Modulation of SCE frequencies in cell lines derived from human B and T lymphocytes

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The frequency of sister chromatid exchanges (SCEs) in correlation to the concentration of bromodeoxyuridine (BrdUrd) was studied in rosetting and non-rosetting human peripheral lymphocytes, EBV-lymphoid and lymphoma cell lines, T-lymphoid and lymphoma cell lines. An euploid T-cell line and the rosetting cells had a higher mean frequency of SCEs than the euploid B-cell lines and the non-rosetting cells. In the B-cell lineage, but not in the T-cell derived lines, the yield of SCEs was increased by the addition of deoxycytidine. This hints at an epigenetic difference in the DNA precursor metabolism that also might be the underlying reason for the overall level of SCEs in the two cell types. A higher incidence of SCEs is not an inevitable consequence of malignancy and aneuploidy; an increased level seems to be a property only of malignant cells of the B-cell lineage.

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Phytohemagglutinin (PHA) stimulated human lymphocyte cultures consist of distinct populations of lymphocytes, with different properties and immunological functions. We have shown previously that the heterogeneity of these cell cultures can be demonstrated with the technique for detecting sister chromatid exchanges (SCEs) on metaphase chromosomes (SANTESSON et al. 1979). As a rule, cell populations enriched in cells forming rosettes with sheep red blood cells (SRBC) (here referred to as R-cells) show higher frequencies of SCEs than non-rosetting cells (referred to as NR-cells). NR-cells also need higher concentrations of the thymidine analogue bromodeoxyuridine (BrdUrd) in the growth medium to show differential staining of sister-chromatids by the method of ALVES and JONASSON (1980). Furthermore, the toxic effects of BrdUrd on cell cycle kinetics seem to differ in the two cell populations, NR-cells being less affected by mitotic delay than R-cells at low (<50 μM) concentrations of BrdUrd. These observations indicate that less BrdUrd is incorporated into nuclear DNA in NR-cells as compared with R-cell populations. However, R-cells can survive much higher concentrations of BrdUrd with correspondingly increased frequencies of SCE without showing growth arrest. As a consequence, the total number of SCEs detectable in normal NR-cells will always remain relatively low whereas R-cells show a much wider range dependent on the BrdUrd concentration in the growth medium. The reason for this difference is not known.

The overwhelming majority of the R-cells, activated by PHA, are most likely T-cells. The NR-cell population includes B-cells as well as a high percentage of esterase positive cells, e.g., monocytes (unpublished results). B-cells are known to become activated by lectin in the presence of a few T-cells (PHILIPS and ROITT 1973) but monocytes are not considered to respond to PHA. It was therefore tentatively assumed that differences between T- and B-cells constitute the underlying reason for the low frequency of SCE in NR-cells compared to R-cells (SANTESSON et al. 1979).

The aim of the present study was to investigate whether the modulation of SCE frequencies that we have observed in normal NR- and R-lympho-
cytome is present also in cell lines derived from T- and B-cells and is consistent with their origin.

Materials and methods

B-cell derived cell lines

Two Burkitt lymphoma (Raji, U-984), one lymphocytic lymphoma (U-698) and three non-neoplastic EBV-carrying lymphoblastoid cell lines (U-1171, U-1706, H.W.-B) were used.

T-cell derived cell lines

Two cell lines (JM and SKW-3), derived from an acute lymphoblastic leukemia, were used plus one derived with T-cell growth factor (TCGF) from normal peripheral blood (H.W.-T.).

The sources of these cells are given in Table 1. The normal R- and NR-lymphocyte populations analyzed originated from the peripheral blood of healthy individuals. They were separated by the AET-SRBC rosetting technique (KAPLAN and CLARK 1974). The fraction referred to as R-cells contained only rosetting cells while the NR-cell fraction usually contained 5–10% rosette-forming cells. A high percentage were esterase positive cells.

Culture methods

All cell lines have been cultured in RPMI 1640 supplemented by 10% foetal calf serum and antibiotics (100 IU/ml of penicillin and 50 µg/ml of streptomycin). They were incubated at 37°C in the dark. BrdUrd at different concentrations from 2 to 300 µg/ml was included in the growth medium the appropriate length of time for the majority of cells in each individual line to reach mitosis after 2 cycles of DNA replication. The separation and cultivation of R- and NR-cells have been described earlier (SANTESSON et al. 1979).

Chromosome staining

Sister chromatid differentiation was induced either by the FPG technique (PERRY and WOLFF 1974) or by the staining method described by ALVES and JONASSON (1980). Briefly, the slides were stained with fluorochrome Hoechst 33258, exposed to visible light for 5 min., incubated at 65°C for 1–2 h or stained directly at high pH, and finally counterstained with Giemsa.

Results

SCE and cellular growth in relation to BrdUrd-concentration

The three malignant B-cell-derived lymphoma lines all displayed elevated SCE frequencies as compared to normal NR-cells (Table 1). They also showed differential staining of sister chromatids at BrdUrd-concentrations below that required by NR-cells (5 µg/ml). At high BrdUrd-concentrations the cells divided only once. Such first generation cells often showed fragmented chromosomes (Table 1).

The two EBV-induced lymphoblastoid cell lines had slightly increased frequencies of SCE as compared to NR-cells but, nevertheless, lower than those exhibited by the lymphomas (Table 1). High concentrations of BrdUrd caused growth arrest after one cell division but no chromosome fragmentation. These non-malignant lymphoblasts thus reacted towards BrdUrd in the same manner as the original NR-cells, e.g., their tentative parental cell.

In the T-cell-derived acute lymphoblastic leukemia line J.M. the SCE frequency was, however, much lower than that of normal R-cells (Table 1). Furthermore, this cell line was exceedingly susceptible to the inhibitory effects of BrdUrd, growth being arrested after one cell cycle at 15 µg/ml, whereas normal R cells grow well in 150 µg/ml. The second T-cell line, SKW-3 showed similar properties (Table 1).

The TCGF-dependent T-cell line (H.W.-T., normal peripheral blood) was cultivated with 15 pg BrdUrd. The frequency of SCE was found to be 0.25 ± 0.01 per chromosome, e.g., within the range for normal R-cells.

At the same time an EBV-line from the same donor (H.W.-B., normal peripheral blood) was tested with 15 µg/ml BrdUrd/ml. It had 0.13 ± 0.01 SCEs per chromosome. Both cell lines were diploid.

Deoxycytidine as a suppressor of the toxic effects of BrdUrd

Experiments were performed to determine if deoxycytidine (dCyd) can counteract the effects of BrdUrd on cell proliferation and SCE frequency in normal lymphocytes and in our cell lines. U-1171, J.M. and SKW 3, together with NR- and R-cells were studied.

It was found that the addition of dCyd (45 µg/ml) to the culture medium largely suppressed
**Table 1. SCE frequencies in human lymphoid cells**

| Cell type      | EBV genome | Mean no. of chrom./ metaphase | Conc. BrdUrd, μg/ml | SCE per chrom. ± S.E. | No. of cells analysed | Ploidy       |
|----------------|------------|-------------------------------|---------------------|-----------------------|----------------------|--------------|
| Normal NR-cells | –          | 46                            | 2                   | No. diff.             | –                    | normal       |
|                |            |                               | 5                   | 0.07±0.01             | 42                   | diploid      |
|                |            |                               | 15                  | 0.14±0.01             | 62                   |             |
|                |            |                               | 60                  | 0.20±0.01             | 74                   |             |
|                |            |                               | 150                 | only 1st gen.         | –                    |             |
| U-171          | +          | 46                            | 2                   | 0.12±0.01             | 25                   |             |
|                |            |                               | 15                  | 0.21±0.01             | 44                   | normal       |
|                |            |                               | 45                  | 0.23±0.02             | 20                   | diploid      |
|                |            |                               | 60                  | 0.21±0.01             | 6                    |             |
| U-1706         | +          | 46                            | 2                   | 0.12                 | 20                   | normal       |
|                |            |                               | 15                  | 0.20                 | 31                   | diploid      |
|                |            |                               | 45                  | only 1st gen.         | –                    |             |
| U-698          | –          | 47                            | 2                   | 0.26±0.02             | 22                   | aneuploid    |
|                |            |                               | 15                  | 0.30±0.02             | 38                   |             |
|                |            |                               | 60                  | only 1st gen. fragm.  | –                    |             |
| Raji           | +          | 48                            | 2                   | 0.26±0.02             | 16                   | aneuploid    |
|                |            |                               | 15                  | 0.34±0.02             | 30                   |             |
|                |            |                               | 60                  | only 1st gen. fragm.  | –                    |             |
| U-984          | +          | 45                            | 2                   | 0.26                 | 28                   | aneuploid    |
|                |            |                               | 15                  | 0.33                 | 51                   |             |
|                |            |                               | 60                  | only 1st gen. fragm.  | –                    |             |
| Normal R-cells | –          | 46                            | 2                   | 0.20±0.01             | 71                   | normal       |
|                |            |                               | 15                  | 0.26±0.01             | 160                  | normal       |
|                |            |                               | 60                  | 0.36±0.01             | 67                   | diploid      |
|                |            |                               | 150                 | 0.44±0.02             | 17                   |             |
|                |            |                               | 300                 | 0.64±0.05             | 8                    |             |
| J.M.           | –          | 72                            | 2                   | 0.14±0.01             | 20                   | aneuploid    |
|                |            |                               | 10                  | 0.11±0.01             | 20                   |             |
|                |            |                               | 15                  | only 1st gen.         | –                    |             |
| U-SKW-3        | –          | –                             | 1                   | 0.09±0.004            | 24                   | aneuploid    |
|                |            |                               | 2                   | 0.15±0.004            | 40                   |             |
|                |            |                               | 7.5                 | 0.17±0.01             | 30                   |             |

No. diff. = no differential staining  
Only 1st gen. = only 1st cell generation metaphases

References to cell lines:

**Line** Source of reference

**B-cell-derived cell lines:**

U-1171 C. SUNDBRÖM et al. (1980). – J. Clin. Lab. Immunol. 3: 99
U-1706 K. NILSSON, unpublished.
U-698 K. NILSSON and C. SUNDBRÖM (1974). – Int. J. Cancer 13: 808
Raji R. J. V. PULVERTAFT (1965). – J. Clin. Pathol. 18: 261
U-984 K. NILSSON and J. PONTEN (1975). – Int. J. Cancer 15: 321
H.W.-B. K. NILSSON, not published

**T-cell-derived cell lines:**

J.M. H.-U. SCHWENK and U. SCHNEIDER (1975). – Blut 31: 299
SKW-3 P. RALPH and K. NILSSON, unpublished
H.W.-T J. T. KURNICK, K.-O. GRÖNVik, A. K. KIMURA, J. B. LINDBLOM, V. T. SKOOG, O. SJÖBERG and H. WIGZELL (1979). – J. Immunol. 122: 1224
the inhibitory effect of BrdUrd on cellular growth in all cell types. Second generation cells appeared in the presence of dCyd even at as high a concentration as 150 µg/ml BrdUrd for U-1171 and at least at 45 µg/ml for J.M. and SKW-3 (higher BrdUrd concentrations have not been tested).

The effect of dCyd on the SCE frequency was, however, very different between different cell types. The yield of SCE in R-cells was not influenced at all by dCyd: the same relationship between BrdUrd concentration and SCE frequency was obtained with and without dCyd from 2 pg/ml up to at least 125 µg/ml of BrdUrd. In NR-cells the presence of dCyd caused an elevated level of SCE at the two BrdUrd concentrations tested. There was none or only a small increase in the T-cell-derived lines J.M. and SKW-3, but up to a three-fold increase in the B-cell-derived line U-1171 (Table 2).

Effect of mitomycin C

We wanted to know whether the comparatively low frequency of SCE inducible in J.M. and SKW-3 cells by BrdUrd in spite of its non-toxicity in the presence of dCyd was due to some inherent upper limit of SCE in these cells. Mitomycin C, which is a well-known inducer of SCE (Latt et al. 1975) was therefore included in the culture medium. As in normal cells mitomycin C did cause a clear increase in the number of SCE, in J.M. and SKW-3 cells as well as in U-1171. For example, in J.M. cells 0.1 µM of mitomycin C caused 0.69±0.05 SCEs per chromosome at a BrdUrd concentration of 2 µg/ml (control value 0.17±0.01). Inclusion of dCyd, 45 µg/ml, reduced this figure to 0.52±0.04.

Discussion

This investigation was undertaken in an attempt to clarify our original observation that NR-cells have fewer SCEs than R-cells. We wanted to know if the level of SCE is a distinct property of the B- and T-cell lineage.

Increasing concentrations of BrdUrd usually causes an increased frequency of SCE in mammalian cells (McFEE and SHERRIL 1979) including human lymphocytes (Latt 1974). The correlation between dose and response is, however, a question of debate. Kato (1974) maintains that BrdUrd concentrations below 3 µM do not modify the yield of SCEs in Chinese hamster cell cultures, whereas McFEE and SHERRIL (1979), using cow, pig, sheep, and human lymphocytes, find a sharp increase as the BrdUrd level is increased to 1 µg/ml but a slower rate of increase at higher concentrations. Obviously, the technique cannot answer the question if there are true "spontaneous" SCEs as BrdUrd has to be present and may in itself induce SCEs even at low concentrations. The "spontaneous" level of SCE can only be estimated by extrapolation from the slope of the BrdUrd concentration curve.

It is, however, difficult to conduct dose response curves over a sufficiently broad range of BrdUrd concentrations for several of our cell types. It is therefore not possible to compare adequately the hypothetical "true" levels of SCE in T- and B-cell-derived cell types or the slope of the response curve for increasing concentrations of BrdUrd. It can only be inferred that diploid

| Table 2. Effect of deoxycytidine (45 µg/ml) on the frequency of SCE at different BrdUrd concentrations (number of cells analyzed, in brackets) |
|------------------|------------------|------------------|
| Cell type        | BrdUrd µg/ml     | SCE per chromosome |
|                  | without dCyd     | with dCyd         |
| T-cell derived:  |                 |                  |
| R-cells          | 15              | 0.20 (37)        | 0.24 (39) |
|                  | 60              | 0.36 (30)        | 0.37 (25) |
|                  | 120             | 0.39 (15)        | 0.45 (20) |
|                  | 150             | 0.43 (5)         | 0.36 (2)  |
| U-SKW-3          | 1               | 0.09 (24)        | nd        |
|                  | 2               | 0.15 (40)        | 0.15 (20) |
|                  | 7.5             | 0.17 (30)        | nd        |
|                  | 15              | 0.16 (20)        | 0.26 (20) |
|                  | 45              | 0.17 (20)        | 0.15 (15) |
|                  | 15              | no 2nd           | 0.17 (15) |
|                  | 45              | no 2nd           | 0.22 (15) |
| B-cell derived:  |                 |                  |
| NR-cells         | 15              | 0.13 (39)        | 0.18 (39) |
|                  | 60              | 0.21 (24)        | 0.28 (22) |
| U-1171           | 15              | 0.21 (44)        | 0.26 (15) |
|                  | 45              | 0.23 (20)        | 0.46 (24) |
|                  | 60              | 0.21 (6)         | nd        |
|                  | 150             | no 2nd           | 0.70 (15) |

no 2nd = no 2nd cell generation metaphases
nd = not done
T-cell-derived cells, including R-cells, have a higher SCE frequency than the diploid B-cell-derived cells, including NR-cells at all workable BrdUrd concentrations, thus corroborating the original hypothesis. But the aneuploid cell lines show the opposite pattern, the T-cell derived lines having fewer SCEs than the B-cell derived lymphomas.

Recently, it has been suggested that the heterogeneity in SCE frequency in PHA lymphocyte cultures should not be attributed to inherent differences between T- and B-cells but to heterogeneous growth patterns (Lindblad and Lambert 1981).

Snope and Rary (1979) have presented data indicating the presence of two different sub-populations in human lymphocyte cultures: one with a high SCE frequency and slow proliferation rate and another with a lower SCE frequency and higher rate of proliferation. This observation is in obvious agreement with our previous results concerning R- and NR-cells (Santesson et al. 1979). However, Lindblad and Lambert (1981) consider this difference in SCE frequency not attributable to properties of T- and B-cells but rather to the proliferation rate as such: in any cell population the SCE frequency would be inversely related to growth rate according to these authors.

In the cell populations now investigated there is no correlation whatsoever between growth rate and SCE frequency. The NR-cells and the B-cell derived lymphoblastoid cell lines all have about the same SCE frequency but their growth rates differ from one cell type to another. The same applies to the T-cell lines. The most BrdUrd susceptible cell lines, J.M. and SKW-3, have low numbers of SCE. Addition of dCyd usually improves the growth rate but at the same time it also increases the SCE-frequency in some cell lines, but not in others. It seems safe to conclude that there is a difference between R-cells and T-cell-derived lines on the one side, and NR-cells and B-derived lines on the other, in the way they respond to BrdUrd and dCyd. Can the characteristics responsible for this difference in response to dCyd plus BrdUrd possibly explain our original observation that NR-cells from human lymphocytes have fewer SCE than R-cells?

New hypotheses concerning the mechanism of SCE maintain that the replication fork is the site of SCE formation (Kato 1977; Cleaver 1981). Both modulation of the chain elongation rate (Painter 1980; Ishii and Bender 1980) and the number of initiation points (Kato 1980) have been implicated with the frequency of SCE. Cleaver (1981) maintains that cells with larger replicons have higher base line SCE frequencies. These hypotheses could well be compatible with our observations on differences between B- and T-cell lineages. The intricate mechanisms at the replication fork may be the target of many different stimuli, some of which are specific for certain cell types. Such reactions could involve the metabolic events determining the deoxynucleotide pools, and thereby account for the effects of dCyd together with BrdUrd.

In conclusion: our original suggestion (Santesson et al. 1979) that the lower mean frequency of SCEs in non-rosetting cells compared to rosetting cells reflected an inherent difference between B- and T-cells is compatible with our present observation: euploid EBV-lymphoid cells have fewer SCEs than normal lectin activated T-cells and the only euploid T-cell line tested so far.

This is not, however, true for the aneuploid lymphoma B- and T-cell lines, the B-lymphomas having a greatly elevated level of SCEs. Consequently, malignancy and aneuploidy per se does not create conditions for a higher incidence of SCEs.

The response to dCyd and increasing concentrations of BrdUrd points to an inherent difference in nucleotide metabolism between the B- and T-cell lineages that might also influence the overall yield of SCE in response to ordinary concentrations of BrdUrd.

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