Dimorphism of Sister Chromatid Exchange in Bloom's Syndrome B- and T-Cell Lines Transformed with Epstein-Barr and Adult T-Cell Leukemia Viruses

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ABSTRACT

The present study describes the establishment of both B- and T-cell lines from the peripheral blood cells of two Bloom's syndrome (BS) patients and one healthy female by using Epstein-Barr (EBV) and adult T-cell leukemia viruses (ATLV). The cell lines from normal and BS subjects exhibited cell surface markers compatible with B- and T-cell origin; in addition, the BS B- and T-cell lines retained the original cytogenetic characteristics of the syndrome. Even though phytohemagglutinin-stimulated BS lymphocytes from the two BS patients studied all showed high levels of sister chromatid exchange (SCE), the established BS B-lines with EBV yielded two separate lines each, i.e., one with increased SCE and another with normal levels of SCE; also, one of the BS T-lines retained high SCE levels in 100% of the cells, whereas the other BS T-line contained two populations, one with high SCE (70%) and the other with normal SCE levels (30%), at a relatively constant frequency over a period of 6 months. Neither EBV nor ATLV caused a significant increase in chromosome instability in the established lines compared to fresh lymphocytes. Reinfection of the BS B- and T-cell lines with EBV or ATLV did not alter the SCE or karyotypes. These results strongly suggest that BS patients have two populations in vivo, one with high and another with normal levels of SCE, at least in the lymphoid cell system.

INTRODUCTION

EBV has been widely used to transform B-lymphocytes from healthy donors and patients with a variety of diseases (2, 6, 12). The majority of these cell lines possess characteristics of normal B-lymphocytes, such as surface immunoglobulin and complement receptors (2, 12, 13). However, the establishment of B-cell lines with abnormal characteristics, including leukemic and genetic markers, has been difficult. Furthermore, only T-cell lines of neoplastic origin have been characterized (2, 12, 13). The majority of these cell lines possess characteristics of normal lymphocytes with a lethally irradiated ATLV-carrying human T-cell line has successfully led to the continuous growth of T-lymphocytes without T-cell growth factor (8, 10, 11). The establishment of both B- and T-lymphoid cell lines is particularly well suited to a number of studies, including those on cytogenetics. In the present study, we wish to report on the establishment of both B- and T-cell lines derived from the cells of 2 BS patients, which have retained the original cytogenetic characteristics of the syndrome, i.e., chromosome instability and increased SCEs. To our knowledge, this is the first report of the establishment of BS B- and T-cell lines, utilizing EBV and ATLV, which retain the characteristics of the original BS cells.

MATERIALS AND METHODS

Leukocytes were separated by Ficoll-Conray gradient centrifugation from 20 to 40 ml of peripheral blood of 2 BS patients with features characteristic of the syndrome (1, 3, 16) and one normal female. Establishment of B-cell lines followed methods described previously (5, 16). Briefly, the lymphocyte suspension (1 x 10^6 cells) was infected with EBV (B95-8 strain) for 2 hr at 37°C, then washed, and centrifuged, and the sedimented cells were cultured in 35-mm Petri dishes with Roswell Park Memorial Institute Tissue Culture Medium 1640 supplemented with 10% human cord serum and 10% fetal calf serum at 37°C in a 5% CO2 incubator. Three cultures were set up from each sample, and the cultures were fed once a week by addition of about 1 ml of fresh medium. During the fourth week of incubation, it became apparent that the cell cultures were proliferating slowly, as they began to form cell aggregates of various sizes. In the second month of incubation, the proliferation of the cultured cells became steady, and subcultures of B-cell lines were established from both normal and BS patients.

The transformation of T-lymphocytes followed methods described previously (8, 10, 11). The cells were cultured at a density of 1 x 10^6 cells/ml in three 35-mm Petri dishes with Roswell Park Memorial Institute Tissue Culture Medium 1640 supplemented with 10% human cord serum and 10% fetal calf serum for all subjects. After 2 days of culture, 1 x 10^6 lethally irradiated (10,000 R) MT-2 cells were added to 2 of the 3 dishes. MT-2 is a T-cell line derived from normal human cord lymphocytes by cocultivation with leukemic T-cells from a patient with ATL (8). This cell line is persistently infected with abundant type C virus particles (8). The type C virus (so-called ATLV) is presumed to have originated from ATL cells, since cultured ATL cells were shown to harbor type C virus particles. Control cultures included one dish of irradiated MT-2 cells without coculture and one dish of leukocytes alone from each BS patient and the normal female. All cultures were incubated at 37°C in a 5% CO2 atmosphere, and approximately one-half of the growth medium was replaced with fresh medium twice a week. One week after coculture, scattered foci of cell aggregates were observed on the sheets of macrophages. These cell aggregates slowly increased in size and number over the next 2 weeks. The first cell transfer was made 18 days after coculture. The cells grew in suspension, forming clumps of cells, and were serially subcultured every 7 days. The irradiated MT-2 cells did not
RESULTS AND DISCUSSION

Lymphocytes from the peripheral blood of 2 BS patients and one healthy female subject were stimulated with B95-8EBV; 3 cultures were set up from each sample. B-lymphoid lines were successfully established from all of 3 normal cultures set up and from 2 of 3 BS cultures in each BS patient. All cell lines established with EBV were positive for surface immunoglobulin and EBV nuclear antigen. Three B-lymphoid lines from the normal subject were established by the beginning of the second month; cell lines (3 cultures) of the same origin were designated by the same symbol (NB), since they had a normal karyotype. The cells of the 2 BS patients (BS, and BS2) yielded 2 cell lines each (BS1, BS1-2, BS2, and BS2,2) following EBV treatment. The establishment of the cell lines BS1, BS1-2, and BS2,2 was delayed a few weeks and completed by the end of the second month, although the other 2 BS lines (BS1, and BS2,2) were established by the beginning of the second month.

The cocultivation technique led to the establishment of a normal T-cell line (NT) and BS T-cell lines (BST-SY1, and BST-SY2) which carried ATLV and ATLV-associated antigens. All these T-cell lines have now been maintained in continuous culture for over 6 months. A high proportion of cells from the 3 cell lines (NT, BST-SY1, and BST-SY2) formed spontaneous rosettes with sheep erythrocytes and reacted with a monoclonal antibody to T-cells (Leu 1). The results are summarized in Table 1. These surface marker and antigenic characteristics of NT, BST-SY1, and BST-SY2 are almost identical to those of MT-2. Almost 100% of BST-SY1, and BST-SY2 cells were shown to be positive for ATLV-associated antigens when reacted with ATLV patient’s serum by indirect immunofluorescence (Fig. 1). Thus, the type C virus was apparently transmitted from the MT-2 cells to the BS lymphocytes during cocultivation. Neither the irradiated MT-2 cells nor the normal and BS leukocytes cultured alone were alive for more than 2 months.

The normal B- and T-cell lines (NB, NT) showed a normal karyotype. Banding karyotype analyses of BS B- and T-cell lines showed that 4 lines (BS1, BS1-2, BST-SY1, and BST-SY2) had a normal karyotype, whereas 2 cell lines, BS1, and BS2,2, were 100% abnormal karyotypically, consisting of 2 major clones, respectively. In BS1,2, the modal chromosome number was clearly 46. Q-bandng studies of 41 cells with 46 chromosomes showed that 32 cells were 46,XY,7p+,12q+,+t(15;15)(p11;q12), and 9 cells were 46,XY,12q+,+t(15;15)(p11;q12); all 7p+ and 12q+ markers had identical patterns and size (Fig. 2). However, we could not trace the exact genesis of the marker chromosome, although we suspect that it may have originated primarily from chromosome 1. In BS2,2, the cells had 46 (29%) or 47 (71%) chromosomes. Q-bandng analyses of 18 cells with 47 chromosomes revealed them to have the following karyotype: 47,XY,t(3;15)(p25;q15),t(7;11)(q22; p15),m1 (Fig. 3). On the other hand, the karyotype of the cells with 46 chromosomes (5 cells) was shown to be: 46,XY,t(3;15)(p25;q15),t(7;11)(q22;p15). The exact origin of m1 could not be identified, although it had identical patterns and size in all the cells.

The values for spontaneous chromosome breaks in PHA-stimulated BS lymphocytes (BS1, BS2) were 0.19 and 0.21 breaks/cell, whereas that of the cells from the normal subject was 0.03 break/cell (p < 0.01). In BS B- and T-cell lines, a significant increase in spontaneous chromosome breaks was noted in BS1-2 (0.20/cell), BST-SY1 (0.24/cell), BS2,2 (0.21/cell), and BST-SY2 (0.23/cell), although the frequency of breaks was of a normal level in BS1, and BS2,2. Quadriradials and endomito
toses were also seen at a relatively constant frequency (approximately 0.04/cell) in BS1, BST-SY1, BS2,2, and BST-SY2 lines. Neither EBV nor ATLV caused a significant increase in breaks in established BS B- and T-cells compared to the values in fresh lymphocytes.

In the PHA-stimulated BS lymphocyte cultures, the frequency of SCE was increased more than 10-fold over that of normal cultures (p < 0.001). This increase was present in all mitoses; SCE levels per BS cell (in the PHA culture) varied from 41 to 97 (mean, 74.7). The SCE frequencies were examined at the time of establishment of these B- and T-cell lines and compared with those obtained after 6 months. As shown in Table 2, no significant changes occurred. These findings indicate that SCE frequencies in these lines are fairly stable. In BS B-cell lines, the frequency of SCE was within the normal range in BS1, and BS2,2. However, a significant increase in SCE was detected in cells of BS1, and BS2,2 cell lines, paralleling the high frequency of chromosome aberrations and abnormal karyotypes in both cell lines. Noticeable is the fact that high SCE levels were already detected in 100% of cells at the time of establishment of BS1,2 and BS2,2, although the exact mechanism remains unknown. In BS T-cell lines (Table 2), BST-SY1 retained a high SCE frequency in 100% of the cells (Fig. 4), whereas BST-SY2 contained 2 populations, one with high (70%) and the other with normal (30%) SCE, at a relatively constant frequency over a period of 6 months. It is to be noted that, although PHA-stimulated BS blood lymphocytes showed high levels of SCE with almost no significant difference, 2 BS B-cell lines (BS1,2 and BS2,2) each had 2 populations (one with high and another with normal SCE levels), and one of the T-cell lines (BST-SY2) contained a mixed population. This is compatible with the observation of 2 populations, i.e., one with high and another with low SCE, occasionally seen in PHA-stimulated cultures of lymphocytes from BS cases (4). Other findings have shown that some cells with SCE levels in the normal range were seen in the liposaccharide- and EBV-stimulated short-term culture of lymphocytes of BS patients. Thus, it can be assumed that B-cells with normal SCE levels were present in the blood of the patients studied by us. Neither EBV nor ATLV caused a significant increase in breaks in established normal and BS B- and T-cells compared to the levels in fresh lymphocytes. No karyotypic abnormalities were observed in normal B- and T-cells transformed with EBV and ATLV. Reinfection of the BS B- and T-cell lines with EBV and ATLV did not alter the SCE levels or the karyotypes. These findings strongly suggest that BS B-cell lines with abnormal karyotypes...
reflect in vivo chromosome abnormalities and are not an in vitro effect of EBV. Of special interest was the finding that karyotypic abnormalities observed in the cell lines sometimes corresponded to those observed in PHA- and liposaccharide-stimulated lymphocyte cultures (17). Even though BS T-cell lines with high SCE retained a normal karyotype, the situation was different from first subculture. BS B-cell lines with increased SCE could not be easily transformed with EBV due to cell growth disadvantages. A feasible mechanism explaining this discrepancy may be related to the differential transforming activity of EBV and ATLV in subpopulations of B- and T-lymphocytes. BS B-cell lines in which karyotypically abnormal clones were paralleled by increased SCE. A feasible mechanism explaining this discrepancy may be related to the differential transforming activity of EBV and ATLV in subpopulations of B- and T-lymphocytes. BS B-cell lines with increased SCE could not be easily transformed with EBV due to cell growth disadvantages in vitro, in the absence of karyotypic abnormalities. Therefore, the BS B- and T-cell lines reported here need to be further characterized as to the type of B- and T-cell subpopulations. These results suggest the strong possibility that BS patients have 2 populations in vivo, one with high and another with normal levels of SCE, at least in the lymphoid cell system. The establishment of BS B- and T-cell lines is particularly well suited to a number of studies, including those on the cytogenetic, biochemical, and immunological aspects of cell populations.

REFERENCES


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Table 2

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<tr>
<th>Cell line</th>
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<th>Time (days) to first subculture</th>
<th>Days examined</th>
<th>No. of cells scored</th>
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<td>88 (69)</td>
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a Fresh T-lymphocytes were cultured for 3 days with PHA stimulation.
b Mean ± S.E.
c Numbers in parentheses, percentage of cells scored.
Fig. 1. Immunofluorescence micrograph of acetone-fixed BST-SYi cells showing ATL-associated antigens in the cytoplasm with brighter fluorescence in the periphery. All the cells are fluorescent. The cells were first reacted with serum from an ATL patient and then incubated with fluorescein isothiocyanate-conjugated goat anti-human IgG. x 750.

Fig. 2. Q-banding karyotype showing 46,XY,12q+,+14,t(15;15)(p11;q12) in an EBV-transformed BS B-lymphoid cell line (BS,-2).
Fig. 3. Q-banding karyotype showing 47,XY,2p+,t(3;15)(q25;q15),t(7;11)(q22;p15),+m, in BS2-2.

Fig. 4. Metaphase showing a high incidence (about 70 SCEs) in a BST-SY1 cell.
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