CONCISE REPORT

The Presence of Monoclonal Cytoplasmic Immunoglobulins in Leukemic B Cells from Patients with Chronic Lymphocytic Leukemia

By Tin Han, Howard Ozer, Marvin Bloom, Kimitaka Sagawa, and Jun Minowada

It has generally been assumed that CLL B cells do not contain cytoplasmic immunoglobulin (CyIg), although these cells express surface membrane immunoglobulin (SmIg). The present study, in which SmIg and CyIg determinations were performed simultaneously using leukemic B cells from 20 patients with CLL, demonstrates that CyIg staining was detectable in each CLL cell population examined and that the intensity of cytoplasmic immunofluorescent staining in each instance was moderate to strong. Both SmIg and CyIg light chain determinations indicated monoclonality in all 20 cases. The heavy chain class of SmIg and CyIg in each CLL cell population was not uniformly comparable, however. The majority (15 cases) of CLL B cells contained χ heavy chain CyIg and μ and δ heavy chain CyIg was demonstrated in the remaining 6 cases. In contrast, the SmIg phenotype appeared heterogeneous with both γ and α chain determinants found associated with μ or μ and δ chains on the same leukemic cell populations. This apparent polyclonal SmIg staining pattern was most likely due to nonspecific adsorption of the patient's own serum Ig by Fc receptors on CLL B cells. It is concluded that the great majority of CLL B cells contain detectable CyIg and that CyIg determination is superior to SmIg phenotyping in documenting the monoclonality of CLL.

MATERIALS AND METHODS

Peripheral blood mononuclear cells from patients with histologically diagnosed CLL were isolated by a Ficoll-Hypaque gradient centrifugation technique as previously described. SmIg and CyIg determinations of CLL B cells were performed simultaneously using direct immunofluorescent staining with FITC-conjugated goat antibody to human immunoglobulins (Cappel Lab., Cochranville, Pa.).

The FITC-conjugated goat anti-human immunoglobulin chain-specific reagents were carefully standardized for their specificity in the following way: following reconstitution with the appropriate amount of phosphate-buffered saline (pH 7.2), the reagents were first absorbed with rabbit liver powder (10 mg/ml) (Difco, Cleveland, Ohio) at 4°C for 1 hr. The absorbed reagents were centrifuged at 100,000 g for 1 hr and serial twofold dilutions were prepared. Combinations of selected permanent B-lymphoblastoid cell lines of known immunoglobulin isotypes were used to establish specificity and antibody titer. The antibody titer was defined as the reciprocal of highest reagent dilution giving positive staining of a phenotypically defined lymphoblastoid line in direct immunofluorescence assays for either SmIg or CyIg. For use in the leukemic cell assays, the reagent was prepared at a concentration 4 times higher than the maximum antibody titer. For SmIg staining, 2–3 × 10^6 isolated mononuclear cells in 25 μl were incubated at room temperature with 25 μl of the reagent for 30 min. The cells were washed twice with PBS and were mounted with a drop of 50% (v/v) glycerin in PBS on a microscope slide.

From the Departments of Medical Oncology and Immunology Research, Roswell Park Memorial Institute, New York State Department of Health, Buffalo, N.Y.; and the School of Medicine, State University of New York at Buffalo, Buffalo, N.Y.

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Address reprint requests to Dr. Tin Han, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, N.Y. 14263.

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a microscope slide with a cover slip. For CyIg staining, the isolated
mononuclear cells were smeared on a cover slip and air dried
immediately, followed by acetone fixation at room temperature for 5
min. Direct immunofluorescent staining of the fixed smears was
done with 25 μl of the standardized reagent in a moist chamber at
room temperature for 30 min. The smears were washed individually
in Petri dishes with a large volume of PBS for 10 min at room
temperature with constant lateral rotation. The smears were then
mounted cell-side down with a drop of 50% glycerin in PBS on a
microscope slide. Both SmIg and CyIg preparations were read at
1000× magnification with oil immersion using a Leitz Ortholux II
equipped with Plomepak 2 epi-illumination of u.v. light. A narrow
blue band excitation filter module type I was used to reduce
background autofluorescence.

Twenty patients were studied, of which 15 were males and 5 were
females. The majority of patients were over 60 yr of age. Clinical
staging41 indicated that the patients were well distributed among the
various stages (stage 0, 5; stage I, 1, stage II, 8; stage III, 5; stage
IV, 1 patient). Lymphocytosis within the patient population ranged
from 20,000 to 144,000/cu mm. Fifteen patients were previously
untreated, and the remaining 5 patients were previously treated at
the time of the study.

RESULTS AND DISCUSSION

It has generally been assumed that CyIg is absent in
CLL cells. We found only one recently published
report by Koziner et al. showing the absence of CyIg
in SmIg-positive leukemic cells from 50 patients with
CLL.6 We also encountered the statement made by
Stein et al.4 that CyIg was consistently absent in CLL
cells, although no detailed methodology or results were
given in their report. Our data showing comparative
results of SmIg- and CyIg-positive cells in patients
with CLL are shown in Table 1. It has been previously
reported that, in the majority of CLL patients, the
intensity of the positive immunofluorescent reaction
for SmIg is regarded as weak or moderate.3 In
contrast, in the present study, the intensity of the
immunofluorescent reaction for SmIg in each case was
moderate to strong, as shown in Fig. 1A. Unexpectedly,
CyIg-staining was also present in each of the 20
CLL cell populations examined, and the intensity of
cytoplasmic immunofluorescent staining in each
instance was moderately strong, as shown in Fig. 1B.

A comparison of the results of SmIg light chain
determination with CyIg determination for identical
demographic is shown in Table 1 and indicates monoclonal-
ity in all 20 cases. It is generally accepted that the
monoclonality represents the proliferative expansion
of the neoplastic clone of B cells arising from a single
cell. The results of heavy chain phenotyping for both
SmIg and CyIg of each cell population were not
uniformly comparable. The majority (32%-100%) of
CLL B cells expressed μ-type heavy chain SmIg. A
significant number (more than 20%) of leukemic cells
carried δ-type heavy chain SmIg (14 cases), and γ-type
heavy chain SmIg (11 cases), and α-type heavy
chain SmIg (3 cases). These observations suggest that
the CLL B cells not infrequently demonstrate polyclonal
SmIg staining patterns. Similar polyclonal
SmIg staining patterns in CLL have previously been
reported.7 In contrast, the majority (40%-100%) of
CLL B cells expressed μ heavy chain CyIg alone (15
cases) or μ heavy chains associated with δ heavy
chain CyIg in the remaining 5 cases (nos. 1, 8, 13, 15, and

<table>
<thead>
<tr>
<th>No.</th>
<th>Stage</th>
<th>SmIg-Positive Cells (%)</th>
<th>CyIg-Positive Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ϵ  λ  α  γ  δ  μ</td>
<td>ϵ  λ  α  γ  δ  μ</td>
</tr>
<tr>
<td>1.</td>
<td>III</td>
<td>95  0  50  95  82</td>
<td>&lt;1  95  0  90  95</td>
</tr>
<tr>
<td>2.</td>
<td>0</td>
<td>58  4  63  15  41</td>
<td>80  0  2  0  0  70</td>
</tr>
<tr>
<td>3.</td>
<td>III</td>
<td>71  19  39  50  44</td>
<td>50  0  0  0  50  50</td>
</tr>
<tr>
<td>4.</td>
<td>II</td>
<td>6  69  53  5  83</td>
<td>0  10  0  1  0  70</td>
</tr>
<tr>
<td>5.</td>
<td>II</td>
<td>6  83  66  79  83</td>
<td>20  80  10  15 &lt;1  95</td>
</tr>
<tr>
<td>6.</td>
<td>III</td>
<td>58  4  5  20  74</td>
<td>90  2  2  0  0  70</td>
</tr>
<tr>
<td>7.</td>
<td>0</td>
<td>50  0  2  24  32</td>
<td>80 &lt;1  0  5  30  95</td>
</tr>
<tr>
<td>8.</td>
<td>0</td>
<td>60  0  0  0  60</td>
<td>50  5  10  1  0  75</td>
</tr>
<tr>
<td>9.</td>
<td>II</td>
<td>2  80  6  70  95</td>
<td>0  60  0  10  0  80</td>
</tr>
<tr>
<td>10.</td>
<td>II</td>
<td>58  4  10  20  76</td>
<td>100  0  0  0  100 100</td>
</tr>
<tr>
<td>11.</td>
<td>III</td>
<td>58  4  10  20  76</td>
<td>100  0  0  0  100 100</td>
</tr>
<tr>
<td>12.</td>
<td>III</td>
<td>0  95  0  79  85</td>
<td>0  100  0  15  50  100</td>
</tr>
<tr>
<td>13.</td>
<td>II</td>
<td>8  69  51  76  100</td>
<td>0  100  0  10  0  90</td>
</tr>
<tr>
<td>14.</td>
<td>IV</td>
<td>77  0  18  56  63</td>
<td>100  0  0  0  50  50</td>
</tr>
<tr>
<td>15.</td>
<td>0</td>
<td>1  52  0  29  55  60</td>
<td>0  60  0  0  30  40</td>
</tr>
<tr>
<td>16.</td>
<td>II</td>
<td>53  0  32  24  41</td>
<td>70  10  0  0  0  80</td>
</tr>
<tr>
<td>17.</td>
<td>II</td>
<td>17  42  56  58  80</td>
<td>0  70  0  0  0  80</td>
</tr>
<tr>
<td>18.</td>
<td>0</td>
<td>90 &lt;1  43  56  95</td>
<td>90  0  0  0  0  90</td>
</tr>
<tr>
<td>19.</td>
<td>II</td>
<td>93  0  17  23  81</td>
<td>60  0  0  0  0  70</td>
</tr>
</tbody>
</table>
Observations that the CLL B cells from some patients may express two heavy chains, e.g., \( \mu \) and \( \delta \), SmIg or CyIg still qualify for monoclonality on the basis of a sequential switch in the production of heavy chain Ig controlled at a gene level (C region genes). Of interest is the fact that a significant number of CLL B cells also expressed \( \gamma \)-type heavy chain SmIg (nos. 1–5, 11, 14, 16–19) and \( \alpha \)-type heavy chain SmIg (3, 5, and 14). These polyclonal staining patterns of SmIg are most likely the result of nonspecific adsorption of autologous serum Ig by Fc receptors on CLL B cells. Incidentally, the presence of strong Fc receptors on the CLL B cells is a common feature. This nonspecific adsorption of Ig can be eliminated by enzymatic stripping or incubation of the cells at 37°C for 2–24 hr, during which time nonspecifically bound Ig will be shed from the cell surface Fc receptors. Intrinsic SmIg will be unaffected or will be resynthesized. Use of F(ab')_2 antibody fragments will additionally avoid much of the problem caused by nonspecific binding of the staining antibodies.

It has previously been recognized that the leukemic CLL lymphocytes that are morphologically characteristic of the disease but which fail to express SmIg may frequently represent a significant problem in patient phenotyping. Koziner et al. recently reported that SmIg could not be visualized on CLL cell membranes in 21 of 71 patients with histologically typical CLL. These conflicting results between our data and those of Koziner et al. may be partially explained by differences in the sensitivity of the method used.

Our data unequivocally demonstrate that, in each instance, the great majority of CLL B cells contain detectable CyIg and that CyIg determination is superior to SmIg phenotyping in documenting the monoclonality of CLL. Similar results showing greater sensitivity of CyIg determination in patients with hairy cell leukemia have recently been obtained. Another advantage of CyIg assays over SmIg assays is that CyIg can be demonstrated with fixed cells that lack Fc receptor activity whereas SmIg expression requires the use of viable cells bearing functional Fc receptors and thus leading to potentially confusing results if cytophilic Ig is present. It should be emphasized that the actual methods of direct immunofluorescence determinations for both CyIg and SmIg are identical. These results therefore suggest the possibility of improving both sensitivity and specificity of phenotyping techniques in CLL by the use of CyIg determinations.
REFERENCES

12. Han T: Unpublished data
The presence of monoclonal cytoplasmic immunoglobulins in leukemic B cells from patients with chronic lymphocytic leukemia

T Han, H Ozer, M Bloom, K Sagawa and J Minowada