Comparison of the Morphology and Enzyme Activity of Mononuclear Cells from Fischer 344 Rats with Either Spontaneous or Transplanted Leukemia

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ABSTRACT

Mononuclear cell (MNC) leukemia was identified in 26-month-old F344 rats by splenomegaly, reduced red blood cell counts, and elevated white blood cell counts. Atypical MNC were predominant in spleen and blood with acentric nuclei and red cytoplasmic granules. Pentose shunt, glycolytic, and Krebs cycle enzymes were elevated 2- to 11-fold in the enriched MNC fraction (Ficoll-Paque density gradients, 1.077 g/ml) isolated from spleen. A leukemic MNC line was derived from one of the spontaneously leukemic donors and then maintained in vivo by s.c. transfer of 2 × 10^7 spleen cells into 7-8-week-old syngeneic recipients. In these serial transplantation experiments leukemia that was clinically and morphologically indistinguishable from spontaneous leukemia in 104-week-old rats was induced in 22-24-week-old rats. Enhanced enzyme activity in MNC was not essential to maintain the phenotypic expression of Fischer rat leukemia. The pattern of biochemical response in spleen MNC from transplanted cases was the opposite of that previously noted in spontaneously leukemic rats, with 50-70% decreases in the specific activities of pentose shunt enzymes and malate dehydrogenase. Reversal of the expression of these enzymes in MNC may be related to a difference in the growth rate of the tumors or to selective proliferation of the transplanted leukemic cells. In addition acetylcholinesterase activity decreased 35-85% in MNC of spleen and blood. Transplantable MNC from F344 rats provide abundant tumorigenic material with a novel biochemical expression that may be useful in the study of chemotherapeutic intervention.

INTRODUCTION

Fischer rat leukemia was described by Moloney (1) as a spontaneous MNC leukemia originating in the spleen (2) with secondary involvement of many tissues in the body. The incidence of spontaneous leukemia in the Fischer strain was 11 of 43 in females and 10 of 43 in males, averaging 24.4% (1, 3). The duration of the disease after appearance of leukemic cells in peripheral blood averaged 5 weeks; age at death ranged between 14 and 30 months with a median of 25 months. Other cases of Fischer rat leukemia that developed leukemia between 91 and 292 days posttransplantation.

Toward this end we have separated, quantitated, and utilized discrete populations of MNC derived from spontaneous cases of Fischer rat leukemia. An in vivo cellular transplant model was subsequently developed that compressed the time course for expression of leukemia to 103-112 days, thereby enabling production of large amounts of biological material in a reasonable length of time to carefully validate the model and to determine the value of these cellular biochemical responses as markers for leukemia. Toward this end we have separated, quantitated, and identified an enriched MNC population from the peripheral blood and spleens of F344 rats and report here a comparison of the morphology and enzyme activities of cells derived from hosts with either spontaneous or transplanted MNC leukemia.

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1 The abbreviations used are: MNC, mononuclear cell; PCV, packed cell volume; HB, hemoglobin; G6PD, glucose-6-phosphate dehydrogenase; 6-PGD, 6-phospho-gluconic dehydrogenase; HIMS, hexose monophosphate shunt pathway; IDH, isocitric dehydrogenase; MDH, malate dehydrogenase; TCA, tricarboxylic acid cycle; ACHE, acetylcholinesterase; HBSS, Hanks' balanced salt solution.

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MATERIALS AND METHODS

One of our contract laboratories, Southern Research Institute in Birmingham, AL, assisted us in identifying leukemic rats by preparing blood smears from control F344 rats being used in 2-year carcinogenicity tests. Fifty control rats were sampled and by hematological examination we selected six to eight positive or negative cases for leukemia that were subsequently verified at necropsy. At termination 4-ml heparinized blood samples from the inferior vena cava and single cell suspensions from the spleen were collected from each rat, placed on ice, and transported by plane to the National Institute of Environmental Health Sciences, Research Triangle Park, NC, for further processing the following day. In addition body weights, organ weights, spleen impressions, and selected tissues for histopathological analysis were collected from each of the rats.

The whole spleen from controls or about 2 g of leukemic spleens were crushed in a Petri dish with heparinized HBSS and the slurry was passed through gauze. Single cell suspensions were prepared by expressing the coarsely filtered material through a 25-gauge needle. Either 3 ml of these cell suspensions or 3 ml of blood were layered on the top of Ficoll-Paque gradients and centrifuged at 400 x g in a swinging bucket rotor at 5°C for 20 min, and the enriched MNC fraction at a density of 1.077 g/ml was removed, washed twice in HBSS, counted, and resuspended at appropriate concentrations.

MNC were identified by light microscopy as normal or leukemic in blood smears, spleen smears, and in concentrated single cell cytopsin preparations after Wright-Giemsa staining. Cells for transplantation or enzymatic analyses were counted on an Ortho ELT-8 hematology analyzer.

Cell suspensions from the blood and spleen of one selected leukemic rat were washed, counted, and suspended on Ficoll-Paque gradients and an enriched MNC fraction was separated, identified, and quantitated.

Syngeneic 6-7-week-old male F344 rats were given s.c. injections of 0.25 ml of 2 x 10^7 of these leukemic mononuclear spleen cells in HBSS. When animals exhibited unequivocal signs of leukemia, including weight loss, pale eyes, and icterus, they were sacrificed and an aliquot from a pool of their leukemic mononuclear spleen cells was used for transplantation into the next group of syngeneic recipients. The original leukemic mononuclear spleen cell line has thus been maintained by serial transplantation. The remainder of these cells, as well as mononuclear blood cells from leukemic rats, were cryopreserved at -70°C at each transplantation passage.

RBC and WBC, PCV, and HB concentration were measured by the Bradford-Coomassie blue dye-binding method (22) with a commercial reagent (Bio-Rad Laboratories, Richmond, CA) and run against a standard curve of bovine serum albumin. The cellular supernatant was assayed for enzyme activities that included G6PD (EC 1.1.1.49) and 6-PGD (EC 1.1.1.43) from the HMS pathway, pyruvate kinase (EC 2.7.1.40) and lactate dehydrogenase (EC 1.1.1.27) from the Embden-Meyerhof pathway, iCDH (EC 1.1.1.42) and MDH (EC 1.1.1.37) from the TCA cycle, and the membrane-bound enzyme ACHE (EC 3.1.1.7).

All of the enzyme activities were measured under linear conditions so that activities were proportional to amount of added supernatant and elapsed time. If possible enzymes were analyzed in a batch mode utilizing a centrifugal analyzer with automatic pipetor (Centrifichem; Baker Chemical Co.), but, when necessary, enzymes with lower activities were measured on an automated recording Model 250 spectrophotometer (Gilford Instruments Corp.). Within the limits of sensitivity enzyme activities attained with the two instruments were identical. All of the enzyme assays except ACHE used coenzyme-linked standard methods (23) that measured the oxidation or reduction of NAD-NADP at 340 nm. ACHE was measured at 405 nm using the method of Ellman et al. (24). Enzyme activities were related to protein concentration in the supernatant and were expressed as specific activities (nmol substrate transformed per min per mg protein). Data were analyzed for statistical significance using Student’s t test.

RESULTS

Spontaneous Leukemia

Body Weight, Spleen Weight, and Hematology. The body weight of rats developing spontaneous leukemia was less than 10% below that of normal rats, but absolute spleen weight and spleen/body weight ratios were 4-fold greater in leukemic rats (Table 1). There was a 13-fold increase in WBC and 30-50% decreases in RBC, PCV, and HB concentration in spontaneously leukemic rats compared to the 26-month-old controls.

Serum Chemistry. There were no clinical chemistry determinations made on animals with spontaneous leukemia. However, the typical clinical pathology profile of hypoglycemia, hyperbili-rubinemia, and elevated serum enzyme responses in spontaneously leukemic F344 rats were reported previously (9), and the responses detailed later in this report for transplanted leukemic cases were identical.

Pathology. The spontaneous MNC leukemia has been adequately described in the Fischer 344 rat (1, 3, 9) and presented no differently in this study. Large indented acenric nuclei, decreased cytoplasmic/nuclear ratios, and numerous red-stained, cytoplasmic granules are typical features of these leukemic cells (Figs. 1 and 2). The spleen and the liver were infiltrated by leukemic MNC in all of the cases and the lung, lymph nodes, adrenals, kidneys, and bone marrow were usually infiltrated as well.

Cellular Biochemistry. The activities of six enzymes in the

| Table 1 |
| Body weight, spleen weight, and hematology in F344 rats with spontaneous leukemia (mean ± SE, N = 6) |
| Body (g) | Spleen (g) | Spleen/Body × 100 | WBC (10^3/mm^3) | RBC (10^6/mm^3) | PCV (%) | HB (g/dl) |
| Controls | 470 ± 19 | 1.70 ± 0.28 | 0.36 ± 0.06 | 9.9 ± 1.9 | 8.1 ± 0.9 | 44.0 ± 4.1 | 16.5 ± 1.5 |
| Leukemic | 433 ± 13 | 6.81 ± 1.71 | 1.63 ± 0.52 | 133 ± 48 | 4.0 ± 0.9 | 26.1 ± 4.0 | 11.3 ± 0.9 |
| Change | 0.9 ± 1.9 | 4.1 ± 1.7 | 4.5 ± 1.3 | 13 ± 6.8 | 0.5 ± 1.0 | 0.8 ± 0.4 | 0.7 ± 0.2 |

* NS, not significant.
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Table 2
Enzyme activity in mononuclear cells from F344 rats with spontaneous leukemia (mean ± SE, N = 6–8)

<table>
<thead>
<tr>
<th>Enzyme Activity (milliunits/mg protein)</th>
<th>Normal</th>
<th>Leukemic</th>
<th>Change *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen mononuclear cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G6PD</td>
<td>32 ± 10</td>
<td>8 ± 4</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>6PGD</td>
<td>54 ± 6</td>
<td>1829 ± 171</td>
<td>4 ± 3</td>
</tr>
<tr>
<td>PK</td>
<td>40±1</td>
<td>110±20</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>LDH</td>
<td>16±8</td>
<td>272±216</td>
<td>2.3±x</td>
</tr>
<tr>
<td>ICDH</td>
<td>20±3</td>
<td>118±110</td>
<td>2.3±x</td>
</tr>
<tr>
<td>MDH</td>
<td>21±4</td>
<td>1296±441</td>
<td>2.1±x</td>
</tr>
<tr>
<td>Blood mononuclear cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>112 ± 21</td>
<td>47 ± 8</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>Leukemic</td>
<td>119 ± 17</td>
<td>41 ± 6</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>Change</td>
<td>11±1</td>
<td>0.8±x</td>
<td>4 ± 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\* PK, pyruvate kinase; LDH, lactate dehydrogenase.

MNC from spleen and peripheral blood are shown in Table 2. The specific activities of all enzymes were elevated from 2- to 11-fold in leukemic spleen MNC compared to normal splenocytes. By comparison there were variable and smaller increases (2–3-fold) in leukemic MNC from blood that were restricted to enzymes from the Embden-Meyerhof pathway and the tricarboxylic acid cycle.

Transplanted Leukemia

Body Weight and Organ Weight. In the first serial transplantation of leukemic mononuclear spleen cells recipients were killed at 112 days posttransplantation. There was a 6% decrease in body weight, a 15-fold increase in absolute spleen weight, and a 16-fold increase in spleen/body weight ratio (Table 3). In the second serial transplantation recipients were killed 103 days after cell transfer. These rats weighed 34% less than controls and there were 20- and 30-fold increases in absolute spleen weight and spleen/body weight ratio, respectively.

Hematology. Table 4 illustrates hematograms from the serial transplantation experiments. The WBC in leukemic rats increased 8–27-fold while the RBC decreased more than 60% below controls. The PCV and HB concentration exhibited comparable decreases.

Serum Chemistry. Table 5 depicts clinical pathology in the leukemic MNC recipients, characterized by hypoglycemia and marked hyperbilirubinemia, with total bilirubin concentrations 6–10-fold greater than controls. There were 3–6-fold increases in alanine aminotransferase, 2–3-fold increases in alkaline phosphatase and sorbitol dehydrogenase, and a 2-fold increase in lactate dehydrogenase activity. There were no detectable changes in the serum enzyme activities of α-hydroxybutyric dehydrogenase and creatine kinase or in the concentrations of serum urea nitrogen or serum protein (data not included).

Pathology. The transplanted cases of MNC leukemia were fundamentally identical to the spontaneous cases and it was impossible to delineate between them by histopathological examination. In all transplanted cases the spleen and liver were extensively involved. In addition all lymph nodes, and particularly the mesenteric lymph nodes, were moderately to severely infiltrated by leukemic MNC. All other tissues known to be affected in spontaneous leukemia were similarly infiltrated by leukemic MNC in the transplantation cases.

Cellular Biochemistry. Enzyme activities in spleen MNC from rats with transplanted leukemia were lower than controls (transplant cases 1 and 2 in Table 6) in direct contrast to the 2.3–10.5-fold increases in the same cellular enzymes from spontaneously leukemic rats (Table 2). These decreases were 50–70% below controls in the serial transplantation experiments (P < 0.01) with respect to glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase, and malate dehydrogenase.

The pattern of enzyme responses in blood MNC from rats with transplanted leukemia were not as uniform. The decreases below controls (P < 0.05) in glucose-6-phosphate and 6-phosphogluconate dehydrogenases and in isocitrate dehydrogenase enzyme activities were not reproduced in blood MNC in the second serial transplantation, but the 2–3-fold significant increases in malate dehydrogenase activity were present in blood MNC in both transplantation experiments (Table 6). The latter increase was the only cellular enzyme response in rats with induced leukemia.

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Table 5
Clinical chemistry in F344 rats with transplanted leukemia (mean ± SE, N = 12)

<table>
<thead>
<tr>
<th>Test</th>
<th>Serial transplantation 1</th>
<th>Controls</th>
<th>Leukemic</th>
<th>Change</th>
<th>Serial transplantation 2</th>
<th>Controls</th>
<th>Leukemic</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>198 ± 6</td>
<td>0.34 ± 0.07</td>
<td>216 ± 29</td>
<td>69 ± 6</td>
<td>127 ± 3</td>
<td>28 ± 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>141 ± 5</td>
<td>2.70 ± 0.46</td>
<td>368 ± 47</td>
<td>232 ± 56</td>
<td>242 ± 10</td>
<td>82 ± 11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH*</td>
<td>0.7X</td>
<td>6X</td>
<td>2X</td>
<td>3X</td>
<td>2X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALAT</td>
<td>98 ± 9</td>
<td>4.10 ± 0.81</td>
<td>602 ± 122</td>
<td>441 ± 74</td>
<td>398 ± 14</td>
<td>55 ± 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALK-P</td>
<td>0.5X</td>
<td>16X</td>
<td>6X</td>
<td>3X</td>
<td>2X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDH</td>
<td>28 ± 16</td>
<td>58 ± 11</td>
<td>26 ± 9</td>
<td>55 ± 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* LDH, lactate dehydrogenase; ALAT, alanine aminotransferase; ALK-P, alkaline phosphatase; SDH, sorbitol dehydrogenase.

** Animals in transplantation 1 sacrificed at 112 days and in transplantation 2 at 103 days postinjection with leukemic mononuclear spleen cells. All changes significant, at P < 0.05.

Table 6
Enzymatic activity in mononuclear cells from F344 rats with spontaneous or transplanted leukemia

<table>
<thead>
<tr>
<th>Test</th>
<th>Spontaneous cases</th>
<th>Serial transplant cases</th>
<th>Blood MNC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N = 6-8)</td>
<td>Experiment 1 (N = 10)</td>
<td>Experiment 2 (N = 10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(N = 6-8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Experiment 1 (N = 10)</td>
</tr>
<tr>
<td>G6PD</td>
<td>32 ± 10</td>
<td>56 ± 6</td>
<td>112 ± 21</td>
</tr>
<tr>
<td></td>
<td>127 ± 16</td>
<td>28 ± 2</td>
<td>119 ± 17</td>
</tr>
<tr>
<td>6PD</td>
<td>8 ± 4</td>
<td>18 ± 4</td>
<td>47 ± 8</td>
</tr>
<tr>
<td></td>
<td>54 ± 6</td>
<td>14 ± 1</td>
<td>41 ± 7</td>
</tr>
<tr>
<td>PKd</td>
<td>2 ± 1</td>
<td>5 ± 1</td>
<td>5 ± 2</td>
</tr>
<tr>
<td></td>
<td>20 ± 3</td>
<td>4 ± 0.7</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>LDH</td>
<td>374 ± 83</td>
<td>800 ± 33</td>
<td>560 ± 102</td>
</tr>
<tr>
<td></td>
<td>1629 ± 171</td>
<td>737 ± 45</td>
<td>1295 ± 441</td>
</tr>
<tr>
<td>ICDH</td>
<td>2 ± 2</td>
<td>2 ± 0.5</td>
<td>4 ± 1</td>
</tr>
<tr>
<td></td>
<td>21 ± 4</td>
<td>2 ± 0.6</td>
<td>11 ± 5</td>
</tr>
<tr>
<td>MDH</td>
<td>1181 ± 210</td>
<td>2299 ± 98</td>
<td>696 ± 118</td>
</tr>
<tr>
<td></td>
<td>2721 ± 216</td>
<td>1032 ± 48</td>
<td>1481 ± 369</td>
</tr>
</tbody>
</table>

* Rats were 104 weeks old.
** Rats were 22-24 weeks old.
† Significantly different from controls, at P < 0.05.
∥ PK, pyruvate kinase; LDH, lactate dehydrogenase.
* One sample only.

The specific enzyme activity of acetylcholinesterase in rats with transplanted leukemia (Table 7) decreased 34% below controls in spleen MNC (P < 0.05) and 85% below controls in blood MNC (P < 0.01). In the second serial transplantation MNC acetylcholinesterase activity was 87% below control values in spleen and 57% below control values in blood (P < 0.01).

** Mononuclear Cell Protein Concentration **

The total protein concentration in the enriched MNC preparations of blood were lower in leukemic than in control rats and the 50% decrease was comparable in both the spontaneous and transplanted cases of leukemia. By contrast the total protein concentration in enriched MNC preparations from spleen was consistently greater in leukemic than in control rats and the 2-fold increase was comparable in both the spontaneous and transplanted cases of leukemia (data not presented).

DISCUSSION

The clinical and morphological presentations of spontaneous and transplanted leukemia in F344 rats were indistinguishable. Leukemic animals lost weight, were pale, and had palpable spleens that were as much as 20-fold larger than normal. WBC were grossly elevated to up to 27 times normal and RBC and PCV and HB concentrations were markedly reduced.

The pattern of MNC enzyme responses from spontaneously leukemic rats describes an elevation of enzymes in glycolysis and the hexose monophosphate shunt pathway similar to that similar to the cellular enzyme responses in spontaneously leukemic rats.
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Table 7

<table>
<thead>
<tr>
<th>Enzyme Activity</th>
<th>Spleen</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific activity (milliunits/mg protein)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylcholinesterase</td>
<td>85 ± 11</td>
<td>44 ± 5</td>
</tr>
<tr>
<td>Change</td>
<td>56 ± 3</td>
<td>6.4 ± 0.7</td>
</tr>
<tr>
<td>Significance</td>
<td>0.7x</td>
<td>0.7x</td>
</tr>
</tbody>
</table>

*Animals were given s.c. injections of 2 x 10^7 spleen mononuclear cells from F344 rats with spontaneous leukemia; animals from transplantations 1 and 2 sacrificed at 112 and 103 days postinjection, respectively.

In subsequent transplantation experiments to be reported in detail in a separate paper, morphological and biochemical responses were measured at monthly intervals. Decreases in glucose-metabolizing enzymes from spleen MNC were not limited to the terminal phases of the disease, but in some enzymes the decreases in activity preceded other diagnostic indices of leukemia, when active tumor proliferation was presumably under way.

There appear to be only a limited number of hypotheses that could explain this dramatic change in expression of enzymatic activity from the leukemic spleen mononuclear cells. The original leukemic MNC from spontaneously leukemic rats may have become gradually altered after serial transplantation through syngeneic hosts, culminating in the enzymatic expression described here.

It is also possible that the preponderant type of MNC isolated from the spleens of rats with induced leukemia was functionally and therefore biochemically different than the preponderant cell type isolated from spleen of rats with spontaneous leukemia. Szelenyi et al. (34) found that acetylcholinesterase activity of normal human peripheral blood lymphocytes was entirely confined to the lower density T-lymphocytes and was deficient in B-lymphocytes. Later the same investigators (35) separated the peripheral blood lymphocytes from normal donors and from patients with chronic lymphocytic leukemia and found a 60–95% decrease in acetylcholinesterase activity in leukemic cells correlated with an increase in cell counts. Functional membrane marker analyses by erythrocyte-forming rosette technique indicated that the decrease in acetylcholinesterase activity was due to decreased T-cell content in the mixed lymphocyte population and was supported by the finding that purification and concentration of the T-cells led to restoration of normal acetylcholinesterase activity.

We have demonstrated 34–87% decreases in acetylcholinesterase activity in the spleen MNC from rats with transplanted leukemia, strongly suggesting that there was a specific decrease in the percentage of T-cells in the mixed mononuclear spleen cell culture. Stromberg et al. (11) previously used identical procedures to isolate mixed mononuclear spleen cells from the same source of animals as studied here. They reported a 69% decrease in T-cells in 2-year-old Fischer 344 rats with spontaneous leukemia. We were not previously assaying acetylcholinesterase activity and thus cannot directly compare MNC acetylcholinesterase activity between rats with spontaneous and transplanted leukemia.

It is more difficult to adequately explain the decrease in HMS and glycolytic enzyme activities in leukemic cells by a change in cellular composition. Ordinarily neoplastic cells display an integrated pattern of biochemical responses characterized by increases in these glucose metabolizing enzymes for energy production, NADPH production for biosynthetic processes, and provision of ribose 5-phosphate for RNA and DNA syntheses (25–28, 36–38).

There are, however, data to indicate that certain biochemical responses vary inversely with the rate of growth of tumor transplants. Denton et al. (38) examined human colon tumors and tumor xenografts in nude mice and reported that HMS and glycolytic enzyme activities were much lower in slowly growing, well differentiated colon tumor xenografts than in rapidly growing, poorly differentiated ones, with primary human colon carci-
nomas showing intermediate activities. For example there were 400% greater increases in pyruvate kinase and 500% greater increases in G6PD activities in the rapidly growing line of human colon carcinoma xenografts carried in nude mice than in the slowly growing tumor cell line, compared to normal colon mucosa enzyme activities. In another comparison there were 150% increases in HEMS enzyme responses observed in spontaneous human colon carcinomas, whereas in chemically induced mouse colon carcinomas there were no changes in G6PD and transaldolase activities and a 40% decrease in the 6-PGD enzyme level (39) as compared to normal colon mucosa enzyme activities.

An analogous situation may exist in the rat leukemia transplant model used here since the growth rate of the splenic leukemic cells should be unchecked and faster in immunologically deficient MNC from transplanted cases may depend more on salvage enzymatic expressions that may be useful in the study of chemotherapeutic intervention.

We conclude that enhanced mononuclear cell enzymatic activity is not essential for the phenotypic expression of Fischer rat leukemia since the enzymatic responses between the spontaneous and transplanted leukemia cases were diametrically opposed. Elevated glycolysis and HEMS enzyme activities may reflect a neoplastic transformation necessary for de novo nucleic acid syntheses in spontaneous cases whereas the neoplastic MNC from transplanted cases may depend more on salvage pathways for their nucleotide requirements. Regardless of the reasons for these metabolic differences, these transplantable rat MNC provide abundant tumorigenic material with novel biochemical expressions that may be useful in the study of chemotherapeutic intervention.

REFERENCES

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Fig. 1. Morphology of F344 rat mononuclear leukemic cell. Wright-Giemsa-stained peripheral blood smear. The acentric nucleus and numerous cytoplasmic granules of the large leukemic cell are evident. × 2,000.

Fig. 2. Ultrastructural morphology of a F344 rat mononuclear leukemic cell. The large indented acentric nucleus, golgi apparatus, mitochondria, and electron-dense granules (red granules of Wright-Giemsa-stained cells) are evident. × 13,600.
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