RAPID COMMUNICATION

Expression of Shared Idiotypes by Paraproteins From Patients With Multiple Myeloma and Monoclonal Gammopathy of Undetermined Significance

By James R. Berenson, Alan Lichtenstein, Sarah Hart, David Palomares, and Richard A. Miller

Twenty-nine murine monoclonal antibodies have been produced that react with shared idiotypes expressed by B-cell lymphomas and leukemias. We tested this panel of antibodies for reactivity with the paraproteins from 32 patients with multiple myeloma and 10 patients with monoclonal gammopathy of undetermined significance (MGUS). Thirteen of 42 paraproteins reacted with at least one antibody in this panel of anti-idiotypic antibodies. Six different anti-idiotypic determinants demonstrated reactivity with the paraproteins. A similar frequency of reactivity was found for both myeloma and MGUS proteins. One antibody, S30-47, reacted with 6 of 32 (19%) of the paraproteins from patients with multiple myeloma, whereas this anti-idiotypic only bound to 3% of non-Hodgkin’s B-cell lymphomas and no cases of chronic lymphocytic leukemia. This anti-idiotypic reacted with both components of a biphenotypic paraprotein (IgG λ and IgG κ) in one patient. In each of nine patients tested, plasma cells isolated from bone marrow were shown to be reactive with the same anti-idiotypic we found to react with the paraprotein. Anti-shared idiotypic antibodies may provide useful reagents for studies of patients with monoclonal gammopathies.

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MATERIALS AND METHODS

Patients. Thirty-two patients with multiple myeloma were studied who fulfilled the diagnostic criteria of the Chronic Leukemia-Myeloma Task Force. Ten patients with MGUS also were evaluated and have been followed for 3 to 9 years without increases in their serum paraprotein levels. This study was approved by Human Subject Protection Committees at both UCLA and the VA Medical Center West Los Angeles.

Preparation of anti-idiotypic antibodies. Id proteins were obtained from specimens of B-cell lymphomas according to previously described procedures using a heterohybridoma fusion technique. Purified Id obtained from B-cell tumors was used to immunize either mice or rats as previously described. Spleen cells were fused with the nonspecific mouse myeloma line SP2/0 Ag 14, and hybridoma supernatants were screened for anti-Id reactivity. Anti-Ids were selected based on reactivity with the patient’s Id but not with a panel of other antibodies, some of which were isotype matched. Anti-Ids reactive with shared Ids were found by using normal human serum to inhibit binding of anti-Id to the Id as previously described. Anti-Ids that reacted with low levels of serum Ig were tested for reactivity with lymphoma biopsy specimens. Antibodies reactive with tumors from more than one patient detected shared idiotypes. Twenty-nine out of a total of 43 anti-Ids identified using these techniques were used in the studies reported here. We have previously reported on the frequency of anti-shared Id reactivity with B-cell lymphomas and leukemias. These results have now been extended to evaluate 306 cases of B-cell lymphoma and 61 cases of chronic lymphocytic leukemia (CLL). The frequency of anti-shared Id reactivity with paraproteins is compared with the results obtained with B-cell lymphomas and leukemias using the panel of 29 anti-Ids.

Detection of shared Ids expressed by paraproteins from patients with myeloma and MGUS. Sera from patients or normal donors were diluted 1:1,000 and further serially diluted in a blocking solution containing 5% Carnation instant milk (Carnation Co, Los Angeles, CA) in phosphate-buffered saline. This large dilution was performed to reduce the concentration of normal polyclonal Ig in the serum samples. Diluted sera from normal donors were tested in parallel with patients in these assays and showed no reactivity with any of the anti-shared Id antibodies. The diluted serum was incubated in the wells of microtiter plates coated with either goat

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F(ab')₂, anti-human Ig κ light chain reagents (Tago, Burlingame, CA) for κ paraproteins or anti-human Ig λ light chains (Tago) for λ paraproteins. The wells were washed, and the murine monoclonal anti-shared Ids were added. After incubation and washing, horseradish peroxidase conjugated goat anti-mouse IgG (Tago) was added to the wells. Bound antibody was detected by washing and adding 2,2'-AZINO-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) substrate. Optical density (OD) was measured using a microtiter plate reader. A paraprotein was considered positive for reactivity with an anti-shared Id if the OD at a 1:1,000 dilution was greater than three times that seen with normal serum. Nonreactive anti-shared Ids served as additional negative controls.

Shared Id expression by bone marrow plasma cells. Mononuclear cells were isolated from the bone marrow aspirates of nine patients by centrifugation through Histopaque 1077 (Pharmacia, Piscataway, NJ). Mononuclear cells were deposited on slides using a cytocentrifuge. The specimens were incubated with either the anti-shared Id that had been shown to react with the patient's paraprotein, or other anti-Ids that were nonreactive with the paraprotein. After washing, the slides were incubated with a biotinylated rabbit anti-mouse Ig reagent (Tago). After additional washing, an alkaline phosphatase conjugated avidin (Tago) was added. The substrate naphthol AS-MX phosphate mixed with Fast Red TR salt (Sigma, St Louis, MO) was added for color development. The slide served as additional negative controls.

Detection of shared Ids in myeloma and MGUS sera. Paraproteins from 13 of 42 patients reacted with anti-shared Id antibodies previously generated against B-cell lymphomas. The reactive paraproteins contained a variety of light and heavy chain types (Table 1). IgG κ proteins from 4 of 21 patients demonstrated reactivity. Five of 11 patients with IgG λ paraproteins showed reactivity with the antibody panel. In one patient with a biphenotypic paraprotein, both the IgG κ and IgG λ paraproteins showed reactivity with the same anti-Id (Tables 1 and 2). One patient each with IgA κ, IgA λ, and IgM κ paraproteins reacted with anti-shared Id antibodies. Overall, one third (10 of 32) of myeloma paraproteins reacted with the anti-shared Ids. No differences were found in the age of patients, sex, stage, or response to therapy between patients with or without reactivity to anti-shared idiotypes. A similar fraction (3 of 10) of paraproteins from patients with MGUS showed reactivity.

Six of the panel of 29 monoclonal anti-Ids reacted with these M-proteins (Table 2). One antibody, S30-47, reacted with paraproteins from six cases of myeloma, including the patient with biphenotypic disease and one patient with MGUS. These patients had paraproteins that contained a variety of IgG heavy chain isotypes and either κ or λ light chains. Two myeloma patients' paraproteins reacted with the J18-76 antibody. Interestingly, proteins from 2 of the 3 MGUS patients demonstrated reactivity with anti-Ids (C31-145 and S26-16) that did not react with any of the myeloma M-proteins.

We compared the frequencies of anti-Id reactivity with paraproteins compared to their reactivity with the Ids expressed by B-cell lymphoma and CLL (Table 3). The overall reactivity of the anti-shared Ids with these three malignancies (31% to 39%) did not differ. However, particular antibodies reacted with each of the types of B-cell tumors at different frequencies. Most notable was S30-47, which

### Table 2. Anti-Shared Ids Reactive With Paraproteins

<table>
<thead>
<tr>
<th>MAB*</th>
<th>Patients Reactive</th>
<th>Isotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>S30-47</td>
<td>7 (6 MM† and 1 MGUS‡)</td>
<td>1 IgG1 λ</td>
</tr>
<tr>
<td>J18-76</td>
<td>2 (2 MM)</td>
<td>1 IgG1 λ</td>
</tr>
<tr>
<td>H101-2</td>
<td>1 (1 MM)</td>
<td>IgA κ</td>
</tr>
<tr>
<td>H-55-4</td>
<td>1 (1 MM)</td>
<td>IgGλ</td>
</tr>
<tr>
<td>C31-145</td>
<td>1 (1 MGUS)</td>
<td>IgM κ</td>
</tr>
<tr>
<td>S26-16</td>
<td>1 (1 MGUS)</td>
<td>IgGλ</td>
</tr>
</tbody>
</table>

* MAB, murine monoclonal anti-shared Id antibody.
† MM, multiple myeloma.
‡ MGUS, monoclonal gammopathy of undetermined significance.
§ IgG not determined.

### Table 3. Reactivity of Anti-Shared Id With Different B-Cell Malignancies

<table>
<thead>
<tr>
<th>MAB*</th>
<th>MM†</th>
<th>NHL‡</th>
<th>CLL§</th>
</tr>
</thead>
<tbody>
<tr>
<td>S30-47</td>
<td>19%</td>
<td>3%</td>
<td>0%</td>
</tr>
<tr>
<td>J18-76</td>
<td>6</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>H-55-4</td>
<td>3</td>
<td>0.4</td>
<td>3</td>
</tr>
<tr>
<td>H101-2</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S37-48</td>
<td>0</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>C33-13</td>
<td>0</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>C45-23</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>C52-22</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>0</td>
<td>20</td>
<td>21</td>
</tr>
</tbody>
</table>

* MAB, anti-shared Id antibody.
† MM, 32 multiple myelomas.
‡ NHL, 306 non-Hodgkin's lymphomas.
§ CLL, 61 chronic lymphocytic leukemias.
| Others, includes 21 other anti-Ids that reacted with lymphomas and nine other anti-Ids that reacted with CLL samples.
reacted with 19% of myeloma but only 3% and 0% of lymphoma and CLL, respectively. Many anti-Ids that were reactive with the lymphoma and/or CLL samples did not show reactivity with any myeloma paraprotein. Thus, a different pattern of reactivity emerged with our myelomas than the other two B-cell tumors.

Demonstration of shared Id expression by the bone marrow plasma cells. In each of nine cases studied, the bone marrow plasma cells showed marked reactivity with the same anti-Id that was found to be reactive with the patient’s paraprotein. Plasma cells and approximately 80% of the lymphoid cells in these patients’ marrow specimens were reactive with the anti-Ids. Figure 1 shows the results of immunocytochemical staining using the H55-4 anti-Id on bone marrow cells from a patient with IgG λ myeloma. The patient’s plasma cells show staining with this antibody, which also reacted with the patient’s paraprotein. In Fig 2, bone marrow plasma cells and lymphocytes from a patient with IgG3 κ myeloma showed marked staining with S30-47. Bone marrow from two patients with MGUS were tested for reactivity with anti-Ids that reacted with their paraprotein. In these two cases, greater than 90% of the plasma cells reacted with the anti-Id.

DISCUSSION

Shared Ids have been described in nonmalignant B-cell disorders, including systemic lupus erythematosus (SLE), rheumatoid arthritis, mixed cryoglobulinemia, and IgA nephropathy, as well as in a number of B-cell malignancies. Davidson et al showed that an anti-Id which recognized a determinant on κ chains of anti-DNA antibodies in SLE patients also reacted with over 10% of myelomas. Recently, Miller et al demonstrated that a high proportion of B-cell lymphomas express shared Ids using a panel of anti-Ids generated against follicular lymphomas. Using these anti-shared idiotype antibodies, we have demonstrated that one third of myeloma and MGUS paraproteins react with anti-Id antibodies that were generated against low-grade follicular B-cell lymphomas.

Two antibodies known as 17.109 and G6 were generated against rheumatoid factors and react with the cells of up to 20% of patients with CLL. The frequent occurrence of Ids reacting with these two anti-Id antibodies has been shown to be the result of the expression of a few Ig germ line V genes. The G6 and 17.109 antibodies show reactivity much less frequently with B-cell lymphomas. In previous studies, B-cell lymphomas were shown to express shared Ids in about one third of cases. However, the frequency of reactivity with particular anti-Ids in lymphoma is lower than that observed in CLL. In our current study of monoclonal gammopathies, we find that one anti-Id, S30-47, reacts with a high proportion (17%) of paraproteins. It is likely that this anti-Id is directed against an Ig heavy chain determinant because it reacts with both the IgG κ and IgG λ paraproteins in our patient with biphenotypic myeloma. The frequency of reactivity of S30-47 with B-cell lymphomas (3.6%) was less than that with the monoclonal gammopathies; however it was one of the most frequently reactive anti-Ids with B-cell lymphomas. Overall, different antibody reactivity was seen with myeloma, CLL, and lymphomas. These results suggest that these B-cell lineage malignancies evolve from different B-cell subsets or are potentially driven by different antigenic stimuli. In support of this, it is known that CLL and B-cell lymphoma use different Vκ genes preferentially. In addition, B-cell lymphomas have an active Ig somatic mutation process, whereas CLL does not exhibit hypermutation. In murine plasmacytomas, frequent somatic mutation occurs. To determine which V genes are used and the rate of somatic mutation, it will be necessary to sequence the V genes expressed by the plasma cells in patients with myeloma.

There is some evidence suggesting that myeloma may originate from an earlier cell in B-cell differentiation than the plasma cell. The S30-47 reacted with both the IgG κ and IgG λ paraprotein components in a patient with biphenotypic myeloma. This suggests that in this patient the tumor is clonal and that the original malignant event occurred in a cell that had not yet undergone light chain rearrangement. Alternatively, a malignant cell may have sustained a mutation that rendered the κ chain gene nonproductive, leading to the productive rearrangement of the λ chain gene.

The frequency of shared Id expression in monoclonal gammopathies of both the malignant form, multiple myeloma, and the relatively benign state, MGUS, appeared to be similar. In some cases, the same anti-Id reacted with both myeloma and MGUS. Two monoclonal anti-Ids reacted only with MGUS patients. It will require a larger series of patients to determine if the shared Ids detected by these two anti-Ids are restricted to MGUS paraproteins. The existence of such Ids that are shared by both myelomas and B-cell lymphomas suggests that some of these tumors may use the same V genes. In CLL which is a malignancy of CD5+ B cells, limited V gene usage is thought to represent the restricted repertoire of CD5+ cells that are used during early B-cell ontogeny. In support of this, it is known that CD5+ cells constitute the predominant B-cell population in human fetal liver and spleen. Some malignant cells in multiple myeloma also have been shown to express CD5.

We found that bone marrow plasma cells isolated from patients reacted with the same anti-Id that bound to their paraproteins. In preliminary studies, we have seen similar reactivity with a subpopulation of peripheral blood lymphocytes from patients with myeloma (unpublished observations, January 1990). These anti-Ids provide an opportunity to identify and isolate the malignant cells from the blood as well as the marrow. The identification of Id-bearing plasma cells in the normal-appearing marrow of MGUS patients suggests that these cells are responsible for production of the paraprotein in this disorder.

In this study, the anti-shared Ids that bind to the myeloma and MGUS paraproteins were prepared against follicular lymphomas. By producing antibodies against myeloma or MGUS paraproteins directly, one may be able to identify anti-Ids with more frequent reactivity with paraproteins. It is possible that the B-cell tumors expressing shared Ids represent a distinct subset of these malignancies with a distinct pathophysiology and clinical course.

Anti-shared Id antibodies should facilitate studies of the biology of B-cell malignancies, including multiple myeloma.
Fig 1. Immunocytochemical staining of a cytocentrifuge specimen from a bone marrow of a patient with IgG1 κ myeloma. The cells were stained with H55-4. Cells are shown both at low (×100) magnification (A) and higher (×400) magnification (B). Many cells react with the anti-id and under higher magnification these reactive cells are shown to be plasma cells. Fig 2. (bottom left) Immunocytochemical staining of a cytocentrifuge specimen from a bone marrow of a patient with IgG3 λ myeloma. The cells were stained with S30-47, and are shown at high magnification (×500). The cells that stain with the anti-id are plasma and lymphoid cells.
These antibodies may be used to investigate relationships between myeloma and MGUS. Using the techniques described in this report, these reagents also may find a valuable role in diagnosis and/or monitoring of monoclonal gammopathies.

REFERENCES

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