Ultraviolet Light-induced Sister Chromatid Exchanges in Xeroderma Pigmentosum and in Cockayne’s Syndrome Lymphocyte Cell Lines

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ABSTRACT

Sister chromatid exchanges (SCE’s) were studied in lymphocyte cell lines derived from 11 patients with xeroderma pigmentosum (XP) and 2 patients with Cockayne’s syndrome (CS), autosomal recessive diseases characterized by sun sensitivity in vivo and by an abnormal sensitivity of the patient’s cells to the killing effects of ultraviolet light (UV) in vitro. XP patients have DNA repair defects and develop numerous tumors on sun-exposed skin. CS patients do not develop such tumors, and the cause of the sensitivity of their cells to UV is not known. Reciprocal SCE’s were evaluated by fluorescence microscopy following staining of the chromosomes with the bisbenzimidazole dye 33258 Hoechst after the cells had passed through two phases of DNA synthesis in the presence of 5-bromodeoxyuridine. In the absence of UV irradiation, the XP and CS cells had the same frequency of “spontaneous” SCE’s as did normal cells. UV irradiation resulted in significant increases in the SCE’s in the normal, XP, and CS cells. The number of UV-induced SCE’s in the XP variant’s cells was not greater than the number in the normal cells. The number of UV-induced SCE’s in the XP Complementation Group B and E cells and in the CS cells, while higher than the number in the normal cells, was not significantly greater at the 0.05 confidence level. However, all the XP Complementation Group A, C, and D cells tested had significantly greater numbers of UV-induced SCE’s than did the normal cells. There were significant differences in the number of UV-induced SCE’s among cells from unrelated XP Group C patients, while cells from 3 XP Group C siblings had almost identical UV-induced responses. The distribution of spontaneous and UV-induced SCE’s among chromosomes of normal and XP cells was determined, and in all lines there was a significantly greater frequency of observable SCE’s per chromosome length in the longer chromosomes than in the shorter chromosomes. Our studies indicate that cells from certain excision-deficient XP patients have abnormally increased numbers of UV-induced SCE’s. Thus, XP is another disease characterized by excessive numbers of cancers and by abnormally high frequencies of SCE’s.

INTRODUCTION

SCE’s have recently become of great interest in cancer biology with the development of improved and accurate methods for their study (26, 35) and with the recognition that SCE’s occur with an abnormally high frequency in certain conditions predisposed to cancer. For example, there are abnormally high frequencies of “spontaneous” SCE’s in phytohemagglutinin-stimulated cultured leukocytes (9) from patients with Bloom’s syndrome (18) and from patients with histories of inorganic trivalent arsenical ingestion followed by development of skin cancer (8). Abnormally increased numbers of SCE’s are found in XP fibroblasts exposed in culture to chemical carcinogens (12, 46).

XP is a rare autosomal recessive disease characterized by sun sensitivity and excessive tumor formation on sunlit-exposed areas of skin (38). XP cells are unable to perform normal repair (13, 38) of DNA damage induced by UV and certain chemical carcinogens. XP was the first human disease shown to be caused by defective DNA repair (11). There are currently 5 forms of XP in which DNA excision repair is deficient, and these forms are classified into XP Complementation Groups A to E (25). In the sixth form of XP, the variant form, excision repair appears normal, but there is a marked impairment in postreplication repair (27). While there is a normal frequency of spontaneous SCE’s in cultured XP fibroblasts (22, 45) and in XP peripheral blood leukocyte cultures stimulated by phytohemagglutinin (43), there can be markedly increased frequencies after cultured XP cells have been irradiated with UV. This abnormal increase in UV-induced SCE’s has been reported to occur in XP leukocytes stimulated with phytohemagglutinin (Ref. 6, note added in proof; Ref. 43), in XP fibroblasts (16) and in LCL’s (10) derived from XP patients’ Epstein-Barr virus-transformed peripheral blood lymphocytes.

We now report in detail on our study (10) of UV-induced SCE’s in LCL’s derived from XP patients representing each of the 6 forms of XP. In addition, we report our findings on UV-induced SCE’s in LCL’s derived from patients with CS, a form of cachectic dwarfism characterized by acute sensitivity without any known predisposition to sunlight-induced carcinogenesis (19). CS is of particular interest because 1 XP patient also has CS (38, 47) and because CS cells (3, 4, 41, 42), like XP cells (3), are more readily killed by UV than are normal cells.

MATERIALS AND METHODS

Origin of the Control Donor LCL’s. Six control donor LCL’s were studied. Each was derived from the peripheral

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blood of apparently normal individuals. These 6 LCL's are designated as follows: C1BE-L1, from a 50-year-old man; C7BE-L2, from a 26-year-old woman; C6BE-L1 and C6BE-L3, 2 lines derived separately from a 22-year-old woman; GM588, from a 26-year-old man; and GM1079, from a 33-year-old woman. LCL's with the designation "BE" were derived at NIH, Bethesda, Md. The designation "L" indicates "lymphocyte" as the cell of origin for the cell line, and the numeral following L identifies the particular line from any other lines derived from the same individual. LCL's designated by "GM" were derived at and obtained from the Human Genetic Mutant Cell Repository, Camden, N. J. Most of the LCL's studied in this report are or will soon be available from this repository.

Origin of the CS LCL's. Two CS LCL's were studied: GM1712, from a 21-year-old man, designated CS2BE (7, 44, 47); and C5KCTO-L1, from a 12-year-old girl (Ref. 1, Case 3; Ref. 32, Patient K. C.) whose peripheral blood was sent to us by Dr. Robert M. Ehrlich, Hospital for Sick Children, Toronto, Ontario, Canada.

Origin of the XP LCL's. All 12 XP LCL's were derived at NIH. LCL's XP1BE-L1, and XP1BE-L3, XP2BE-L1, XP3BE-L3, XP7BE-L1, XP8BE-L1, XP9BE-L2, XP11BE-L1, and XP12BE-L2 were derived, respectively, from Patients XP1, XP2, XP3, XP7, XP8, XP9, XP11, and XP12 of the NIH XP patient series (38); XPPHBE-L1 was derived from a patient with XP who is the brother of the XP variant patient XP13 (38); XP17BE-L1 was derived from a 14-year-old black youth (2); XP3RO-L1 was derived from a man with XP in the same family as XP patient XP2RO (15). Both Patients XP3RO and XP2RO are in XP Complementation Group E (15, 25), and peripheral blood from Patient XP3RO was provided to us by Dr. Dirk Bootsma, Erasmus University, Rotterdam, The Netherlands. The complementation group assignments of the XP patients were determined by cell fusion studies reported in the previously cited references.

Derivation, Culture, and Irradiation of the LCL's. All the control donor and CS LCL's and 10 of the 12 XP LCL's were derived from peripheral blood leukocyte cultures to which a transforming strain of Epstein-Barr virus had been added. Strains XP1BE-L1 and XP3BE-L3 were established without addition of the virus (5). The LCL's were grown at 37°C in a humidified atmosphere of 5% CO2:95% air in RPMI 1715 or RPMI 1640 culture medium containing 20% fetal calf serum. Just prior to irradiation the cells were gently centrifuged and resuspended in Hanks' balanced salt solution at a concentration of 1 to 2 x 10^6 cells/ml. Two ml of the cell suspension were placed in Petri dishes (Tissue Culture Dish 3002, 60- x 15-mm style; Falcon Plastics, Oxnard, Calif.). The dishes were gently agitated during the period of UV irradiation. Irradiation was from a germicidal lamp (General Electric No. G15T8 lamp) emitting predominantly 254 nm UV at an incident flux of 0.9 erg/sq mm/sec. Immediately after irradiation the cells were resuspended at 2 x 10^7 cells/ml of fresh RPMI containing 20% fetal calf serum. Then 0.2 ml of a freshly prepared aqueous solution of 5-bromodeoxyuridine (500 μg/ml) was added to the resuspended cells to a final concentration of 8.2 μg/ml of culture medium. All operations with the bromodeoxyuridine were conducted in the dark, and the culture flasks were wrapped with aluminum foil to prevent exposure to light. The LCL's were then incubated at 37°C for 44 hr in the incubator. During the final 2 hr of incubation, colchicine was added to a final concentration of 0.2 μg/ml to arrest the cells in mitosis.

Preparation and Evaluation of LCL's for SCE's. SCE's were studied with the fluorescent staining method of Latt (26) with several modifications. The cells were centrifuged and resuspended in a hypotonic KCl solution (75 mm) for 10 min. Then a few drops of an acetic acid:ethanol (1:3) fixative were added. After 5 min the cells were centrifuged and resuspended in the fixative. After 20 min the cells were centrifuged and resuspended in a small volume of the fixative, dropped on glass slides that had been pretreated with cold distilled water, and air-dried. The air-dried slides were rehydrated successively with 95% ethanol, 75% ethanol, and then with distilled water. The cells then were stained for 10 min with an aqueous solution of the bisbenzimidazole dye 33258 Hoechst (0.1 μg/ml). The slides were then immersed in a sodium acetate solution (pH 4.4) in the dark for 1 to 7 days. Coverslips were then sealed with Kronig cement (G. T. Gurr, Ltd., London, England), and the cells were examined under a Leitz Ortholux fluorescent microscope equipped with an Orthomat camera. The cells were photographed, and the SCE's were counted from the negative film under magnification from a film viewer. SCE's were recognized as reciprocal exchanges of bright and dull segments along sister chromatids. The only cells scored were those with a diploid chromosome mode that had passed through 2 successive S-phases in the presence of the bromodeoxyuridine. In most cases 30 metaphase preparations were evaluated in each experiment. The chromosomes were identified according to the following classification: Chromosome 1, Chromosome 2, Chromosome 3, Chromosome Group B (Chromosomes 4 and 5), Group C (Chromosomes 6 through 12 and XX or X), Group D (Chromosomes 13, 14, and 15), Group E (Chromosomes 16, 17, and 18), Group F (Chromosomes 19 and 20), and Group G (Chromosomes 21, 22, and Y).

Statistical Analysis of the Data. There was statistically significant variability among the 6 normal individuals with regard to the incidence of SCE's, and the means of their spontaneous SCE's in the absence of UV radiation ranged from 4.03 to 5.83 SCE's/metaphase. Since each line was tested only once, we cannot determine whether this variability was due to genetic or to experimental variations. For estimation of the effect of UV irradiation in the presence of such variation, a mixed-model analysis of variance was used (39). The analysis was performed with the square root of the number of SCE's per metaphase as the dependent variable to minimize the effect of occasional large observations that were atypical of the majority of the observations in a given experiment. The square root transformation acted as a variance-stabilizing transformation for these data (40), permitting the use of analysis-of-variance techniques. The mixed model treats the normal individuals tested as a random sample from the population of all normal individuals and allows the estimation of the mean UV-induced increase in SCE's while adjusting for individual variation and interactions. Confidence intervals for the mean UV-induced SCE increases were calculated by the methods of Scheffé (39). Since 5 XP Complementation Group C patients were tested and showed statistically
significant variation in the incidence of the spontaneous SCE's in the absence of UV irradiation, the same techniques were used to estimate the confidence intervals for the UV-induced increase in SCE's for the Group C patients. The confidence intervals for the mean UV-induced SCE increases for the remainder of the patients were calculated with the Student t distribution after transformation of counts by the square root.

RESULTS

UV-induced SCE's per Metaphase in Normal, XP, and CS LCL's. The mean numbers of "spontaneous" SCE's per metaphase when there was no UV exposure were 4.98 for the 6 control donor LCL's (Chart 1a) and 4.75 for the 11 XP LCL's (Chart 1b). Analysis-of-variance techniques revealed no evidence of a difference in the number of these spontaneous SCE's per metaphase between the control donor and XP LCL's (p > 0.50).

UV irradiation of the control donors' LCL's with 14.4 ergs/sq mm induced a statistically significant number of SCE's (p < 0.05), indicated by the fact that the 95% confidence interval does not contain zero (Table 1, Analysis 1). Results from a representative experiment depicting the SCE's in a control donor line irradiated with 14.4 ergs/sq mm are shown in Chart 1c. Irradiation of the control donor LCL's with only 7.2 ergs/sq mm did not result in a significant increase in the number of SCE's (Analysis 2).

For the 5 XP Complementation Group C LCL's, there were significant increases in the number of SCE's induced by both 14.4 (Analysis 3) and 7.2 ergs/sq mm (Analysis 4). At each dose the UV radiation induced significantly more SCE's in these Group C LCL's than in the control donor LCL's, as indicated by the fact that the confidence intervals for Analyses 3 and 4 do not overlap the intervals of Analyses 1 and 2, respectively.

There were highly significant increases in the number of SCE's at both UV exposure levels in the LCL from the XP Complementation Group A patient (Analyses 5 and 6; Chart 2). These increases were significantly greater than those caused by the UV in the control donor LCL's.

Both LCL's from the XP Complementation Group D patients responded to each of the UV exposures with very similar significant increases in the numbers of SCE's (Analyses 7 to 10). For each of these Group D LCL's, both the high and low UV doses induced SCE increases that were greater than those induced by the high dose in the control donor LCL's. However, with regard to the 95% confidence intervals, only XP7BE-L1 at the low dose and XP17BE-L1 at the high dose responded with significantly greater numbers of SCE's than did the control donor LCL's at the same respective doses.

There were significant increases in SCE's at both exposure levels for the XP Group B LCL (Analyses 11 and 12). These increases were larger than those induced in the control donor LCL's but were not significantly larger at our predesignated 0.05 level.

The XP Group E LCL had a significant increase in SCE's after the 14.4-ergs/sq mm dose (Analysis 13) but had no evidence of any increase whatever at the 7.2-ergs/sq mm dose (Analysis 14). This UV-induced increase in the number of SCE's observed in the Group E LCL after the high UV dose was greater than the UV-induced increase observed in any of the 6 control donors' LCL's (latter individual lines' data not presented). However, the UV-induced increase of this Group E line was not significantly greater than the control donors' increase at the 0.05 level (compare Analyses 13 and 1).

The variant line was tested at only the high UV dose. The UV induced a statistically significant number of SCE's (Analysis 15). This UV-induced number of SCE's was not different from that induced in the control donors' LCL's (compare Analyses 15 and 1).

The 2 CS lines had significantly increased SCE's induced by the high dose of UV (Analyses 16 and 18). The low dose produced no such increase (Analysis 17). While these increases at the high dose were greater for both of the CS lines than for any of the increases observed in the 6 normal lines at the same high dose, they were not significantly greater (compare Analyses 16 and 18 to Analysis 1). A LCL was studied from each of 5 XP Complementation Group C patients. Three of these patients were from the same family. Patient XP2 was the brother of the identical twins XP8 and XP9 (38). As already stated, the 5 Group C lines had significant numbers of SCE's induced by both doses of UV (Table 1, Analyses 3 and 4). Table 2 shows that there were significantly different numbers of UV-induced SCE's among the Group C XP LCL's. Lines XP1BE-L1 and XP3BE-L3 had a greater number of UV-induced SCE's at both UV doses than did lines from the 3 siblings (Table 2; compare Analyses 1 to 4 and Analyses 5 to 10). These increases were significant at the 0.05 level at the high UV dose for XP1BE-L1 (Compare Analysis 1 and Analyses 5, 7,
Table 1

Data and statistical analysis of SCE’s in control donor, XP, and CS irradiated and unirradiated LCL’s

SCE’s were determined and analyzed as described in “Materials and Methods.”

<table>
<thead>
<tr>
<th>LCL’s</th>
<th>Chart depicting histogram of experimental results</th>
<th>Av. no. of SCE’s/metaphase at indicated UV dose (ergs/sq mm)</th>
<th>Mean UV-induced SCE effecta</th>
<th>95% confidence interval for mean UV-induced SCE effecta</th>
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<tbody>
<tr>
<td>6 control donor linesb</td>
<td>1a</td>
<td>6.52 ± 0.47c</td>
<td>4.98 ± 0.33</td>
<td>Analysis 1: 0.318</td>
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</tr>
<tr>
<td>5 control donor linesd</td>
<td>2b</td>
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<td>4.10 ± 0.69</td>
<td>Analysis 2: 0.002</td>
</tr>
<tr>
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<td>2a</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5 XP Group C lines</td>
<td>3b</td>
<td>12.39 ± 2.39</td>
<td>4.34 ± 0.26</td>
<td>Analysis 3: 1.394</td>
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<td>5 XP Group C lines</td>
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<td></td>
</tr>
<tr>
<td>5 XP Group C lines</td>
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<td>10.01 ± 1.65</td>
<td>4.34 ± 0.26</td>
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<td>XP Group A line</td>
<td>4b</td>
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<td>4.10 ± 0.69</td>
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<td>XP Group A line</td>
<td>4b</td>
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<tr>
<td>XP Group A line</td>
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<td>4.10 ± 0.69</td>
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<td>XP Group D lines</td>
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<td>XP7BE-L1</td>
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<td>XP7BE-L1</td>
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<td>5.63 ± 0.49</td>
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<td>XP17BE-L1</td>
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<tr>
<td>XP Group B line</td>
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<td>5.72 ± 0.65</td>
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<td>4.87 ± 0.29</td>
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<td>XP Group E line</td>
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<td>5.03 ± 0.47</td>
<td>4.87 ± 0.29</td>
<td>Analysis 14: 0.008</td>
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<td>XP variant line</td>
<td>9b</td>
<td>5.40 ± 0.43</td>
<td>4.27 ± 0.43</td>
<td>Analysis 15: 0.286</td>
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<tr>
<td>XP variant line</td>
<td>9b</td>
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<td></td>
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<tr>
<td>CS lines</td>
<td>10b</td>
<td>7.33 ± 0.48</td>
<td>4.77 ± 0.34</td>
<td>Analysis 16: 0.514</td>
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<tr>
<td>CSKCTO-L1</td>
<td>10b</td>
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</tr>
<tr>
<td>CSKCTO-L1</td>
<td>10b</td>
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and 9) and at both doses for XP3BE-L3 (compare Analyses 3 and 4 to Analyses 5 to 10). Lines XP1BE-L1 and XP3BE-L3 had very similar responses to the high UV dose (compare Analyses 1 and 3) and differed, although not significantly so, at the low UV dose (compare Analyses 2 and 4). The lines from the 3 siblings had extremely similar numbers of UV-induced SCE's at both the high dose (compare Analyses 5, 7, and 9) and the low dose (Compare Analyses 6, 8, and 10). These results show that LCL's derived from their distribution after irradiation (p > 0.20 for each of the 4 lines).

**Effect of UV on Distribution of SCE's among Chromosomes.** We determined whether the spontaneous SCE's occurring in the control donor LCL's in the absence of any UV had the same relative frequency distribution among the chromosome groups as did the SCE's occurring after the lines were irradiated. For each line the observed frequency distribution of the spontaneous SCE's through the chromosome complement was compared (with a $\chi^2$ test of heterogeneity) to the observed frequency distribution of SCE's after UV, 14.4 ergs/sq mm. Chromosome Groups F and G were combined in these analyses because of the low incidences of SCE's in these groups. The relative frequencies of spontaneous SCE's among the chromosomes of the 6 normal LCL's (C7BE-L2, C1BE-L1, GM588, GM1079, C6BE-L1, and C6BE-L3) were not significantly different from their distribution after irradiation ($p > 0.20$ for each of the 6 lines). A similar analysis was performed comparing the frequency distribution of spontaneous SCE's in 4 XP LCL's with and without irradiation (14.4 ergs/sq mm). The 4 XP LCL's analyzed were those that gave the largest number of SCE's per metaphase after irradiation. These lines were XP3BE-L3, XP12BE-L2, XP1BE-L3, and XP1BE-L1, and 2 experiments were performed on the latter line. As with the control donor lines, the relative frequencies of spontaneous SCE's among the chromosomes of these XP lines were not significantly different from their distribution after irradiation ($p > 0.20$ for each of the 4 lines).

**SCE's as a Function of Chromosome Length in Normal and XP LCL's.** If SCE's are distributed randomly throughout the entire chromosome complement of a cell, then the expected percentage of the total SCE's of a cell found in a given chromosome group should be equal to the diploid chromosome length of that chromosome group expressed as a percentage of the entire diploid length of the cell. For each LCL the expected frequency of SCE's for each chromosome group was calculated under the assumption of such a random distribution. The expected frequencies were calculated with the chromosome length values as reported at the Denver Conference (33, 34) and are represented as *solid horizontal bars* in Chart 3. The frequencies found for each of the 6 unirradiated control donor LCL's are presented in Chart 3a. The average frequencies of SCE's found per chromosome group for these 6 lines are represented by the *broken horizontal bars*. It is apparent from Chart 3 that the average frequencies of SCE's found for Chromosomes 1 and 2 and for Chromosome Groups B, C, and D were always greater than the expected frequencies. The frequencies found for Chromosome Groups E, F, G, and G + Y were always below the expected frequencies. The SCE frequencies found after these 6 LCL's were irradiated with UV (14.4 ergs/sq mm) also showed a greater frequency of SCE's in the longer chromosomes than in the shorter chromosome groups (Chart 3b). Similar results are shown for the unirradiated (Chart 3c) and irradiated (Chart 3d) XP LCL's.

For the 6 unirradiated control donor LCL's and for the 11 unirradiated XP LCL's presented in Tables 1 and 2, the hypothesis of a random distribution of observed SCE's throughout the chromosome complement was tested with a $\chi^2$ goodness-of-fit test. For the 6 control donor LCL's and

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**Table 1—Continued**

<table>
<thead>
<tr>
<th>Chart depicting histogram of experimental results</th>
<th>Av. no. of SCE's/metaphase at indicated UV dose (ergs/sq mm)</th>
<th>Mean UV-induced SCE effect$^a$</th>
<th>95% confidence interval for mean UV-induced SCE effect$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCL's</td>
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</tr>
<tr>
<td>CSKCTO-L1</td>
<td>14.4 ± 0.50</td>
<td>6.10 ± 0.50</td>
<td>Analysis 17: 0.251</td>
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<td>CSKCTO-L1</td>
<td>7.2 ± 0.48</td>
<td>4.77 ± 0.34</td>
<td>-0.048, 0.550</td>
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<td>GM1712</td>
<td>7.40 ± 0.48</td>
<td>4.93 ± 0.50</td>
<td>Analysis 18: 0.543</td>
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<td>GM1712</td>
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<td>0.252, 0.834</td>
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</tbody>
</table>

$^a$The analysis-of-variance formulation as elaborated by Schäffé (39) utilizes a parameter, $b_\alpha$, corresponding to the mean spontaneous frequency of SCE's, a parameter, $b_\beta$, corresponding to the mean frequency of SCE's at the 7.2-ergs/sq mm dose of UV, and a parameter, $b_\gamma$, corresponding to the mean frequency of SCE's at the 14.4 ergs/sq mm dose. The numbers in this column are the estimates of $b_\gamma - b_\beta$ (for the 7.2-ergs/sq mm dose) and of $b_\beta - b_\alpha$ (for the 14.4-ergs/sq mm dose).

$^b$C7BE-L2, C1BE-L1, C6BE-L1, C6BE-L3, GM 588, and GM1079.

$^c$Mean ± S.E.

$^d$All lines immediately above except C1BE-L1.

$^e$XP1BE-L1, XP3BE-L3, XP2BE-L1, XP8BE-L1, and XP9BE-L2.

$^f$XP12BE-L2.

$^g$XP1BE-L1.

$^h$XP3RO-L1.

$^i$XPPHBE-L1.

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*UV-induced SCE's in XP and CS Lymphocyte Cell Lines*
significant evidence against a random distribution of SCE’s in the short chromosomes (i.e., Chromosomes 16 to 22 and the Y chromosome). This consistent underrepresentation of SCE’s in the short chromosomes could be the result of a nonrandom distribution of SCE’s throughout the chromosome complement or the result of observation bias.

DISCUSSION

LCL’s derived from XP and from CS patients are suitable cells in which to study the patients’ inherited defect(s) as evidenced by the following observations: (a) XP (5, 37) and CS (A. D. Andrews and J. H. Robbins, unpublished data) LCL’s, like XP and CS fibroblasts (3), are more readily killed by UV than are normal cells; (b) LCL’s from DNA repair excision-defective XP patients have decreased rates of UV-induced unscheduled DNA synthesis (37), one reflection of defective excision repair; and (c) XP LCL’s give essentially the same results with regard to UV-induced SCE formation as do XP fibroblasts when the XP complementation group assignment of the cells is considered. Thus, it has been reported (16) that, compared with results from normal fibroblasts, significantly greater numbers of UV-induced SCE’s were found in XP fibroblasts representing XP Complementation Groups A, B, C, and D but not in those representing XP Groups E or the XP variant. Furthermore, these investigators (16) also reported that XP strains in the same complementation group could have different frequencies of UV-induced SCE’s. An advantage of the use of LCL’s rather than of fibroblasts in SCE studies is that LCL’s rather than of fibroblasts in SCE studies is that LCL’s generally provide greater and more readily accessible numbers of cells in mitosis. A disadvantage in the use of LCL’s results from the fact that LCL’s are Epstein-Barr virus-transformed lymphocytes. Since this virus is considered by some to be a potential oncogenic agent for humans (23, 31), work with LCL’s must be conducted with appropriate biohazard safeguards. Such safeguards may be of particular importance when the LCL’s are subjected to UV and 5-bromodeoxyuridine as well as to other physical and chemical mutagens that might activate the virus and that can alter the nucleic acid not only of the LCL but also of the virus present in the LCL. The results of such damage are currently not fully known.

The precise molecular events involved in SCE formation are unknown (for recent review, see Ref. 21). However, considerable information is being obtained concerning the somatic genetics (14, 24, 25, 38), mutability (28, 29), post-UV colony-forming ability (3), and chemical basis of the DNA repair defects (30) of XP. Therefore, it is likely that in the not too distant future the molecular basis for UV-induced SCE’s in XP cells will be understood with regard to the DNA repair defect(s) of XP. From the results concerning UV-induced SCE formation in our XP LCL’s, we conclude that the XP DNA repair defects do not appear to prevent either spontaneous or UV-induced SCE’s. We can recognize a partial relationship between UV-induced SCE’s and the patients’ rates of excision repair. UV-induced unscheduled

Chart 2. Histograms of the frequency distribution of SCE’s in unirradiated and irradiated LCL’s from each of the 6 forms of XP and from a CS patient. Groups A to E refer to the XP complementation groups of excision-deficient XP patients (25). UV, 14.4 ergs/sq mm. Numbered arrows, mean number of SCE’s per metaphase cell. Thirty metaphase cells were examined for all histograms, except that only 28 metaphases were examined for Histograms f and h and 25 were examined for c. Further details as in “Materials and Methods.”

for 10 of the 11 XP LCL’s, the observed distribution of SCE’s departed significantly from a random SCE distribution ($p < 0.01$ for each of the 6 control donor LCL’s, and $p < 0.02$ for each of the 10 XP LCL’s). Only XP1BE-L1 did not give significant evidence against a random distribution of SCE’s ($p = 0.25$).

Using the experimental data in Table I of Galloway and Evans (17) and the length values as reported at the Denver Conference (33, 34), we calculated (under the assumption of a random distribution of SCE’s throughout the chromosome complement) the expected frequency of SCE’s for each chromosome group presented in Table II of the report of Galloway and Evans (17). A $x^2$ goodness-of-fit test indi-
Table 2

Data and statistical analysis of SCE's in irradiated and unirradiated XP Complementation Group C LCL's

SCE's were determined and analyzed as described in "Materials and Methods."

<table>
<thead>
<tr>
<th>LCL's</th>
<th>Av. no. of SCE's/metaphase at indicated UV dose (ergs/sq mm)</th>
<th>Mean UV-induced SCE effect&lt;sup&gt;a&lt;/sup&gt;</th>
<th>95% confidence interval for mean UV-induced SCE effect&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14.4</td>
<td>7.2</td>
<td>0</td>
</tr>
<tr>
<td>XP1BE-L1</td>
<td>17.10 ± 0.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.53 ± 0.26</td>
<td>Analysis 1: 2.012 1.763, 2.262</td>
</tr>
<tr>
<td>XP1BE-L1</td>
<td>11.80 ± 1.13</td>
<td>4.53 ± 0.26</td>
<td>Analysis 2: 1.245 0.907, 1.583</td>
</tr>
<tr>
<td>XP3BE-L3</td>
<td>19.14 ± 1.40</td>
<td>5.07 ± 0.46</td>
<td>Analysis 3: 2.129 1.727, 2.531</td>
</tr>
<tr>
<td>XP3BE-L3</td>
<td>15.60 ± 0.90</td>
<td>5.07 ± 0.46</td>
<td>Analysis 4: 1.747 1.438, 2.057</td>
</tr>
<tr>
<td>XP2BE-L1</td>
<td>7.23 ± 0.60</td>
<td>3.48 ± 0.36</td>
<td>Analysis 5: 0.851 0.535, 1.167</td>
</tr>
<tr>
<td>XP2BE-L1</td>
<td>6.37 ± 0.60</td>
<td>3.48 ± 0.36</td>
<td>Analysis 6: 0.678 0.359, 0.997</td>
</tr>
<tr>
<td>XP8BE-L1</td>
<td>9.23 ± 0.59</td>
<td>4.30 ± 0.38</td>
<td>Analysis 7: 0.986 0.708, 1.269</td>
</tr>
<tr>
<td>XP8BE-L1</td>
<td>8.06 ± 0.57</td>
<td>4.30 ± 0.38</td>
<td>Analysis 8: 0.807 0.515, 1.099</td>
</tr>
<tr>
<td>XP9BE-L2</td>
<td>9.25 ± 0.64</td>
<td>4.33 ± 0.41</td>
<td>Analysis 9: 0.978 0.693, 1.263</td>
</tr>
<tr>
<td>XP9BE-L2</td>
<td>8.20 ± 0.97</td>
<td>4.33 ± 0.41</td>
<td>Analysis 10: 0.751 0.413, 1.089</td>
</tr>
</tbody>
</table>

<sup>a</sup> As in Table 1.

<sup>b</sup> Mean ± S.E.

DNA synthesis occurs at the following relative, initial rates in XP cells (24, 25, 38): XP variant (100% of normal) > Group E (>60%) > Group D (25 to 50%) > Group C (10 to 20%) > Group B (3 to 7%) > typical Group A (0.4 to 1.3%) (36). In our SCE studies the lines from XP patients with the higher rates of unscheduled DNA synthesis had the fewer UV-induced SCE's with 1 clear exception. The Group B LCL's had no significant increase in UV-induced SCE's compared to those induced in normal LCL's, despite the fact that Group B fibroblasts have only 3 to 7% of the normal rate of unscheduled DNA synthesis (24, 38). However, the Group B patient is unusual in that she has coincident CS (38, 47). While CS cells have normal rates of unscheduled DNA synthesis (4), they have decreased post-UV colony-forming ability (3, 4, 41, 42). We have found that our Group B patients' LCL's are extremely sensitive to the killing effects of UV (A. D. Andrews and J. H. Robbins, unpublished data). Thus there is considerable complexity involved in attempting to relate the XP Group B patient's LCL's to SCE formation in a meaningful way at this time. With the exception of the low frequency of UV-induced SCE's in the XP Group B patient's LCL's, it appears as though XP cells with the greatest inability to repair their UV-damaged DNA (at least with regard to unscheduled DNA synthesis) have the highest frequencies of UV-induced SCE's. This correlation suggests that the unrepai red (unexcised) DNA damage may be the cause of the UV-induced SCE's as has been suggested by Cleaver (12) in the case of...
REFERENCES


UV-induced SCE's in XP and CS Lymphocyte Cell Lines


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