ABSTRACT

The frequency of sister chromatid exchange (SCE) was analyzed in bone marrow cells of 16 patients with chronic myeloid leukemia. We have compared the SCE frequency in these patients, in both chronic and blast phases of the disease, to that of normal individuals. The frequency of SCE in chronic-phase chronic myeloid leukemia patients (mean, 1.86/cell) was significantly lower than that in chronic phase (mean, 2.96/cell), which was in turn lower than that in normal individuals (mean, 4.108/cell). We have used the SCE data to compare the rates of division of the bone marrow cells. Cells from chronic myeloid leukemia patients divided more slowly than did those from normal individuals, those from blast-phase patients being slowest.

INTRODUCTION

SCE is a symmetrical interchange between sister chromatids. This phenomenon was originally described by Taylor et al. (27) with an autoradiography technique. Latt (18) introduced the current BrdUrd-labeling method which allows more precise and reliable analysis of this phenomenon. Various chemicals, ionizing radiation, and UV cause an increased incidence of SCE (16). Even though the mechanism leading to SCE is not yet fully understood, the highly increased incidence of SCE after treatment of cells with DNA-damaging agents suggests that SCE is a reflection of DNA damage and subsequent repair processes (16). The analysis of the SCE incidence after treatment with various types of mutagen-carcinogens also provides evidence for possible relevance of SCE to mutagenesis-carcinogenesis (3, 9, 21, 22).

SCE had been analyzed in cells from patients with various genetic disorders highly characterized by a susceptibility to genetic damage and proneness to develop cancer. These studies include patients with Bloom's syndrome, Fanconi's anemia, ataxia telangiectasia, and xeroderma pigmentosum (5). A greatly increased incidence of SCEs has been demonstrated in cells from patients with Bloom's syndrome (10), but cells from patients with Fanconi's anemia, ataxia telangiectasia, and xeroderma pigmentosum have displayed normal levels of SCE. When certain strains of xeroderma pigmentosum cells were treated with chemical agents, irradiation, or UV, an increased SCE frequency was observed (31).

MATERIALS AND METHODS

Patients. Bone marrow aspirates were taken from 16 patients with CML in the chronic phase of the disease and again during blastic transformation. Two other patients were first examined cytogenetically during the blast phase of the disease.

Patients in the chronic phase of the disease were studied before treatment. The 16 patients who were seen first in chronic phase received busulfan (4 to 8 mg/day) after cytogenetic examination over 4 to 48 months. All patients were untreated for at least 6 months prior to the onset of the blast phase. During blastic crisis, the samples were taken before treatment of the transformation.

Controls. Bone marrow aspirates were obtained from 10 healthy bone marrow donors.

Apart from genetic diseases, there are very few SCE studies on cells from human neoplasia (1, 2, 6, 7, 15, 17, 23). The scarcity of SCE studies in neoplastic cells seems to be partly attributable to the difficulty in obtaining a sufficient number of proliferating neoplastic cells under culture conditions. However, bone marrow cells from leukemia patients appear to readily overcome this difficulty and make it feasible not only to study SCE in leukemic cells (1, 15, 17) but also to analyze the growth pattern of such cells (1, 2, 7) with an in vitro BrdUrd-labeling technique (28).

The visualization of SCE requires that the BrdUrd be incorporated into the chromosomal DNA through at least 2 cell cycles. We studied the cells of Ph1-positive CML, since these cells are known to divide in vitro without much difficulty. The presence of the Ph1 leaves little doubt that the cells which are being examined are leukemic cells.

The patients were studied during the chronic phase of the disease and again during blastic crisis. As far as we know, this is the first comparative study of SCE and cell cycle time in the chronic and acute phases of CML in the same patients.

Received August 25, 1981; accepted May 4, 1982.

1 This work was supported in part by grants from the Fondation pour la Recherche Médicale Française, the Federation Nationale des Centres de Lutte contre le Cancer and UER des Sciences Biomédicales de Strasbourg (Legacy Ziring).

2 To whom requests for reprints should be addressed, at Institut de Puériculture, Hôpital Civil, 23, rue de la Porte de l'Hôpital, 67000 Strasbourg, France.

3 The abbreviations used are: SCE, sister chromatid exchange; BrdUrd, bromodeoxyuridine; Ph1, Philadelphia chromosome; CML, chronic myelocytic leukemia.

Claude Stoll, Franci Oberling, and Marie-Paule Roth
University of Strasbourg, School of Medicine, 67000 Strasbourg, France
karyotype was 46,XX or XY,Ph1. Cells were scored from one culture. Methods for chromosome preparation and sister chromatid differentiation by means of an acridine orange fluorescence technique were the same as those described in our previous paper (24). Metaphases were classified as first (M1), second (M2), and third (M3) generation after exposure to BrdUrd, according to the pattern of sister chromatid differentiation (18). Between 18 and 50 metaphases were evaluated per study. SCE frequency and growth kinetics were evaluated on different slides from the same sample.

RESULTS

Chromosomal Studies. In our patients in the chronic phase of the disease, we found the common type of Ph1 translocation, t(9;22)(q34;q11), in 100% of the bone marrow cells. At the blastic phase, other karyotypic aberrations were observed in addition to the Ph1 (25).

The 2 patients first examined during the blastic phase had the common type of Ph1 with additional aberrations (25).

SCE Incidence. In controls, the SCE ranged from 2.52 to 5.12 (mean, 4.108; Table 1). A lower SCE was found during the chronic phase (Table 2), ranging from 2.16 to 4.04 per cell (mean, 2.97; by Student's t test, t = 4.42, 24 d.f., p < 0.001) and again during the blastic crisis in the same patients (Table 2) ranging from 1.16 to 2.32 (mean, 1.86). Comparison of the rate of SCE during chronic and acute phases of CML shows a statistically significant difference (Student's t test for matched pairs: t = 7.51, 15 d.f., P < 0.001). The rates of SCE of the 2 patients who were studied only during blastic crisis were 3.28 and 1.78.

Cell Kinetics. In the blastic phase, the distribution of metaphases ranged from 52 to 64% for M1 metaphases and 32 to 48% for M2 metaphases. The number of M3 metaphases was very low, between 0 and 8% (Table 3).

DISCUSSION

We studied CML because the presence of the Ph1 chromosome guarantees that the metaphases which are analyzed are actually leukemic cells and not healthy cells which may be accidentally isolated due to the technical difficulties encountered. Studies of SCE in phytohemagglutinin-stimulated lymphocytes from leukemic patients were performed by Cheng et al. (11), McDonald et al. (19), and Otter et al. (20). Fonatsch et al. (13) and Wang et al. (30) studied cultured cell lines. Knuutila et al. (17), Kakati et al. (15), and Abe et al. (1) investigated acute leukemia. Knuutila et al. (17) found SCE incidence in the leukemic patients in the same range as in the controls. Kakati et al. (15) investigated acute leukemia. Knuutila et al. (17) found SCE incidence in the leukemic patients in the same range as in the controls.

SCE frequency in bone marrow from patients with CML

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>No. of metaphases</th>
<th>No. of SCEs</th>
<th>SCEs/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>F</td>
<td>50</td>
<td>25</td>
<td>5.12 ± 2.17</td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>F</td>
<td>50</td>
<td>184</td>
<td>3.66 ± 1.86</td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td>F</td>
<td>50</td>
<td>224</td>
<td>4.48 ± 2.01</td>
</tr>
<tr>
<td>4</td>
<td>56</td>
<td>F</td>
<td>50</td>
<td>126</td>
<td>2.52 ± 1.21</td>
</tr>
<tr>
<td>5</td>
<td>61</td>
<td>F</td>
<td>50</td>
<td>206</td>
<td>4.12 ± 1.83</td>
</tr>
<tr>
<td>6</td>
<td>51</td>
<td>M</td>
<td>50</td>
<td>172</td>
<td>3.44 ± 1.72</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>M</td>
<td>50</td>
<td>236</td>
<td>4.72 ± 1.87</td>
</tr>
<tr>
<td>8</td>
<td>46</td>
<td>M</td>
<td>50</td>
<td>218</td>
<td>3.98 ± 1.96</td>
</tr>
<tr>
<td>9</td>
<td>47</td>
<td>M</td>
<td>50</td>
<td>198</td>
<td>3.96 ± 1.32</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>M</td>
<td>50</td>
<td>234</td>
<td>4.68 ± 2.09</td>
</tr>
</tbody>
</table>

* Mean ± S.D.
et al. (15) evaluated SCE in Ph1-positive leukemia. They found a high SCE rate in 2 patients with the blastic phase of CML. In the other patients, the SCE values were within the normal range. Abe et al. (1) studied SCE rates of 17 patients with acute leukemia before and during chemotherapy. In 6 cases analyzed before treatment, the SCE frequency was between 3.17 and 4.54. The SCE rates of 8 nonhealthy controls (7 malignant lymphoma, 1 polycythemia vera) were not different from those of the leukemia patients. Becher et al. (7) found significantly lower SCE rates in 8 cases of acute leukemia and an increased number of M2 metaphases when compared to their control group.

SCEs were not studied in CML bone marrow cells except by Knuutila et al. (17), Kakati et al. (15), and Becher et al. (6). The patients of Knuutila et al. (17) and those of Kakati et al. (15) had had cytostatic treatment. Becher et al. (6) studied untreated patients with CML in chronic phase. They observed a statistically significant decrease in SCE frequency compared with that in their healthy controls. We have found similar results. Becher et al. (6) did not study patients with CML during blastic crisis. We found that during blastic crisis SCE rates are lower than during chronic phase in the same patients. Therefore, the SCE incidence is lower in CML than in controls and even lower in acute phase than in chronic phase. A different contraction status which might influence SCE incidence did not account for the different SCE frequencies (7). An influence on SCE frequency caused by different cell cycle times is questionable (14). It should be noted that all our patients had additional chromosomal abnormalities in the blastic phase.

Interpretation of the low SCE rates observed in CML is difficult since little is known about the biological significance of SCE. Our results might suggest that DNA repair mechanisms are less active in the acute phase than in the chronic phase of the disease since we have demonstrated that SCE incidence is lower during the blastic crisis than during the chronic phase of CML.

The growth pattern of bone marrow cells was evaluated by studying metaphases which had undergone one, 2, or 3 cycles in the presence of BrdUrd. In the normal bone marrow, a high percentage of M2 metaphases and a lower percentage of M1, and of M3 metaphases was found. Comparable percentages were calculated by Becher et al. (7): M2 = 61 to 71%, M1 = 13 to 28%; and M3 = 11.1 to 17.5%. We can therefore estimate that the fastest-growing cells which complete 3 cell cycles within 52 hr have a cell cycle time about 17 hr. More cells had passed only 2 cell cycles within 52 hr, giving a cell cycle time shorter than 26 hr. Boecker et al. (8) and Dormer (12), using autoradiographic methods, concluded that the cell cycle time of bone marrow cells was shorter. Selection of fast-growing cells in culture could explain this discrepancy (7).

Cell cycle times in the acute phase of CML were longer, inasmuch as a high number of M1 metaphases and only a few M3 metaphases were found. The results of Abe et al. (1) and Becher et al. (7) obtained by the same method agree with our conclusions. Vincent (29), who studied the cell kinetics by autoradiographic techniques, also showed that the cell cycle time is increased in acute leukemia. Abe and Sandberg (2) studied growth kinetics of bone marrow cells of acute nonlymphoid leukemia patients with diploid and aneuploid clones.
They found that the cell cycle time is longer in aneuploid leukemic cells than in diploid cells. However, except for 3 cases, their examinations were done after chemotherapy, and no marker such as the Ph1 guaranteed that the diploid cells observed were leukemic cells. In our study, the same patients were studied during the chronic phase and during the acute phase of CML. The Ph1 enabled us to consider only leukemic cells. In the same patients, we observed a longer cell cycle during the acute phase, when additional chromosomal abnormalities were present, than during the chronic phase.

REFERENCES


Sister chromatid Exchange and Growth Kinetics in Chronic Myeloid Leukemia

Claude Stoll, Francis Oberling and Marie-Paule Roth


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/42/8/3240

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.