Sister Chromatid Exchange in Philadelphia Chromosome (Ph')-positive Leukemia

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ABSTRACT

The sister chromatid exchange (SCE) frequency was studied in the leukemic cells of 12 patients, 10 with Philadelphia chromosome (Ph')-positive chronic myelocytic leukemia (CML), 1 with Ph'-negative CML, and 1 with acute myeloblastic leukemia. Except for two patients in the blastic phase of CML, the SCE values were within the normal range [3.8 ± 6.4 (S.D.) SCE/cell; normal is 3.3 ± 2.2 SCE/cell]. In the two cases with the blastic phase of CML, the values were 7.6 ± 3.2 and 8.9 ± 4.7 SCE/cell, a statistically significant difference from the control values. However, in the patient with acute myeloblastic leukemia, the SCE incidence increased from 3.6 to 24.4 SCE per cell when therapy was changed to daunorubicin and vincristine and the disease became progressive. Further studies on SCE and leukemia may prove the usefulness of this determination for therapeutic and clinical purposes.

INTRODUCTION

The introduction of a method for the detection of SCE in mammalian cells by Latt (4) led to the demonstration that various chemicals, ionizing radiation, and UV cause an increased incidence of SCE (3). Subsequently, it was shown that a high rate of "spontaneous" SCE occurs in the cells of patients with Bloom's syndrome (1, 6), which to date remains the only clinical condition with this phenomenon (2, 7). These findings led us to study the SCE incidence in the bone marrow cells of leukemic patients with the possibility for its potential use in following the course, clinical and therapeutic, of the disease.

Since the visualization of SCE requires that the bromodeoxyuridine be incorporated into the chromosomal DNA through at least 2 cell cycles, we chose the cells of Ph'-positive CML for our initial study, since these cells are known to divide in vitro without much difficulty. Furthermore, the presence of the Ph' left little doubt that the SCE was being examined in a leukemic cell population.

This report is of a preliminary nature, but it indicates the feasibility of such studies in cells of human leukemia and the possible significance and usefulness of the findings observed. As far as we know, this is the first report on SCE in human leukemic cells.

MATERIALS AND METHODS

Bone marrow samples were obtained from 11 patients with CML and without prior knowledge of the state of the disease, i.e., whether the patients were in the blastic phase, remission, or the chronic phase of CML. Short-term (20- to 24-hr) cultures of the marrow cells were made in Roswell Park Memorial Institute Medium 1640 with 20% fetal calf serum. Bromodeoxyuridine at a concentration of 1 μg/ml was added after 20 to 24 hr, and the culture continued for another 48 to 50 hr at 37°C. During the last 2 hr, Colcemid (0.015 μg/ml) was added, and then the cultures were terminated. The blood cells from the normal individuals were grown in the presence of PHA, whereas the latter was not added to the blood or bone marrow samples from the patients with CML or AML.

Air-dried slides were prepared at least 24 hr prior to staining. The latter was accomplished by placing the slides in 0.01% acridine orange in Sorensen's buffer at pH 6.8 to 7.0 for 6 min; the slides were then rinsed thoroughly for 6 to 7 min, mounted in the buffer, and scanned under UV optics with a Carl Zeiss photomicroscope III. The SCE frequency of normal blood cells obtained from 1 normal female and 2 normal males constituted the "control" value to which the data of the patients were compared.

RESULTS

Some of the clinical and chemotherapeutic data on the patients with leukemia and the incidence of SCE in all the individuals studied are given in Table 1. All but 1 of the CML patients (Case 6) had a Ph' chromosome. Two of the patients with CML (Cases 9 and 11) were in the blastic phase. The SCE incidence in these 2 patients was increased, with Case 9 having 8.9/cell and Case 11 having 7.6/cell. When the results of these 2 patients were compared to those in the control cultures (3.3 ± 2.2 SCE/cell), the differences were statistically significant (p = 0.05). In 3 other patients (Cases 2, 5, and 6), the incidence of SCE was somewhat higher than the control value, i.e., 5.9, 6.4, and 5.1/cell, respectively. However, in the remaining patients (Cases 1, 3, 4, 7, 8, and 10) the SCE incidence appeared to be comparable to that of the normal subjects. When the above study was completed, we had an opportunity to study the incidence of SCE in the blood cells of an AML patient having 10% Ph'-positive cells. The first study was made on blood cells cultured for 70 hr without PHA, at which time the patient had 92% myeloblasts in the blood and had started on treatment with 1-β-D-arabinofuranosylcytosine, Adriamycin, and azacytidine. The incidence of SCE was
### Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age and sex</th>
<th>Past therapy</th>
<th>Present therapy</th>
<th>Phase of CML/blast cells (%)</th>
<th>Ph+ scored</th>
<th>SCE scored</th>
<th>SCE/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal blood pooled from 3 adults</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>43</td>
<td>141</td>
<td>3.28 ± 2.24</td>
</tr>
</tbody>
</table>
| 1. AHJ   | 43 M        | Allopurinol  | Hydroxyurea     | Remission/0.0                | +          | 20         | 77       | 3.8 ± 2.2
| 2. CA    | 14 M        | Myleran      | Myleran         | Active/0.0                   | +          | 20         | 118      | 5.9 ± 2.2
| 3. FP    | 73 M        | Myleran      | Myleran         | Remission/0.0                | +          | 20         | 81       | 4.0 ± 2.5
| 4. KJ    | 45 M        | BCG          | Myleran         | Remission/2.0                | +          | 20         | 80       | 4.0 ± 2.8
| 5. OEE   | 44 M        | Myleran, BCG| Uracil mustard  | Remission/2.0                | +          | 20         | 127      | 6.4 ± 2.3
| 6. SF    | 59 M        | Vincristine  | Hydroxyurea     | Remission/4.6                | -          | 20         | 101      | 5.1 ± 2.7
| 7. SM    | 60 F        | Myleran, BCG| Hydroxyurea     | Active/3.2                   | +          | 20         | 82       | 4.1 ± 2.4
| 8. TO    | 67 M        | Hydroxyurea  | Hydroxyurea     | Remission/1.2                | +          | 20         | 79       | 4.0 ± 2.2
| 9. WCA   | 30 M        | Myleran, BCG| 6MP             | Blastic/80.4                 | +          | 20         | 178      | 8.9 ± 4.7
| 10. YJ   | 22 M        | Hydroxyurea  | Uracil mustard  | Active/5.6                   | +          | 20         | 69       | 3.4 ± 2.1
| 11. LP   | 52 M        | Myleran      | Vinblastine     | Blastic/18.0                 | +          | 20         | 163      | 7.6 ± 3.2
| 12. KR(a) | 43 M        | ara-C        | ara-C           | Blastic/92                   | 10%+       | 20         | 71       | 3.6 ± 1.4
| KR(b)    | 43 M        | Vincristine  | Vincristine     | Blastic/90                   | 10%+       | 20         | 487      | 24.4 ± 4.7

- **a** Mean ± S.D.
- **b** The abbreviations used are: BCG, Bacillus Calmette-Guérin; 6MP, 6-mercaptopurine; 5-FU, 5-fluorouracil; ara-C, 1-β-d-arabinofuranoslycytosine.
- **c** Patient with Ph+ AML.

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found to be only 3.6/cell, which is comparable to our control data. One week later, at which time the patient still had 90% myeloblasts in the blood but had been started on vincristine and daunorubicin, the incidence of SCE had risen to 24.4/cell. No new chromosomal aberrations were detected in the cells. Two subsequent attempts failed to reveal any mitoses in the blood cells, even when PHA was added. The patient died 2 weeks after our second study.
DISCUSSION

The salient features of the study presented, although they are of a preliminary nature, are as follows.

SCE can be demonstrated readily in leukemic cells, at least those of Ph1-positive CML and AML. With the exception of Case 5, the incidence of SCE in such cells appears to be within normal limits in uncomplicated chronic phase of the disease, whether compared to the incidence obtained in the blood lymphocytes of normal subjects or to the SCE frequency observed in nonleukemic marrow (6).

The presence of a Ph1 did not appear to affect the SCE incidence.

Since the blastic phase of CML or an accelerated form of the disease was not necessarily accompanied by an increased incidence of SCE, the value of this determination for prognostic and diagnostic purposes in leukemia remains to be determined.

A very high incidence of SCE can be observed in leukemic cells, which may be the result of either certain forms of chemotherapy and/or a change in the biology of the leukemic state. We tend to think that it was probably the chemotherapy that led to the greatly increased SCE incidence in Case 12 and, if so, that this points to the feasibility of using SCE as a sensitive index for chemotherapeutic parameters.

In an in vitro study with vincristine, a significant decrease of SCE in human lymphocytes was observed (8). On the other hand, in an in vitro study of bone marrow and blood cells, we have observed a 3-fold increase in the SCE incidence when daunorubicin (0.002 μg/ml) was added to the culture.

However, it is possible that in the patient with AML the increased rate of SCE in vivo was caused either by daunorubicin, by vincristine, or by the combination of the drugs.

Perry and Evans (5) allude to a significant increase in SCE in the blood cells of patients treated with Adriamycin at dose levels that produced only a negligible increase in the frequency of chromosomal aberrations. Our observations support this view, i.e., that SCE frequency may be a more sensitive index of chromosomal damage than are other cytogenetic parameters. This is particularly underlined by the fact that even in patients having a high incidence of SCE, e.g., our AML patient with nearly 25 SCE/cell, no evidence of chromosomal aberrations was encountered. Thus, SCE could turn out to be an excellent assay system for monitoring doses and/or effectiveness of various chemotherapeutic agents in leukemia.

Although much more data must be collected on many more patients with various leukemias, from our observations it would appear that the determination of SCE frequency in leukemic cells may turn out to be a useful tool. Obviously, correlations between the SCE incidence and the type of chemotherapy used, doses administered, and the time sequence, as well as other clinical parameters, must be determined in order to establish the significance of SCE changes on a firmer ground.

REFERENCES

Fig. 1. a, normal level of SCE in a Ph¹-positive cell from a patient with CML; b, high incidence of SCE in a cell from a patient with Ph¹-positive AML.
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