CONCISE REPORT

Selective Sensitivity of Chronic Myelogenous Leukemia Cell Populations to Alkyl-Lysophospholipids

By Reinhard Andreesen, Manuel Modolell, and Paul Gerhard Munder

In order to evaluate the specificity of alkyl-lysophospholipid-induced cell destruction, peripheral blood leukocytes from chronic myelogenous leukemia (CML) patients in chronic phase of the disease as well as in blastic crisis have been separated by density centrifugation. These subpopulations, enriched for the different maturation stages, were tested for their sensitivity to alkyl-lysophospholipids. It is shown that myelocytes in chronic phase CML are resistant, but blast cells from both clinical stages as well as maturational defective myelocytes from blastic crisis CML are highly sensitive to these antimetabolites. In contrast to chronic phase CML myelocytes, these sensitive cells show a high lysophospholipid adsorption rate and lack an O-alkyl-cleavage enzyme.

Alkyl-lysophospholipids represent a new class of antitumor agents. We have shown previously that these compounds, when adsorbed to serum proteins, induce progressive destruction of freshly isolated human leukemic cells in vitro. Due to a lack of O-alkyl-cleavage enzyme in leukemic cells, these alkyl-lysophospholipids accumulate and interfere with the normal phospholipid metabolism, which is responsible for the continuous renewal of important membrane components.

In contrast, normal cells are able to metabolize these compounds and are affected to a much smaller extent. In this study, we report separation experiments of CML cells that evaluate the specificity of the cell destruction induced by alkyl-lysophospholipids. It is shown that the attack is directed exclusively on neoplastic hematopoietic cells and is correlated with a high adsorption rate and an inability to degrade alkyl-lysophospholipids.

MATERIALS AND METHODS

Alkyl-lysophospholipids

Dr. H. U. Weltzien of the Max-Planck-Institut kindly provided 1-Octadecyl-2-methyl-sn-glycero-3-phosphocholine and 1-[8-3H] octadecyl-2-methyl-sn-glycero-3-phosphocholine.

Cell Separation

Leukocytes from the peripheral blood of CML patients were isolated as described previously. After the last washing, they were resuspended in 2 ml of 1.13 g/ml Percoll stock solution (Pharmacia, Freiburg, F.R.G.). A 3-step discontinuous density gradient (3 ml of densities 1.07, 1.06, and 1.05 g/ml) was prepared by mixing the stock solution with appropriate volumes of phosphate-buffered saline (PBS).
This was then layered on top, and the tubes were centrifuged at room temperature for 40 min at 1000 g. Interphase cells were collected, washed 3 times in PBS, and resuspended in Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal calf serum (FCS) and supplemented as described.1

Cell Cultures

Cells were grown as suspension cultures at 10⁶ cells/ml in microtiter plates (Falcon Plastics, Oxnard, Calif.) or on gas-permeable membranes (Petriperm, W. C. Heraeus, 6450 Hanau, F.R.G.). As described elsewhere,2 cell proliferation was measured by a 24-hr incorporation of ³H-thymidine (Amersham and Buchler, 3300 Braunschweig, F.R.G.; specific activity 21 Ci/m mole).

Lipid Adsorption and Analysis

One-milliliter samples from the cultures were centrifuged, the cell pellet washed once in PBS, and the radioactivity of ³H-ET-18-OCH₃ measured in the sediment and in the supernatant. O-alkyl-cleavage activity* was measured as described elsewhere.2

RESULTS

Peripheral blood leukocytes of CML patients in blastic crisis as well as in the chronic phase of the disease represent a heterogeneous cell population. Cells at all stages of maturation are present. We have used density centrifugation to separate these different subpopulations. In all nine patients tested (four with blastic crisis, five in the chronic phase), three fractions with different cell densities could be obtained (Table 1). All blast cells were recovered in the light-density fraction (I) and myelocytes, although seen in all 3 fractions, concentrate in the <1.07 g/ml fraction. At present, we are strongly involved in the further purification of the myelocytes by high-speed centrifugation over a continuous gradient. Both cell types, blast cells as well as myelocytes, belong to the proliferative compartment of granulopoiesis7 and show a high ³H-thymidine incorporation (Table 2).

In CML blastic crisis, the unseparated cells, as well as the three subpopulations, were all highly sensitive to the cell destruction induced by ET-18-OCH₃. In contrast, when the cells were tested in the chronic phase of the disease only the blast-cell-enriched fraction was affected by ET-18-OCH₃, but ³H-thymidine incorporation into cells of fraction II was not changed (Table 2). This demonstrates that the ³H-thymidine-incorporating granulocyte precursors (myelocytes7) are presum-

<table>
<thead>
<tr>
<th>CML</th>
<th>Cell Fraction Density (g/ml)</th>
<th>Percent of Total Cells</th>
<th>Percent of Cells</th>
<th>Blast</th>
<th>Myelocyte†</th>
<th>Band</th>
<th>Granulocyte</th>
<th>Lymphocyte/Monocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blastic crisis</td>
<td>—</td>
<td>&lt;1.06</td>
<td>25</td>
<td>40</td>
<td>23</td>
<td>—</td>
<td>—</td>
<td>7/3</td>
</tr>
<tr>
<td></td>
<td>&gt;1.07</td>
<td>—</td>
<td>9</td>
<td>75</td>
<td>12</td>
<td>—</td>
<td>—</td>
<td>3/—</td>
</tr>
</tbody>
</table>

| Chronic phase | —                           | <1.06                  | 100             | 9     | 41         | 24   | 18          | 3/3                 |
|              | >1.07                       | —                      | 53              | 56    | 28         | —    | —           | 3/12                |

*Erythrocytes and the majority of mature granulocytes had been removed by prior centrifugation over Ficoll-Hypaque.
†Includes promyelocytes and metamyelocytes.
Table 2. Cytotoxic Effect of ET-18-OCH₃ on Subpopulations of Chronic Myelogenous Leukemic Cells

<table>
<thead>
<tr>
<th>CML</th>
<th>Cell Fraction (Density g/ml)</th>
<th>Predominating Cell Type</th>
<th>Control* (cpm)</th>
<th>ET-18-OCH₃ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blastic crisis</td>
<td>---</td>
<td>Unseparated</td>
<td>43,996†</td>
<td>11‡</td>
</tr>
<tr>
<td>I</td>
<td>&lt; 1.06</td>
<td>Blast</td>
<td>41,708</td>
<td>15</td>
</tr>
<tr>
<td>II</td>
<td>&lt; 1.07</td>
<td>Myelocyte, blast</td>
<td>25,056</td>
<td>12</td>
</tr>
<tr>
<td>III</td>
<td>&gt; 1.07</td>
<td>Myelocyte, band</td>
<td>3,078</td>
<td>6</td>
</tr>
<tr>
<td>Chronic phase</td>
<td>---</td>
<td>Unseparated</td>
<td>26,846</td>
<td>8</td>
</tr>
<tr>
<td>I</td>
<td>&lt; 1.06</td>
<td>Blast</td>
<td>25,544</td>
<td>17</td>
</tr>
<tr>
<td>II</td>
<td>&lt; 1.07</td>
<td>Myelocyte, band</td>
<td>11,480</td>
<td>122</td>
</tr>
<tr>
<td>III</td>
<td>&gt; 1.07</td>
<td>Band, granulocyte</td>
<td>3,185</td>
<td>166</td>
</tr>
</tbody>
</table>

* Quadruplicate cultures of 2 × 10⁵ cells were grown in 0.2 ml supplemented with DMEM + 10% FCS in microtiter plates with and without 5 μg/ml ET-18-OCH₃. After 48 hr in culture 0.2 μCi ³H-thymidine was added in 0.05 ml. After 24 hr, incorporation into cellular DNA was measured.
† Mean of 4 cultures, SD < 10%.
‡ Data expressed as percentage of the control.

Fig. 1. Adsorption and cleavage of ET-18-OCH₃ in CML subpopulations in chronic phase of the disease (e — e density < 1.06 g/ml; — — density < 1.07 g/ml; v — v density > 1.07 g/ml); 2 × 10⁵ cells/ml were grown in the presence of 2 μg/ml ET-18-OCH₃. (A) Adsorption rates are given as μg ET-18-OCH₃/10⁵ cells. (B) Cleavage is given as percentage of adsorbed ET-18-OCH₃. For detail of lipid analysis see ref. 2.
ably not affected by alkyl-lysophospholipids. Morphological observation of the unseparated CML cell cultures with ET-18-OCH₃ revealed that after 4 days these cultures are completely depleted of blast cells. Myelocytes and the other more mature precursors were unaffected.

The overall decrease in ³H-thymidine incorporation in unseparated cells might be explained by the liberation of metabolic thymidine by phagocytic cells⁸ or by the release of growth inhibitory substances from destroyed blast cells.⁹ We have previously demonstrated¹² that human leukemic cells adsorb lysophospholipids at a high rate and are unable to metabolize alkyl-lysophospholipids. Therefore, we examined these parameters on the described subpopulations of chronic phase CML. As shown in Fig. 1 (A and B), only the blast cells of the light-density fraction show a high adsorption rate and are nearly unable to cleave the O-alkyl-bond of the ET-18-OCH₃ molecules (Fig. 1B). In contrast, myelocytes and more mature forms adsorb much less, but metabolize these compounds with an increasing rate.

**DISCUSSION**

Chronic myelocytic leukemia can be divided into a chronic phase and a blastic crisis stage. Chronic phase CML is thought to reflect a defect in stem cell proliferation.¹⁰¹¹ This results in a mass production of myeloblasts, which are capable of maturing into normal cells both in vitro and in vivo.¹²⁻¹⁴ In contrast, CML cells in blastic crisis are characterized by a defect in cellular maturation, which accompanies the excessive proliferation of the transformed stem cell clone.¹²⁻¹⁴ By density centrifugation, we could separate CML cells into subpopulations enriched for the different maturation stages and thereby showed that only blast cells and maturationally defective forms were sensitive to the cell destruction induced by alkyl-lysophospholipid (Table 1). The sensitivity of the neoplastic hematopoietic cells to the antimetabolic effect of ET-18-OCH₃ was correlated with a high adsorption rate and the inability of the cells to metabolize these adsorbed lysophospholipids (Fig. 1). The absence of an O-alkyl-cleavage enzyme in neoplastic cells has been reported by Soodsma et al.¹⁵ and confirmed by us for different human leukemias.² In addition, we have shown that human leukemic cells differ from normal cells by a high adsorption rate of alkyl-lysophospholipids.³ Therefore, these alkyl-compounds accumulate and inhibit the synthesis of 3-sn-phosphatidylcholine in neoplastic cells.⁴

The defective cellular maturation of the blast cells in blastic crisis results in myelocytes and young granulocytes, which are still “leukemic” in terms of alkyl-lysophospholipid adsorption and metabolism. The cause of this high adsorption rate in neoplastic cells is still under investigation but might already be considered as a characteristic of tumor cell membranes.

In contrast, in chronic phase CML, myelocytic cells that develop from leukemic blast cells are considered to be morphologically and functionally normal. They adsorb less of the alkyl-lysophospholipid, while showing a high O-alkyl-cleavage activity and are rather stimulated in the presence of ET-18-OCH₃ (Table 1). Thus, the observed low adsorption as well as high metabolic rate of the myelocytes in chronic phase CML provide additional proof of the normality of these cells. Furthermore, the maturation of leukemic cells along the normal pathway seems to be accompanied by the induction or derepression of the O-alkyl-cleavage enzyme.
The differential sensitivity of leukemic and normal myelocytes to alkyl-lysophospholipids might add further support to the value of these compounds as possible specific antimetabolites in tumor therapy.

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