Changes in Lymphocytes as an Early Sign of Relapse in Adult Acute Leukemia

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Kawamura, S., Abe, I., Saitoh, A., Yamaya, T., Tsuchida, S., Chiba, Y. and Yoshida, Y. Changes in Lymphocytes as an Early Sign of Relapse in Adult Acute Leukemia. Tohoku J. Exp. Med., 1981, 135 (3), 237-245 — This study was undertaken to find out some clues to detect early relapse of adult acute leukemia which had been in remission. Laboratory data such as LDH, erythrocyte sedimentation, or immunoglobulin level did not show any difference at the time of relapse from the remission period. Complete blood count with reticulocyte count was evaluated every two weeks retrospectively for 12 weeks before relapse. No significant change was observed at the time of relapse. However, both the percentage and absolute number of peripheral lymphocytes significantly increased, whereas percentages of both T and B cells markedly decreased at the time of relapse, suggesting some derangement of lymphocyte function. Results in bone marrow culture did not yield any remarkable difference. It was suggested from this study that it is the time of prerelapse of adult acute leukemia when lymphocytes show an increase in either percentage or absolute number in the patients who are followed under their maintenance therapy schedule.

Clinical sign; early relapse; adult acute leukemia; percentage of lymphocyte; T lymphocyte

Nationwide studies on the remission induction therapy of adult acute leukemia have been performed, but the mechanism of relapse in acute leukemia remains to be elucidated. In our previous reports, we examined the factors of hosts, types and nature of leukemia, and the methods of therapy to discover the most influential factor for long survival of adult patients with acute leukemia. It was concluded that maintenance therapy in remission was the most important factor (Kawamura et al. 1977). This implies that elongation of remission is the most important for longer survival. Therefore, it is of clinical importance to predict the relapse and to resume the treatment as early as possible.

In order to detect early signs of relapse in adult leukemia without frequent bone marrow punctures, hematological and immunological examinations and tissue culture studies of bone marrow cells were undertaken in the present study.

It was found that the relapse of adult acute leukemia was indicated when lymphocyte counts exceeded 40 to 50% or T and B cells decreased significantly in the patients on maintenance therapy.

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Patients and Methods

Patients. Of 101 patients with acute leukemia admitted to our Department from January 1970 to December 1978, 23 relapsed after complete remission and were subjected to this study. These 23 patients consisted of 17 males and 6 females with an age range from 18 to 53 years (Table 1). Fifteen patients had acute myelocytic leukemia (AML) and 8 acute lymphocytic leukemia (ALL). The complete remission (CR) was defined according to Kimura's criteria (Kimura et al. 1957). Relapse was indicated when leukemia cells were over 5% of the nucleated cells in the bone marrow aspirate. In 2 patients, however, relapse was diagnosed by the appearance of leukemia cells in the peripheral blood while the bone marrow contained less than 5% leukemia cells.

Biochemical and clinical examinations of blood. Total protein (TP), the ratio of albumin to globulin (A/G), lactate dehydrogenase activity (LDH), zinc sulfate test (ZST), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and immunoglobulin levels were determined at the time of remission and relapse.

Hematological examinations. Red cell counts, white cell counts with differentials, hemoglobin levels, platelet counts and reticulocyte counts in the peripheral blood were checked every 2 weeks after remission until the relapse. The data were retrospectively reviewed at the time of relapse.

Non-specific cell-mediated immunity. T-cells and B-cells were counted by E-rosette and EAC-rosette formation. The blastogenesis of lymphocytes was measured by the incorporation of tritium-uridine (3H-uridine) after stimulation with phytohemagglutinin-P (PHA-P), and was expressed as stimulation index (SI).

Cultures of bone marrow cells. Bone marrow cells aspirated from the sternum or ilium were cultured for 14 days in the modified McCoy's 5A medium containing 15% fetal calf serum with 2 x 10⁵ cells/ml at 37°C, 5% CO₂ in air (Pike and Robinson 1970). After the harvest, aggregates over 40 cells were scored as colonies, and aggregates less than 40 cells as clusters. Numbers of colonies and clusters were counted per dish (Falcon plastic, 35 x 10 mm²).

Statistical analysis. Statistical analysis was performed by the t-test.

Results

Biochemical examinations of blood. The results of biochemical and other examinations of blood are shown in Table 2. There were no significant differences between the value of TP, A/G, ZST, LDH, ESR or immunoglobulin level obtained at relapse and that obtained during the complete remission.

Hematological examinations. As shown in Fig. 1, at relapse the nucleated cell counts in the bone marrow of all 23 patients were 21.8 ± 18.2 (×10⁴) cells/mm³, leukemia cells 37.5 ± 31.8% of the nucleated cells, and erythroblasts 16.4 ± 12.1% (mean ± s.d.). The mean percentage of leukemia cells in peripheral blood smears was 9.7 ± 21.3% of white blood cells at relapse. In 12 patients, no leukemia
Early Sign of Relapse in Acute Leukemia

TABLE 2. Laboratory findings

<table>
<thead>
<tr>
<th></th>
<th>Complete remission</th>
<th>Relapse</th>
<th>Level of significance</th>
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</thead>
<tbody>
<tr>
<td>TP (g/100 ml)</td>
<td>6.7±0.4</td>
<td>6.7±0.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>A/G</td>
<td>1.7±0.1</td>
<td>1.7±0.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>ZST</td>
<td>4.8±0.8</td>
<td>6.4±3.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>LDH</td>
<td>341±108</td>
<td>417±244</td>
<td>n.s.</td>
</tr>
<tr>
<td>Sedimentation rate</td>
<td>17±14</td>
<td>32±37</td>
<td>n.s.</td>
</tr>
<tr>
<td>IgG (mg/100 ml)</td>
<td>1201±271</td>
<td>1273±518</td>
<td>n.s.</td>
</tr>
<tr>
<td>IgA</td>
<td>205±66</td>
<td>200±118</td>
<td>n.s.</td>
</tr>
<tr>
<td>IgM</td>
<td>176±50</td>
<td>173±73</td>
<td>n.s.</td>
</tr>
<tr>
<td>CRP (+)</td>
<td></td>
<td>1</td>
<td>n.s.</td>
</tr>
<tr>
<td>CRP (−)</td>
<td></td>
<td>8</td>
<td>n.s.</td>
</tr>
<tr>
<td>CRP (+)</td>
<td></td>
<td>3</td>
<td>n.s.</td>
</tr>
<tr>
<td>P.P.D (−)</td>
<td></td>
<td>4</td>
<td>n.s.</td>
</tr>
<tr>
<td>P.P.D (±)</td>
<td></td>
<td>1</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

n.s., not significant.

Fig. 1. Leukemia cells in peripheral blood and bone marrow at relapse. NCC, the nucleated cell counts.

Tumor cells were recognized in the peripheral blood smears. Until 12 weeks prior to the relapse, no significant changes in red cell count, hemoglobin level, platelet count, white cell count or reticulocyte count was recognized (Fig. 2). The percentage of lymphocytes and lymphocyte count at relapse were significantly
Fig. 2. Hematological examination — Variation of WBC, platelets (Pl) and Hb before relapse.

Fig. 3. Hematological examination — Variation of the rate of lymphocytes.
higher than those 6 weeks before relapse ($p<0.01$). (Figs. 3, 4) Three laboratory data, i.e. hemoglobin level, platelet count and reticulocyte count, at the time of relapse were compared with those 2 weeks prior to it. Decreases in all the three values were recognized in only 1 patient out of 23; the hemoglobin level by over 1 g/100 ml, platelet count by over $5\times 10^4$ /mm$^3$ and reticulocyte count by over 10
Decreases in two values and in one value were recognized in 2 and in 6 patients, respectively. No decrease in any of the three values was recognized in 10 patients. In the remaining 4, an increase in one or more test values was seen (Fig. 5).

**Non-specific cell-mediated immunity.** The percentage of T cells, quantitated by E-rosette formation, decreased significantly at relapse (45.1±28.3%, \(p<0.01\), Fig. 6) as compared with that during the remission (68.3±12.7%) (normal control 61.1±10.7%). Also, the percentage of B cells decreased at relapse (17.5±10.4%, \(p<0.05\), Fig. 7) as compared with that during the remission (25.6±10.5%) (normal control 25.9±6.9%). The stimulating indices (SI) induced by PHA-P were 3.28±2.07 at relapse and 2.36±1.68 in remission. These two values were not statistically different, but significantly low as compared with normal control values (Fig. 8).

**Cultures of bone marrow cells.** The number of colonies were 10±13/dish in remission, and 15±8/dish at relapse. The clusters were 30±29 in remission and 28±21 at relapse. The number of colonies or clusters in remission was not different from at relapse (Fig. 10).

![Fig. 6. E-rosette formation of lymphocytes.](image)

![Fig. 7. EAC-rosette formation of lymphocytes.](image)
DISCUSSION

To find some clues for detecting early relapse of adult acute leukemia without frequent bone marrow punctures, we compared the biochemical findings on sera, hematological observations, non-specific cell-mediated immunity, and the results
of bone marrow cell culture in remission with those at relapse. In some cases, the LDH level increased markedly at relapse, but the mean value for all the patients did not change from the value in remission (Table 2).

At relapse 9 of 23 patients showed decreased reticulocyte count, hemoglobin level and/or platelet count, but 10 other patients showed no decrease in any of these three values.

These tests were not useful for detecting early relapse (Fig. 5). Uzuka et al. (1978) reported that the counts of red blood cells and platelets in many leukemic patients just before relapse were within normal limits.

The rate of lymphocytes to the total white blood cells and the absolute number of lymphocytes increased significantly at relapse as compared with those 6 weeks prior to relapse (Figs. 3, 4). In many patients the lymphocyte rate was 20–30% in the remission period, and over 40% at the second week prior to the relapse. At the early phase of relapse leukemia cells were 37.5±31.8% in the bone marrow, even though no leukemia cells were recognized in peripheral blood. These findings suggested that the count and rate of lymphocytes were useful for detecting the early stage of relapse.

Hersh et al. (1971) reported that 60% of patients showing delayed hypersensitivity after immunization entered into a complete remission, as compared with only 37% of those without this reactivity. Patients converting from immunoincompetence to immunocompetence during therapy achieved remission, whereas those converting from immunocompetence to immunoincompetence did not. Go et al. (1978) discovered an inhibitor of cell-mediated immunity in sera from patients with leukemia. The inhibitory activity was especially prominent at the proliferative stage of acute myelocytic leukemia. Both effector T cells and suppressor T cells were reported to be significantly low in preleukemia according to Hoshino and Takahashi (1979).

Concerning the culture study of bone marrow cells from patients with acute myelocytic leukemia, many investigators pointed out diminished colony counts at onset or at relapse (Robinson and Pike 1970; Greenberger et al. 1971; Brown and Carbone 1971; Moore and Metcalf 1973). Chiyoda et al. (1975) suggested the presence of a suppressive factor for colony formation in sera of AML.

In our study the mean count of colony or cluster did not change significantly throughout the induction stage, remission and relapse (Fig. 9).

Furusawa et al. (1976) reported an increase in cluster count in 72% of patients with AML, and in 3 of 4 patients with preleukemia. They concluded that the marrow agar cluster and maturation defect in myeloid cells measured by the liquid culture in patients suspected of preleukemia frequently indicated an early sign of leukemic transformation. Senn and Pinkerton (1972) reported that in 3 patients a reduced colony formation was found for 27, 15 and 5 months before they were definitely diagnosed as acute leukemia.

In our cases, a few patients who showed no colony formation relapsed within 2 to 4 months, while others whose colony counts were very low maintained
remission over a year without relapse. Although Spitzer et al. (1977) suggested that the colony formation frequently returned to normal before morphological evidence of remission, the validity of colony formation as an early marker of relapse seemed to be elucidated.

In conclusion, when a decrease in T and B lymphocytes or an increase in peripheral blood lymphocytes over 40 to 45% is recognized during maintenance therapy, the bone marrow puncture should be immediately performed even though there are no leukemia cells in the peripheral smear.

References


