Clonal Chromosome Changes in Stimulated Lymphocytes of Untreated Human B-Cell Leukemias

Naoki Sadamori, Tin Han, Jun Minowada, Sei-ichi Matsui, and Avery A. Sandberg

ABSTRACT

The chromosome constitution of peripheral blood lymphocytes from nine cases of B-cell chronic lymphocytic leukemia and one case of B-cell prolymphocytic leukemia were studied at diagnosis following stimulation by B- and T-cell activators. Chromosome analysis with banding techniques revealed an extra chromosome 12 (trisomy 12) in 4 B-cell chronic lymphocytic leukemia cases and complicated abnormalities, i.e., trisomy 12, 6q−, 14q+, and a translocation between chromosomes 6 and 12, [t(6;12)] in the prolymphocytic leukemia case. These findings suggest that trisomy 12 is a prototypic change in B-cell leukemia, particularly in B-cell chronic lymphocytic leukemia. Based on sister chromatid exchange studies of unstimulated lymphocytes, it appears that some leukemia cells with a normal karyotype not only divide but also proliferate in vitro.

INTRODUCTION

Until recently, the cytogenetic aspects of B-cell leukemia were unclear, primarily because the leukemic cells in more than 90% of the cases with this disease are of the B-cell type and thus have a very low spontaneous mitotic index; in addition, these cells are poorly stimulated by most mitogens used previously. With the availability of so-called PBA, such as EBV and LPS, cytogenetic studies in B-cell leukemia entered a new era. The use of PBA revealed that the most common abnormality in B-CLL is trisomy 12 (3, 12). However, there have been few cytogenetic studies of B-cell leukemia using PBA and banding techniques.

In the present study, we tried to clarify the chromosome abnormalities in untreated B-cell leukemia cases, differences in differentiation and proliferation induced by various B- and T-cell activators, and character of the cells with normal karyotypes.

MATERIALS AND METHODS

Blood lymphocytes were obtained from 9 typical B-CLL (Cases 1 to 9) and one prolymphocytic leukemia (Case 10) patients whose ages ranged from 54 to 76 years; the average age was 66 years. The diagnoses were based on accepted clinical and laboratory criteria. All samples were obtained at diagnosis and before therapy was given. Case 10, although untreated for the leukemia before the cytogenetic studies, had been exposed to radiation for a number of years during his practice as a general surgeon (20). Clinical, hematological, and immunological data are shown in Table 1; the staging classification is that of Rai et al. (16).

Spontaneous rosette formation with sheep erythrocytes, rosette formation with IgG-sensitized bovine erythrocytes, and rosette formation with IgM-complement-sensitized bovine erythrocytes were used as T- and B-cell markers as described previously (11). Surface membrane immunoglobulins of the separated lymphocytes were also examined by direct immunofluorescent staining as described previously (4). Fluorescein isothiocyanate-labeled goat anti-human immunoglobulin (κ, λ, α, δ, γ, μ) sera were used.

Peripheral blood lymphocytes were isolated by centrifugation over Ficoll-Hypaque gradient. Separated lymphocytes (1 × 10^6 cells/ml) were cultured in Roswell Park Memorial Institute Medium 1640 supplemented with 10% fetal bovine serum and antibiotics [penicillin (100 units/ml) and streptomycin (50 μg/ml)] with Staphylococcus bacteria strain Cowan I (Cowan I, 100 μg/ml; Calbiochem-Behring Corp., La Jolla, Calif.), PWM (Grand Island Biological Co., Grand Island, N. Y.) at 1:100 dilution, EBV [supernatant from an EBV-producing permanent cell line B-95-8 at 1:9 (v/v) of culture] after filtration through 0.22-μm Millipore filters, LPS (40 μg/ml; Difco Laboratories, Detroit, Mich.), PHA (10 μg/ml; Wellcome, Ltd., Beckenham, Kent, England), and NA at 37° in an atmosphere of 5% CO2. After 3 days in culture, the cells were harvested; data on the cell cycles of the stimulated lymphocytes were based on a SCE technique (19). Colcemid was added 2 hr before harvest. Karyotype analyses were performed by G- and Q-banding techniques (21). Twenty banded metaphases were checked in each sample.

The procedure for SCE was essentially the same as that described by Latt (6) using a final bromodeoxyuridine concentration of 10 μg/ml of culture medium.

RESULTS

Of 10 samples processed with each mitogen, successful cultures containing metaphases were obtained in 6 with Cowan I, 8 with PWM, 7 with EBV, 8 with LPS, 8 with PHA, and 3 with NA (Table 2). The M1, i.e., the number of metaphases/1000 cells (M1 × 10^−3 cells), varied depending on the cases and activators. The mean M1 was highest with PWM (4.97), and that with PHA (3.67) and Cowan I (2.07) followed. The mean M1s with EBV and LPS were similar but not as high (1.52 and 0.92, respectively) as those of PWM and PHA. The value of M1 in prolymphocytic leukemia (Case 10) was similar to that of B-CLL.

As shown in Table 3, of 9 B-CLL cases, 4 (44.4%) contained abnormal metaphases with trisomy 12 (Fig. 1). Case 10 with prolymphocytic leukemia showed a complicated karyotype, 48,XY,+t(6;12)(q15;p13),+12,−13,+mar,t(1;?)p36.7t(6;6;14)p21;q21;q32), which will be reported in detail elsewhere (20). The complicated rearrangements in Case 10 led to the formation of a 6q- and a 14q+ as shown in Fig. 2.

The percentage of metaphases with abnormal chromosomes also varied depending on the sample. Of the 10 samples, cultures
the former mitogens was lower than that of the latter. This fact was considered by Gahrton and Robert (2).

Some investigators (3, 18) have reported that the cells in many B-CLL cases show a poor response to even those mitogens not produced by the mitogenic agents. The incidence of CLL cases with abnormal chromosomes has differed from report to report. Schröder et al. (22) found an abnormal clone in one (3.2%) of 31 untreated cases and in 4 (11.4%) of 35 treated cases with CLL including cases without any mitoses. Gahrton et al. (3) reported that 8 (72.7%) of 11 untreated and treated CLL cases showed an abnormal clone. Morita et al. (12) found karyotypic aberrations in 9 (33.3%) of 27 CLL cases including cases without any metaphases. The data of the above papers, including CLL cases, are based on those containing some metaphases with abnormal chromosomes. The number of cases with abnormal chromosomes using the former mitogens was lower than that of the latter. This fact may indicate that EBV and LPS can lead more selectively to differentiation and proliferation of leukemic cells with chromosome aberrations in B-cell leukemia.

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The prolymphocytic leukemia patient (Case 10) studied by us showed a poor response to those mitogens capable of stimulating B-cells. The findings of Cases 1 and 2 in the present study support this observation. However, this is not always the case, as shown in Table 2; i.e., the MI varied depending on the sample and activator.

In this study, we used activators that act specifically on B-cells (EBV and LPS), others on both T- and B-cells (Cowan I and PWM), and one T-cell-dependent B-cell activator (PHA), as reviewed by Gahrton and Robért (2). As shown in Tables 2 and 3, even though the mean MI of Cowan I, PWM, and PHA were higher than those of EBV and LPS, the number of cases with abnormal chromosomes using the former mitogens was lower than that of the latter. This fact may indicate that EBV and LPS can lead more selectively to differentiation and proliferation of leukemic cells with chromosome aberrations in B-cell leukemia.

The question naturally arises as to whether cells with normal karyotypes are or are not leukemic. As shown in Table 3, normal karyotypes are or are not leukemic. As shown in Table 2; i.e., the MI varied depending on the sample and activator.

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### DISCUSSION

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Table 3

Number of metaphases with or without abnormal chromosomes in the various samples

<table>
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<tr>
<th>Case</th>
<th>Karyotype</th>
<th>Cowan I</th>
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<th>EBV</th>
<th>LPS</th>
<th>PHA</th>
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* This exact karyotype is 48,XY, +t(6;12)(q15;p13), +12, 13, +mar, t(1;17)(p36;?), t(6;6;14)(p21;q21;q32).

* No activator used.

Fig. 1. Karyotype of CLL lymphocyte stimulated with EBV and showing trisomy 12 (arrow) in Case 7.

but also to proliferate in vitro as shown in Fig. 3.

We found no correlation between the type of chromosome abnormalities and surface membrane immunoglobulins or between the value of IgG-sensitized and IgM-complement-sensitized bovine erythrocyte rosettes and incidence of chromosome abnormalities as reported by Morita et al. (12).

ACKNOWLEDGMENTS

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REFERENCES

Fig. 2. Karyotype of CLL lymphocyte stimulated with LPS in Case 10, showing 48, XY,+t(6;12)(q15;p13),+12,-13,+mar, t(1;7)(p36;q7), t(6;6;14)(p21;q21;q32).

Fig. 3. Leukemic cell with normal karyotype in the second cell cycle after Day 3 of bromodeoxyuridine exposure in Case 7.
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