAb in diffuse lymphomas. This might be attributable in part to an active somatic mutational process in follicular lymphoma23 that normally generates Ig diversity in B lymphocytes. Expression of V, and V, gene may be less diverse in diffuse lymphoma, and the shared idiotypes may be more constant and less subject to mutational drift. To address this issue, further studies on the V region sequences within the cross-reactive tumor populations will be important.

A high proportion of individuals with CLL react with an anti-Id MoAb raised against an IgM rheumatoid factor cryoglobulin that recognizes a λ light chain cross-reactive Id.24 Twenty-five percent of CLL cases expressing λ light chain were shown to react with this MoAb. Kipps et al showed that this high frequency is related to a conserved variable region (V-gene) of the λ IIIb sub-subgroup25; they also demonstrated that 20% of the CLL cases they studied reacted with an anti-Id MoAb that recognized an antibody heavy chain-associated cross-reactive Id present on rheumatoid factor paraprotein.26 We did not find a similar repetitive pattern with CLL with any of the anti-SIds from our panel. We would not exclude this possibility, however, until a much larger number of individuals with CLL are screened.

Our findings also indicate that CLL cells express SIds, but at a lower frequency than that observed for lymphomas. The anti-SId panel we used was generated primarily against follicular lymphoma. Perhaps a higher percentage of CLL
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-Id 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-Id MoAbs.

To be a candidate for treatment with anti-Id MoAb, a patient must have less than 50 μg/mL circulating Id in the serum or the murine anti-Id 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 Id-positive CLL and diffuse lymphoma patients. Only two patients had serum levels greater than 50 μg/mL, making them unsuitable for initiating anti-Id therapy.

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