A Study of Leukemic Cell Injury by Physical Agents


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SUMMARY

Human leukemic cells were tested for fragility by physical agents. The results obtained from this study are summarized as follows: (a) acute monocytic leukemia was relatively resistant to the hyperthermic and hypotonic states; (b) acute myeloblastic leukemia was fragile to the hyperthermic state and resistant to the hypotonic state; (c) acute lymphoblastic leukemia was fragile to both the hyperthermic and hypotonic states; (d) two chronic lymphatic leukemias were highly resistant to the hyperthermic state and fragile to the hypotonic state, and the other two chronic lymphatic leukemias were fragile to both states. The data indicate that leukemic cells of various types have their own biophysical properties.

Utilizing these properties of various types of leukemic cells, we may be able to obtain useful information regarding the nature of leukemic cells and thus determine the biophysical criteria for the classification of leukemias.

The mechanisms or factors effecting differences in biophysical properties among the various types of leukemias are also discussed.

INTRODUCTION

Although the biophysical properties of erythrocytes are well understood and are considered in the diagnosis of various hemopoietic diseases (3–5, 9, 15, 22), little previous work has touched on the similar properties of leukocytes (6, 14, 17, 20, 23). Since there is no pigment released from the leukocyte to act as an index of destruction, as in the lysis of the erythrocyte, leukocytolysis is more difficult to demonstrate than erythrocytoysis.

The osmotic fragility of leukemic cells has been studied by several investigators (1, 13, 16, 19, 26). Morrow et al. (16), Magalini and Djerassi (13), and Westring and Brittin (26) reported that human leukemic lymphocytes were more resistant to hypotonic cytolysis than were normal leukocytes. Also, Reif and Allen (19) studied the fragility of normal and leukemic lymphocytes of AKR mice and found that newly derived leukemias from AKR mice possessed a resistance to injury by several physical agents that was higher than the resistance of various types of normal lymphocytes. These data suggest that the biophysical properties of leukocytes make it possible to differentiate normal leukocytes from pathological ones (or leukemic cells).

In this study, the biophysical properties of leukemic cells have been investigated in the hope that useful information might be provided in regard to their nature or that such data might be of value in determining the criteria for the biophysical classification of leukemias. The fragility of leukemic cells of various types to cytolysis by physical agents such as hypotonic media and the hyperthermic state was evaluated by the technique of vital dye exclusion.

MATERIALS AND METHODS

Leukemic Cells. Leukemic cells of various types were obtained from 33 nontreated leukemic patients with more than 80% leukemic cells in peripheral leukocytes (Table 1). According to morphological (12) and histochemical criteria (24), 7 AMoL's, 13 AML's, 9 ALL's, and 4 CLL's were found.

Separation of Leukemic Cells from Peripheral Blood. Nontreated leukemic patients with more than 80% leukemic cells in peripheral leukocytes were selected for this study. Blood was drawn from the cubital vein with a heparinized syringe [10 ml blood; 200 i.u. Heparin Novo (Novo Industrials, Copenhagen, Denmark)] and was allowed to stand at room temperature until there was complete separation of the red blood cell layer from the leukocyte-platelet-rich plasma layer. Then the leukocyte-platelet-rich plasma layer was decanted into another tube, washed once with Earle's solution, and centrifuged at 1500 rpm for 15 min to remove the platelet and plasma. Sedimented leukocytes thus collected were resuspended with Earle's solution and adjusted to 6 X 10^7/ml. Cell suspension containing more than 95% viable cells were used for this experiment.

Table 1

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of leukemic patients</th>
<th>AMoL</th>
<th>AML</th>
<th>ALL</th>
<th>CLL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–9</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>10–19</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>20–29</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30–39</td>
<td>8</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>40–49</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50&gt;</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

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1 The abbreviations used are: AMoL, acute monocytic leukemia; AML, acute myeloblastic leukemia; ALL, acute lymphoblastic leukemia; CLL, chronic lymphatic leukemia; OFI, osmotic fragility index; HFI, hyperthermic fragility index.
Osmotic Cytolysis Test. Earle's solution consisting of 6.80 g NaCl, 0.40 g KCl, 0.20 g CaCl₂, 0.20 g MgCl₂·6H₂O, 0.15 g NaH₂PO₄·2H₂O, 1.00 g glucose, and 2.00 g NaHCO₃ in 1000 ml distilled water was assigned a tonicity of 1.00 unit. The solution was diluted stepwise with distilled water, and the following mixtures of stock solutions of Earle's solution: distilled water with tonicities from 1.00 to 0.10 unit were made and stocked under refrigeration: 1.00 unit, 1.00:0.00; 0.70 unit, 0.70:0.30; 0.50 unit, 0.50:0.50; 0.30 unit, 0.30:0.70; 0.20 unit, 0.20:0.80; 0.10 unit, 0.10:0.90; 0.00 unit, 0.00:1.00. Whenever the study was performed, the same lots of these buffered solutions were used.

Aliquots of 0.40-ml buffered solutions with the various tonicities described above were pipetted into a series of test tubes. Cell suspension (0.10 ml) was then added to each tube; the contents were mixed and the tubes were set at 37° for 2 hr. The percentage of cell viability was determined by the vital dye exclusion technique (7). The percentage of stained cells in each tube was plotted on a graph in which abscissa and ordinate were expressed as tonicity and stained cell percentage, respectively. The end point for the hypotonic cytolysis test (OFI) was the tonicity below 1.00 unit required to produce vital staining of one-half of the viable cells present in the original cell suspension. For a check of the reliability of the buffered solutions and for a control standard for cytolysis, the erythrocytes obtained from healthy subjects were used as the control. Complete hemolysis occurred in a range from 0.3 to 0.2 unit hypotonic Earle's solution. The value of OFI lower

![Chart 1](image-url)

Chart 1. Differences in hyperthermic and osmotic fragility among various types of leukemias. A test tube containing 0.1 ml leukemic cell suspension (6 × 10⁶ cells) and 0.4 ml Earle's solution was placed at 56°. At various time intervals, the stained cell percentage in the cell suspension was determined. The end point (HFI) which was obtained from the graph as described in "Materials and Methods" is plotted in A. HFI is expressed as the time required for loss of integrity of the cell membrane, as judged by vital staining of one-half of the viable cells present before heating at 56°.

Aliquots of 0.4 ml buffered solutions with the various tonicities described in "Materials and Methods" and 0.1 ml leukemic cell suspension were placed at 37° for 2 hr. Then, the percentage of stained cells in each tube was determined. The end point (OFI) for the hypotonic cytolysis which was obtained by the procedure described in "Materials and Methods" is plotted in B. OFI is expressed as the tonicity below 1.00 unit required to produce vital staining of one-half of the viable cells present in the original cell suspension.
Leukemic Cell Injury by Physical Agents

Hyperthermic fragility

Osmotic fragility

Chart 2. Differences in hyperthermic and osmotic fragility between peroxidase-negative and -positive acute leukemic cells. Leukemic cells were divided into 2 groups (peroxidase positive and peroxidase negative) according to the peroxidase reactivity of these cells.

<table>
<thead>
<tr>
<th>Peroxidase</th>
<th>Positive</th>
<th>Negative</th>
<th>Peroxidase</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean of fragility index</td>
<td>9.02</td>
<td>5.08</td>
<td>0.09</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Standard error</td>
<td>1.73</td>
<td>1.24</td>
<td>0.03</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Statistical analysis (F-test)</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

Chart 1A shows the hyperthermic fragility of 4 types of leukemic cells. AMoL was more resistant to the hyperthermic state than were AML and ALL. However, it was shown that the difference in HFI between AMoL and AML, AMoL and ALL, or AML and ALL was not statistically significant (p > 0.05). Two CLL's were extremely resistant, and the other 2 were as fragile as ALL was to the hyperthermic state.

Chart 1B shows the osmotic fragility of 4 types of leukemic cells. ALL was more fragile to the hypotonic state than were AMoL and AML. The difference in OFI between ALL and AMoL or between ALL and AML was statistically significant (p < 0.01 and 0.01 < p < 0.05, respectively).

Chart 2 shows the difference in hyperthermic or hypotonic fragility between peroxidase-negative and -positive leukemias. As to hyperthermic fragility, peroxidase-positive and -negative leukemias had mean values of 9.02 (S.E. 1.73) and 5.08 (S.E.
DISCUSSION

Technically, many methods have been developed to evaluate the leukocyte fragility to various biophysical conditions such as the hyperthermic and cryoscopic states (19), hypo- or hypertonic media (1, 6, 14, 19, 20, 26), and ultrasonic vibration (16). Some methods used were biochemical assay of enzyme of nucleoprotein eluted from leukocytes (13, 26), fragilograph (17), leukocytolysis (20, 23), and vital dye exclusion technique (19). The vital dye exclusion technique used in this study has been generally approved as the best method for the evaluation of loss of integrity of the cell membrane.

AMoL tended to be more resistant to the hyperthermic condition than were AML and ALL, although there was no statistical difference among them (p > 0.05). It is debatable, however, whether the resistance of AMoL cells to the hyperthermic condition is specific for AMoL. AMoL cells consist of monoblasts and promonocytes, and the ratio of monoblasts to promonocytes differs according to the particular case. Four cases of AMoL with a HFI above the average value had more than 50% monoblasts in peripheral leukemic cells, and the remaining 3 with a HFI below the average value had more than 90% monoblasts in them. These findings lead to an assumption that the promonocyte is more resistant to the hyperthermic state than is the monoblast. Therefore, promonocyte-predominant AMoL tends to be more resistant to this state than monoblast-predominant AMoL.

Two out of 4 CLL’s were highly resistant, and the remaining 2 were fragile to the hyperthermic state. Richards and Richards (20) and Nir et al. (17) found (as we did) that, while in most of CLL cells there was increasing osmotic resistance, increased fragility was demonstrated in some. These observations mean that there might be 2 groups in CLL’s as regards their biophysical properties; one is highly resistant and another is fragile to the hyperthermic state. The biophysical heterogeneity of CLL cells might be brought about by the differences in aging and biochemical, enzymatic, morphological, or antigenic properties.

With the osmotic cytolysis test, the biophysical characteristics of the leukemic cells determined by the hyperthermic cytolysis test are not compatible with those obtained by the osmotic cytolysis test. The differences are as follows: (a) AMoL and AML were equally resistant to the hypotonic state, while AML was fragile and AMoL tended to be resistant to the hyperthermic state; (b) AML and AMoL were more resistant to the hypotonic state than was ALL, while AML was as fragile as ALL to the hyperthermic state; (c) although some CLL’s were resistant to the hyperthermic state, they were fragile to the hypotonic state. In this respect, it is very likely that hypotonic and hyperthermic conditions might affect a different point on the cell for the loss of integrity of cell membrane.

As shown in Chart 2, the peroxidase-negative group was more fragile to the hypotonic state than was the peroxidase-positive one. This result means that ALL and AML with peroxidase-negative staining are more fragile to the hypotonic state than are peroxidase-positive AML and AMoL. Considering the close correlation of the biophysical fragility of
ALL to its sensitivity to the antileukemic agent, a possibility might exist that certain biophysical agents in which leukemic cells are placed affect leukemic cell injury or destruction in the same way that the antileukemic drugs do. In addition, a cause of spontaneous remission in the leukemic patient, rarely seen on and after severe infection, might be associated with the cell fragility to biophysical agents such as high fever, plasmin activity elevation, pH change, local osmotic pressure change, etc.

Because of the great differences in chemotherapeutic effects between adult and childhood leukemias, analysis concerning the relationship between hyperthermic and osmotic fragility of acute leukemic cells and age of the acute leukemic patient was carried out. The correlation between hyperthermic fragility and age of the acute leukemic patient was statistically significant (0.02 < p < 0.05). However, as shown in Table 1, childhood leukemias examined in this study consisted of all ALL’s, AML’s predominated in adults and adolescents, and AMoL’s predominated in the old. Thus it can be supposed that the close correlation between them is due to the close relationship between the type of leukemia and the age of the leukemic patient.

From the results obtained in this study, it is obvious that the biophysical properties of leukemic cells are closely associated with leukemic type rather than age of the patient. Utilizing the differences in the properties of the various types of leukemias, one might derive criteria for the classification of acute leukemias as follows: (a) AMoL is relatively resistant to both the hyperthermic and hypotonic states; (b) AML is rather fragile to the hyperthermic state and resistant to the hypotonic state; and (c) ALL is fragile to both physical conditions.

The mechanisms of the hemolytic response of erythrocyte to hypotonic stress are well known. Complex enzyme systems are required by the cell to maintain membrane integrity during exposure to the hypotonic solutions (8). When certain of these enzymes are defective, erythrocyte osmotic fragility is altered (2, 21, 25). An analogy might exist concerning the hypotonic or hyperthermic fragility of the leukocyte or leukemic cell. In fact, several investigators (10, 11, 18) have reported that there are either qualitative or quantitative differences in several enzymes among various types of leukemic cells. It seems possible, therefore, that a difference in the biophysical properties among various types of leukemias might be one result of the enzyme-associated metabolic dysfunction.

There might be certain differences in several properties among various types of human leukemic cells such as cell aging and morphological, biochemical, and antigenic properties. Further investigation on the relationship of biophysical properties of these cells to the other properties is in progress.

REFERENCES


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Moriji Miura, Kohei Kawashima, Hiroshi Nishiwaki, et al.


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