Clonal Chromosome Abnormalities in Patients With Waldenström’s and CLL-Associated Macroglobulinemia: Significance of Trisomy 12

By Tin Han, N. Sadamori, J. Takeuchi, H. Ozer, E. S. Henderson, A. Bhargava, J. Fitzpatrick, and A. A. Sandberg

We performed cytogenetic analyses by G- and Q-banding techniques of unstimulated or B-mitogen-stimulated spleen, bone marrow, and peripheral blood cells from six patients with malignant macroglobulinemia (two with Waldenström’s macroglobulinemia [WM] and four with chronic lymphocytic leukemia associated macroglobulinemia [CLL-M]). Normal karyotypes were obtained in two of the treated patients (one with WM in remission and the other with CLL-M in relapse). An extra chromosome 12 (trisomy 12) was observed in all four untreated patients. In patient no. 2 (K.R.) and no. 3 (F.G.) with CLL-M, an abnormal karyotype, with trisomy 12 as the only abnormality, was identified. In patient no. 1 (C.C.) with WM, there were two clonal chromosome changes, identified: 47,XX, -9, +12, plus marker chromosome and 48,XX, -9, +12, plus both marker and minute chromosomes. In patient no. 4 (R.M.) with CLL-M, a minute chromosome with or without loss of a G-group chromosome was seen in some metaphases without trisomy 12, in addition to metaphases with trisomy 12 alone. Each of the four untreated patients with WM or CLL-M had clonal chromosome abnormalities, suggesting that chromosome changes may be more frequently associated with WM or CLL-M than with typical CLL without macroglobulinemia. These observations also suggest that trisomy 12 may be the primary karyotypic change in malignant macroglobulinemia, whereas the appearance of the minute or marker chromosome as well as the loss of G-group chromosomes or chromosome no. 9 may be secondary karyotypic changes resulting from clonal evolution in these malignancies.

INTRODUCTION

THE TERM “macroglobulinemia” refers to an increase in the serum content of the immunoglobulin M (IgM) isotype. Primary, idiopathic, or essential macroglobulinemia refers to a syndrome first described by Waldenström in 1944; since then, patients with this syndrome have been labeled as having Waldenström’s macroglobulinemia (WM). WM is a rare disease characterized by an excessive proliferation of an IgM-producing malignant B-cell clone. To some extent, WM has features suggestive of chronic lymphocytic leukemia (CLL). The leukocyte counts in WM are typically normal, but in some instances, neutropenia as part of a general pancytopenia has been observed. Atypical cases of WM with a leukemic blood picture have also been reported. CLL, on the other hand, is a more common disease, characterized by the proliferation of monoclonal small lymphocytes of B-cell origin in almost all instances. A majority of patients with CLL have either normal serum levels of IgG, IgA, and IgM or hypogammaglobulinemia (IgG alone or combined with IgA and/or IgM). However, in a few CLL cases, secondary macroglobulinemia (CLL-M) has been observed.

Since Bouttura et al. described the first case of WM with a chromosome abnormality in 1961, a number of such cases have subsequently been reported. In the majority of the published papers on the chromosome constitution in WM, the presence of a marker chromosome resulted in a chromosome count of 47 per metaphase. This marker chromosome was termed the “W” or “MG” chromosome. However, to date, no chromosome change specific for WM has been identified and the exact origin of the extra chromosome has remained unclear, because only three reported cases of WM with karyotypic abnormalities have been analyzed by G-banding techniques.

Until recently, cytogenetic abnormalities in CLL were also poorly defined, primarily because of the very low spontaneous mitotic index of leukemic B cells; in addition, these cells are very poorly stimulated by phytohemagglutinin (PHA), which is primarily a T-cell mitogen. With the availability of primarily B-cell mitogens, such as Epstein-Barr virus (EBV), lipopolysaccharides (LPS), and of combined T/B mitogens such as protein A (PA) and pokeweed mitogen (PWM), cytogenetic analyses of leukemic B cells can now be readily performed. We and others recently reported clonal chromosome changes in stimulated lymphocytes of patients with CLL. The present study describes clonal chromosome abnormalities in both unstimulated and mitogen-stimulated lymphocytes from the peripheral blood, bone marrow, and/or spleen of four patients with malignant macroglobulinemia (one with WM and three with CLL-M).

MATERIALS AND METHODS

The clinical and immunologic data at the time of the cytogenetic analyses for the four patients are presented in Table 1. There were three males and one female; all were over 60 yr old. Three of these patients were treated patients (one with WM in remission and the other with CLL-M in relapse). An extra chromosome 12 (trisomy 12) was observed in all four untreated patients.
Table 1. Clinical and Immunologic Data in Four Patients With WM or CLL-M

<table>
<thead>
<tr>
<th>Clinical and Immunologic Data</th>
<th>Diagnosis</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Duration of disease</th>
<th>Previous therapy</th>
<th>Hemoglobin (g/dl)</th>
<th>Platelets x 10^12/cu mm</th>
<th>Lymphocytes x 10^3/cu mm</th>
<th>Splicenemaly</th>
<th>Serum Immunoglobulins* (mg/dl)</th>
<th>Serum Electrophoresis</th>
<th>Serum Immunoelectrophoresis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>No. 1</td>
<td>62</td>
<td>M</td>
<td>2 mo</td>
<td>None</td>
<td>9.0</td>
<td>182.0</td>
<td>2.5</td>
<td>No</td>
<td>8,350</td>
<td>M spike</td>
<td>Monoclonal</td>
</tr>
<tr>
<td>(C.C.)</td>
<td>No. 2</td>
<td>68</td>
<td>F</td>
<td>3 mo</td>
<td>None</td>
<td>14.1</td>
<td>275.0</td>
<td>28.0</td>
<td>No</td>
<td>3,470</td>
<td>M spike</td>
<td>Monoclonal</td>
</tr>
<tr>
<td>Patient</td>
<td>No. 3</td>
<td>71</td>
<td>M</td>
<td>7 mo</td>
<td>None</td>
<td>15.5</td>
<td>273.0</td>
<td>22.0</td>
<td>No</td>
<td>3,032</td>
<td>M spike</td>
<td>Monoclonal</td>
</tr>
<tr>
<td>(K.R.)</td>
<td>No. 4</td>
<td>63</td>
<td>M</td>
<td>1 mo</td>
<td>None</td>
<td>11.1</td>
<td>140.0</td>
<td>10.0</td>
<td>Postsplenectomy</td>
<td>2,480</td>
<td>M spike</td>
<td>Monoclonal</td>
</tr>
</tbody>
</table>

| Patient                      | No. 1     | 62      | M   | 2 mo                | None             | 14.1             | 170                      | 28                        | Postsplenectomy | 2 cm                           | M spike             | Monoclonal                   |
| (C.C.)                       | No. 2     | 68      | F   | 3 mo                | None             | 17.9             | 283                      | 13                        | No         | No                            | M spike             | Monoclonal                   |
| Patient                      | No. 3     | 71      | M   | 7 mo                | None             | 11.1             | 283                      | 13                        | No         | No                            | M spike             | Monoclonal                   |
| (K.R.)                       | No. 4     | 63      | M   | 1 mo                | None             | 11.1             | 140.0                    | 10.0                      | No         | No                            | M spike             | Monoclonal                   |

*Serum electrophoresis was performed by standard methods. Serum immunoelectrophoresis and serum or urine free light chain analysis were performed by a standard immunoelectrophoresis (IEP). Serum immunoglobulin determinations were performed by a radial immunodiffusion (RID) method. Normal ranges: IgG, 565–1,765 mg/dl; IgA, 85–835; IgM, 55–375.

†Patient C.C. had massive splenomegaly and patient F.G. had an 8-cm spleen at the time of diagnosis.

RESULTS

Normal diploid karyotypes were observed in the two treated patients with WM or CLL-M. The karyotypic abnormalities, defined by Q- and G-banding techniques in four untreated patients with WM and CLL-M, are presented in Table 2. In patient no. 1 (C.C.) with WM, two types of clonal abnormalities were identified in low proportions of uncultured bone marrow metaphases: 47, XX, +12, −9, plus marker chromosome 48, XX, +12, −9, plus marker chromosome, plus minute chromosome. Seventy-one metaphases displayed a normal karyotype of 46,XX, while the remaining metaphases showed random chromosome losses. Of 22 G- and Q-banded metaphases from uncultured spleen cells of patient no. 1, 12 (55%) had the abnormal karyotype 47, XX, +12, −9, plus...
Fig. 1. The "M" spike by serum electrophoresis and respective monoclonal heavy and light chain types by serum immunoelectrophoresis in each patient.

marker chromosome and 3 (14%) were 48, XX, +12, −9, plus marker chromosome, plus minute chromosome. No mitoses were observed in spleen cells cultured with PHA, LPS, or PWM. However, 4 (50%) of 8 banded metaphases from spleen cells cultured with EBV showed the 47, XX, +12, −9, plus marker karyotype. This abnormal karyotype was observed in 2 (33%) of 6 metaphases, in 21 (21%) of 102 metaphases, and in 4 (5%) of 78 metaphases from peripheral blood cells cultured with EBV, LPS, and PHA, respectively. The second clonal abnormality involving a 48, XX, +12, −9, plus marker and minute chromosome (Fig. 2) was observed at a lower frequency in 1 (17%) of 6 metaphases, in 2 (4%) of 102 metaphases, and in 2 (3%) of 78 metaphases from peripheral blood cells cultured with EBV, LPS, and PHA, respectively.

In patient no. 2 (K.R.) with CLL-M, each of 5 metaphases from EBV-stimulated peripheral blood cells, 2 (67%) of 3 LPS-activated metaphases, and 1 of 6 PHA-stimulated metaphases displayed a 47,XY,+12 karyotype (trisomy 12). Similarly, 5 (56%) of 9 metaphases from PWM-stimulated and 3 (50%) of 6 metaphases from EBV-stimulated bone marrow cells showed trisomy 12 (Fig. 3). The trisomy 12 karyotype was also seen in 6 (17%) of 36 metaphases from LPS-stimulated peripheral blood cells of patient no. 3 (F.G.) with CLL-M.

In patient no. 4 (R.M.) with CLL-M, two types of clonal chromosome abnormalities were observed. A minute chromosome, with or without loss of a G-group chromosome, was seen in 3 (14%) of 21 LPS-stimulated metaphases, in 1 (8%) of 12 PA-stimulated
Table 2. The Results of Cytogenetic Analysis by Q- and G-Banding Techniques in Four Patients With WM or CLL-M

<table>
<thead>
<tr>
<th>Patient and Source of Cells</th>
<th>Type of Abnormal Clone*</th>
<th>No. of Metaphases With Abnormal Clone/Total No. of Metaphases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1 C.C., WM Peripheral blood</td>
<td>PHA</td>
<td>LPS</td>
</tr>
<tr>
<td>47, XX, +12, −9, +M</td>
<td>47, XX, +12, −9, +M</td>
<td>47, XX, +12, −9, +M</td>
</tr>
<tr>
<td>4/78 (5%)</td>
<td>2/102 (2%)</td>
<td>2/6 (33%)</td>
</tr>
<tr>
<td>48, XX, +12, −9, +M, +min</td>
<td>48, XX, +12, −9, +M, +min</td>
<td>48, XX, +12, −9, +M, +min</td>
</tr>
<tr>
<td>3/78 (4%)</td>
<td>1/5 (2%)</td>
<td>1/6 (17%)</td>
</tr>
<tr>
<td>Spleen</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>4/8 (50%)</td>
<td>4/8 (50%)</td>
<td>4/8 (50%)</td>
</tr>
<tr>
<td>No. 2 K.R., CLL-M Peripheral blood</td>
<td>47, XY, +12</td>
<td>47, XY, +12</td>
</tr>
<tr>
<td>1/8 (17%)</td>
<td>2/3 (67%)</td>
<td>5/5 (100%)</td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>ND</td>
<td>NM</td>
</tr>
<tr>
<td>3/6 (50%)</td>
<td>3/6 (50%)</td>
<td>3/6 (50%)</td>
</tr>
<tr>
<td>No. 3 F.G., CLL-M Peripheral blood</td>
<td>NM</td>
<td>47, XY, +12</td>
</tr>
<tr>
<td>6/36 (17%)</td>
<td>6/36 (17%)</td>
<td>6/36 (17%)</td>
</tr>
<tr>
<td>No. 4 R.M., CLL-M* Peripheral blood</td>
<td>—</td>
<td>47, XY, +12</td>
</tr>
<tr>
<td>0/8</td>
<td>1/21 (5%)</td>
<td>1/21 (5%)</td>
</tr>
<tr>
<td>—</td>
<td>1/72 (1%)</td>
<td>1/72 (1%)</td>
</tr>
<tr>
<td>—</td>
<td>1/72 (1%)</td>
<td>1/72 (1%)</td>
</tr>
</tbody>
</table>

*Abbreviations used for cytogenetic results are as follows: M, marker chromosome; min, minute chromosome; NM, no mitosis; ND, not done.

Fig. 2. Q-banded karyotype of patient no. 1, showing a 48,XX,+12,−9, plus a marker chromosome, plus a minute chromosome (trisomy 12 with other chromosome changes).
Fig. 3. Q-banded karyotype of patient no. 2, showing a 47.XX,+12 (pure trisomy 12).

metaphases, and in 1 (1%) of 72 EBV-stimulated metaphases from cultured peripheral blood cells. An extra chromosome 12 was also seen in one (5%) of 21 LPS-stimulated metaphases and in 1 (1%) of 72 EBV-stimulated metaphases. No clonal chromosome changes were seen in 18 PHA- or PWM-stimulated cells.

DISCUSSION

Even though a number of cases of WM with chromosome abnormalities were reported prior to the development of banding techniques,7-11 there are only 3 reported cases in which banding techniques have been applied.13,14 The exact incidence of chromosome abnormalities in patients with WM has not been established firmly, primarily because of the small number of cases in the reported studies.7-14 Recently, it has become possible to induce into mitosis leukemic B cells from patients with CLL by polyclonal B-cell mitogens, and clonal chromosome abnormalities have been demonstrated in stimulated leukemic B lymphocytes from these patients.15-21 In our series,15 chromosome abnormalities were seen in 19 (39%) of 49 patients with CLL analyzed by Q- and G-banding techniques, suggesting that the prevalence of clonal chromosome aberrations in CLL is higher than previously believed. Of the four untreated patients with WM or CLL-M in this series, all had clonal chromosome abnormalities, suggesting that karyotypic abnormalities may be more frequently associated with WM or CLL-M than with typical CLL without macroglobulinemia.

Similar chromosome abnormalities in the patients with WM or CLL-M were detected both in unstimulated and in stimulated cells with two or more mitogens, suggesting that these changes were not induced by the mitogenic agents. None of the four patients with WM or CLL-M in the present study had received chemotherapy or radiotherapy prior to or at the time of the cytogenetic studies, and thus, the clonal chromosome abnormalities observed in these patients are disease-specific rather than treatment related. Spengler et al.24 demonstrated chromosome abnormalities in a patient with WM, yet found only normal chromosomes in the healthy monozygotic twin. These observations confirm that chromosome abnormalities may be directly related to WM per se.

Sandberg23 has reviewed the chromosomal data of a total of more than 70 cases of WM examined without banding techniques and described aneuploidy with a
chromosome number of 47 in the great majority of cases; in some cases, the number was 46 pseudodiploid or hypodiploid. Several investigators have previously reported cases of WM with 47 chromosomes, including a marker or "W" chromosome described as either a large metacentric or as a large submetacentric chromosome and related to chromosome no. 2. Contrafatto reported that about 2% of the G-banded metaphases obtained from one female and one male patient with WM showed 47 chromosomes, with an extra or a marker chromosome that appeared to be a chromosome no. 3 with a deletion on the distal part of the short arm. Despite the application of banding techniques, however, neither Contrafatto nor Fortez-Vila et al. observed trisomy 12 in their three reported cases.

In contrast, we observed a 47,XX or XY,+12 karyotype, with or without a minute or marker chromosome, and -9 or -G in varying numbers of metaphases from four untreated patients with WM or CLL-M. This discrepancy likely results from the application of B-cell mitogens to induce larger proportions of malignant B-derived metaphases, although further investigations in a larger series of patients with this malignancy are clearly required to confirm our observations.

The percentage of PHA-induced metaphases with chromosome abnormalities varies between 1.5% and 50%, with an average of 13%. Trisomy 12 was demonstrated in only 3%-5% of PHA-stimulated peripheral blood cells, whereas the abnormality was seen in 21%-50% of EBV- or LPS-stimulated peripheral blood or spleen cells of patient C.C. with WM. EBV and LPS primarily induce B-cell blastogenesis, and thus, metaphases obtained with these mitogens are almost certainly of B-cell origin. It should be emphasized that no chromosome abnormalities were demonstrated in chromosome nos. 2 or 3 (group A) in the present study. It has recently been demonstrated that the most common anomaly in stimulated lymphocytes of patients with CLL is trisomy 12; other abnormalities frequently seen are trisomy 16, trisomy 18, and a Ph-like chromosome or a minute chromosome; a 14q+ is also seen in some patients with CLL. The origin of the minute chromosome found in patients 1 and 4 or the marker chromosome found in patient 1 remains unknown. The fact that all four patients with WM or CLL-M had trisomy 12 and that two of the four patients also had extra chromosomes (minute and marker) and/or the loss of chromosomes (no. 9 and G-group), with or without trisomy 12, in some metaphases suggest the following tentative hypothesis: trisomy 12 may be the primary or the earliest karyotypic change in patients with WM or CLL-M, and the minute or the marker chromosome is generated and the loss of a G-group chromosome or chromosome no. 9 occurs as clonal evolution takes place.

The clinical and immunologic data, including phenotypic analysis, in patient C.C. with WM were similar to those in the three patients with CLL-M, with the exception that plasmacytoid lymphocytes were present in the spleen, bone marrow, and peripheral blood in the WM patient. Of particular interest is the fact that similar clonal chromosome abnormalities, such as trisomy 12 with or without a minute chromosome, were seen in the patient with WM as well as in those with CLL-M. The cytogenetic findings further indicate that WM and CLL-M may not be two distinctly different diseases; both diseases may belong to a single disorder characterized by an excessive proliferation of IgM-producing B cells, with slightly different clinical presentations. It has been demonstrated that clonal chromosome changes correlate with the disease status, stage, and degree of lymphocytosis in patients with typical CLL and may reflect prognostic or clinical significance. However, the clinical significance of clonal chromosome abnormalities in patients with WM or CLL-M has not yet been determined. Nevertheless, the demonstration of such abnormalities in four untreated cases in the present study suggests that cytogenetic analysis may be of value as a supplementary or confirmatory tool in the diagnosis of WM and/or CLL-M. This speculation, however, requires confirmation in a large number of patients with malignant macroglobulinemia.

Preliminary experiments with human hybrid cells have resulted in the assignment of the human genes coding for immunoglobulin heavy chains to chromosome nos. 14 and 6 and of the \( \kappa \) and \( \lambda \) light chains to chromosome nos. 2 and 22, respectively. Kirsch et al. recently demonstrated that the human heavy chain gene region, \( \gamma 4 \), is located on chromosome no. 14 at band q32 (14q32), exactly the band to which the characteristic translocation from other chromosomes occur in certain patients with B-cell lymphoma and leukemia, as well as in multiple myeloma. Recently, Lenoir et al. have emphasized the functional correlates of chromosome translocations in Burkitt’s lymphoma with the demonstration that there is a direct relationship between expression of immunoglobulin light chains and the specific type of translocation of the \( \text{myc} \) oncogene [cells with t(8;22) express \( \lambda \) chains, whereas those with t(1;14) express \( \kappa \) chains]. Although a 14q+ has been associated with some patients with typical CLL, this clonal chromosome abnormality has not yet been observed in patients with WM or CLL-M. It is of interest that a Ph-like (14q-?) or a minute chromosome was observed in two patients with...
CHRONOSOME CHANGES IN WM AND CLL-M

CLL-M in the present study as well as in a few patients with typical CLL.17,19 It should be emphasized that the combination of 14q+ and 22q- has been noted in one patient with CLL.19

Although trisomy no. 12 is most frequently associated with patients with typical CLL and WM or CLL-M, chromosome no. 12 has not yet been demonstrated to code for human immunoglobulin genes. Gahrton et al.31 recently reported a unique chromosome abnormality characterized by the duplicated segment q13 to q22 of chromosome no. 12 in a patient with stage IV lymphocytic non-Hodgkin's lymphoma with lymphocytosis, phenotyped as μκ, and they postulated that the segment q13 to q22 carries the essential genes that are duplicated during leukemogenesis, leading to B-cell lymphoproliferative disorders characterized by trisomy 12. It is tempting to speculate that chromosome no. 12 may be involved in a genetic rearrangement necessary in IgM expression in certain leukemic B lymphocytes.

ACKNOWLEDGMENT

We wish to thank Dr. Jun Minowada for cell phenotyping data, Patricia Maier for immunoglobulin studies, and Gail Walsh for manuscript preparation.

REFERENCES

Clonal chromosome abnormalities in patients with Waldenstrom’s and CLL-associated macroglobulinemia: significance of trisomy 12

T Han, N Sadamori, J Takeuchi, H Ozer, ES Henderson, A Bhargava, J Fitzpatrick and AA Sandberg