Asparagine Metabolism in Some Lymphoproliferative Disorders

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SUMMARY

Lymphocytes obtained from normal individuals and patients with acute lymphocytic leukemia, chronic lymphocytic leukemia, and lymphosarcoma with leukemic blood picture were incubated with uniformly labeled asparagine-14C. The intermediary metabolism was followed by determination of radioactivity in respiratory CO₂, protein, lipids, organic acids, and free amino acids. The greatest quantitative and qualitative changes in asparagine metabolism were found in lymphosarcoma with leukemic blood picture. Chronic lymphocytic leukemia resembled the normal pattern with acute lymphocytic leukemia occupying an intermediate position. With progression of chronic lymphocytic leukemia, changes in asparagine metabolism occurred.

INTRODUCTION

The use of L-asparaginase in the treatment of the lymphocytic leukemias (2, 3, 8, 13, 16) and the development of tests to predict sensitivity to this enzyme (11, 14, 19) emphasize the importance of investigating the intermediary metabolism of asparagine in normal and leukemic lymphocytes. The purpose of the present study was to investigate some aspects of the in vitro metabolism of uniformly labeled L-asparagine-14C by lymphocytes obtained from normal individuals and patients with AcLL, CLL, and LySa. Qualitative and quantitative differences in L-asparagine metabolism were observed between the normal and experimental groups and variations in metabolism were noted.

MATERIALS AND METHODS

Blood was obtained from 4 untreated patients with AcLL, 7 patients with CLL, 5 patients with LySa, and 9 normal individuals. The 4 patients with AcLL and the 3 patients with LySa were children between 3 and 9 years old. The diagnosis of LySa was made on the basis of the clinical presentation and the presence of atypical lymphocytes in the blood classified by some authors as “lymphosarcoma cells (1, 5, 15, 17, 26).

To avoid some of the confusion which exists in the determination and classification of the diseases included in the group of “lymphoproliferative disorders,” we describe more precisely 2 of our experimental groups.

All 4 children in the group of AcLL had similar clinical pictures: absence of local or generalized lymphadenopathy, splenomegaly, or hepatomegaly. All had anemia, thrombocytopenia, and WBC between 8,000 and 22,000/cu mm with 87 to 100% lymphoblasts. Bone marrows showed lymphoblastic infiltration with no mitotic figures. The patients responded to conventional therapy and achieved complete remission. The survival time of all patients is more than 16 months.

All 3 children with LySa had generalized lymphadenopathy and hepatosplenomegaly and subsequently developed anemia and thrombocytopenia with WBC between 154,000 and 510,000/cu mm. Differential count showed 92 to 100% atypical lymphocytes with the characteristics of lymphosarcoma cells. The lymphosarcoma cell is described as a lymphocytic cell which varies in size and shape and contains a nucleus with overlapping borders and one large or several small nucleoli with marked condensation of chromatin at the rim (1, 5, 15, 17, 26). Bone marrow revealed infiltration by the above-described cells and the presence of many mitotic figures. None of these patients had a complete remission, and their survival time was 5 to 7 months.

The 2 adults with LySa had generalized lymphadenopathy, hepatosplenomegaly, and anemia with the subsequent development of a leukemic blood picture. The differential count showed 88 to 92% atypical lymphocytes with the characteristics of lymphosarcoma cells. The lymph node biopsy revealed lymphosarcoma.

Blood was drawn into heparin (30 units/10 ml) and 5% dextran (Sigma Chemical Company, St. Louis, Mo.; M.W., 200,000) solution in a 4:1 proportion for acceleration of red blood cell sedimentation. The WBC suspension was decanted and centrifuged for 5 min at 100 × g. The supernatant was discarded, and the sedimented leukocytes were washed in 0.9% NaCl solution and centrifuged at 60 × g for 5 min. The supernatant was dis-
Lipids were extracted from the original sediment in three steps with acetone, ether, and chloroform:methanol (65:35). The specific lipid composition was not determined.

The remaining residue was primarily protein. The protein fraction was hydrolyzed by means of the proteolytic enzyme Pronase, a product of Calbiochem, Los Angeles, Calif., in order to determine the radioactivity of some of the individual amino acids incorporated into the proteins.

The radioactivity of all metabolic products was determined with a Beckman liquid scintillation spectrometer, Model LS 150. The calculations were expressed as milli moles of asparagine utilized by each fraction.

RESULTS

The metabolic products obtained from L-asparagine metabolism are indicated in Table 1 and Chart 1. Lymphocytes from patients with AcLL and CLL showed an insignificant difference in the conversion of L-asparagine to CO₂ as compared to normal lymphocytes. The production of CO₂ by lymphocytes from LySa patients was significantly greater (p < 0.01) than in any of the other 3 groups. The same difference was found for proteins and organic acids. These data are presented in Chart 1.

In 8 experiments (normal lymphocytes, AcLL, and CLL), lactic acid comprised 12 to 24% of the whole organic acid fraction. In the 3 experiments with lymphocytes from LySa patients, lactic acid was 33 to 47% of the organic acid fraction. Lymphocytes from CLL patients converted L-asparagine to other free amino acids within normal limits, whereas AcLL and LySa showed an increased conversion.

The total utilization of L-asparagine representing the conversion to CO₂, protein, lipids, organic acids, and free amino acids is shown in Chart 2. A marked increase in total utilization was found in LySa group.

The conversion of L-asparagine to specific free amino acids is presented in Table 2. Because of the presence of trace quantities, an analysis of variance was performed only for aspartic acid and arginine (18). The conversion of L-asparagine to aspartic acid was similar for normal, AcLL, and CLL lymphocytes. However, the conversion was increased in lymphocytes from patients with LySa (p < 0.01). Glutamic acid was produced only by lymphocytes from AcLL and LySa, whereas alanine was found in all the leukemic groups. Conversion of asparagine to arginine was slightly increased in all the experimental groups. Unidentified free amino acids were produced only by the leukemic groups.

Segments of the radiograms obtained from the monitor of an amino acid analyzer are presented in Figs. 1 and 2. Glutamic acid was identified in AcLL and LySa and was absent in CLL and normal lymphocytes. The figures also emphasize a greater conversion of L-asparagine to aspartic acid in LySa.

Changes in the utilization of L-asparagine were followed serially in 3 patients with CLL. One of them is considered and the sedimented leukocytes were suspended in autologous serum. Differential WBC indicated that the cell suspension consisted of between 86 and 100% lymphocytes. All preparations were made in siliconized glassware at room temperature.

The incubation and chemical determination of the metabolic products was performed according to methods described elsewhere (6, 20, 25). The cells were incubated in siliconized 50-ml Erlenmeyer flasks containing a center well. Uniformly labeled asparagine-¹⁴C (2.5 μCi) was added to the cells suspended in serum. The radioactive material was purchased from the New England Nuclear Corporation, Boston, Mass. It was assayed by paper chromatography and was found to be radiochemically pure. The incubations were carried out with 100 to 200 × 10⁶ cells (25 × 10⁶/ml serum). The flasks were closed with serum caps and flushed for 2 min with 95% O₂ and 5% CO₂. The flow of the gas mixture was less than 0.5 liter/min, which had no toxic effect on the cells. Incubation mixtures were agitated at 37° for 2 hr. After incubation, 2 ml NCS solubilizer, a basic reagent from the Amersham-Searle Company, Des Plaines, III., were injected into the center well. The reaction was terminated by injection of 1 ml 35% HClO₄ into the main compartment containing the cells. The flasks were then allowed to stand for 3 hr at room temperature to complete the diffusion of CO₂ into the NCS solution. The radioactivity of this solution was then determined. After addition of 15 ml water, the incubation mixture was centrifuged for 10 min at 10,000 × g. The sediment was washed 3 times by suspension in 10 ml 1% HClO₄ followed by centrifugation. The amino acids from the combined supernatant solutions were separated by a Dowex 50 (H⁺ form) column eluted with 1.5 N ammonia. After the eluate was evaporated to dryness, the amino acids were dissolved in 1 ml water and chromatographed (20 µl) on Whatman No. 3MM paper strips in saturated phenol-water solvent and 1-butanol:water:acetic acid (5:4:1) solvent. The chromatograms were monitored for ¹⁴C with a paper strip scanner, Model RSC-363 (Baird Atomic, Cambridge, Mass.). The identity of the amino acids was determined by spraying ninhydrin onto the duplicate strips with known amino acids. Coincidence of radioactivity with known amino acids. Coincidence of radioactivity with known amino acids. Coincidence of radioactivity with known amino acids. Coincidence of radioactivity with known amino acids.

The organic acid fraction of the original eluate obtained after separation of the amino acids was extracted continuously with ether for 48 hr in order to obtain the extractable organic acids. After neutralization, the ether was evaporated, the lactic acid carrier was added, and lactate was purified by chromatography on a Celite column (22, 23). The identification of lactate was determined by means of an organic acid analyzer (silicic acid column) obtained from Waters Associates, Framingham, Mass. (10). The attempt to determine α-keto acids failed.

MAY 1970 1339

Asparagine in Lymphoproliferative Disorders
Table 1

Metabolic products obtained from l-asparagine metabolism

<table>
<thead>
<tr>
<th>Metabolic products are expressed as mmoles/10^6 cells/hr.</th>
<th>CO₂</th>
<th>Protein</th>
<th>Lipids</th>
<th>Organic acids</th>
<th>Amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AcLL</td>
<td>4</td>
<td>0.82 ± 0.28</td>
<td>9.13 ± 3.59</td>
<td>3.59 ± 1.18</td>
<td>1.01 ± 0.72</td>
</tr>
<tr>
<td>CLL</td>
<td>7</td>
<td>1.41 ± 0.66</td>
<td>4.31 ± 1.32</td>
<td>2.82 ± 1.05</td>
<td>2.96 ± 1.28</td>
</tr>
<tr>
<td>LySa</td>
<td>5</td>
<td>5.32 ± 2.93</td>
<td>21.82 ± 11.45</td>
<td>6.06 ± 2.86</td>
<td>11.18 ± 6.77</td>
</tr>
<tr>
<td>Normal lymphocytes</td>
<td>9</td>
<td>1.12 ± 0.45</td>
<td>5.37 ± 2.35</td>
<td>2.92 ± 1.01</td>
<td>2.08 ± 1.22</td>
</tr>
</tbody>
</table>

It is apparent from the data shown in Table 1 and Chart 1 that differences in the various metabolic fractions occurred only in LySa. It is of interest to compare the amount of CO₂ obtained from AcLL and LySa. CO₂ production is significantly increased in the latter group. This can only represent the metabolism of the TCA cycle. The higher conversion of l-asparagine to lactate could also indicate indirectly increased activity of the TCA cycle in LySa. Degradation of the lactic acid molecule would give more information for support of this interpretation. Increased metabolic activity of the TCA cycle in LySa has been previously demonstrated (7, 21).

The results obtained from protein, lipid, and organic acid fractions show only quantitative differences between

DISCUSSION

The known metabolic pathways of asparagine (12) are shown in Chart 4. Asparagine is utilized for protein synthesis, hydrolyzed to aspartic acid by the enzyme L-asparaginase, or converted by transamination reaction to α-ketosuccinamic acid. A specific amidase hydrolyzes α-ketosuccinamic acid to oxaloacetic acid which can then be metabolized via the TCA cycle or converted by another transamination reaction to aspartic acid (9, 12). The main pathways of aspartic acid metabolism are also indicated. Of particular importance is the direct enzymatic conversion of aspartic acid to asparagine by the enzyme asparagine synthetase. The above-mentioned reactions were determined in plants, microorganisms, animal tissues, and cells (9, 12). However, these metabolic pathways have not been studied in either human or leukemic lymphocytes.

It is apparent from the data shown in Table 1 and Chart 1 that differences in the various metabolic fractions occurred only in LySa. It is of interest to compare the amount of CO₂ obtained from AcLL and LySa. CO₂ production is significantly increased in the latter group. This can only represent the metabolism of the TCA cycle. The higher conversion of L-asparagine to lactate could also indicate indirectly increased activity of the TCA cycle in LySa. Degradation of the lactic acid molecule would give more information for support of this interpretation. Increased metabolic activity of the TCA cycle in LySa has been previously demonstrated (7, 21).

The results obtained from protein, lipid, and organic acid fractions show only quantitative differences between
Table 2
Conversion of asparagine to other free amino acids

<table>
<thead>
<tr>
<th></th>
<th>No. of cases</th>
<th>Aspartic acid</th>
<th>Glutamic acid</th>
<th>Arginine</th>
<th>Alanine</th>
<th>Unidentified</th>
</tr>
</thead>
<tbody>
<tr>
<td>AcLL</td>
<td>4</td>
<td>Mean ± S.D.</td>
<td>50.31 ± 4.87</td>
<td>3.82 ± 0.67</td>
<td>4.48 ± 0.82</td>
<td>1.86 ± 0.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S.E.</td>
<td>1.21</td>
<td>0.17</td>
<td>0.20</td>
<td>0.16</td>
</tr>
<tr>
<td>CLL</td>
<td>7</td>
<td>Mean ± S.D.</td>
<td>45.77 ± 4.33</td>
<td>0-traces</td>
<td>4.40 ± 1.09</td>
<td>1.02 ± 0.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S.E.</td>
<td>0.61</td>
<td>3.51 ± 0.70</td>
<td>0.15</td>
<td>0.04</td>
</tr>
<tr>
<td>LySa</td>
<td>5</td>
<td>Mean ± S.D.</td>
<td>74.76 ± 9.39</td>
<td>0.14</td>
<td>3.79 ± 0.64</td>
<td>1.68 ± 0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S.E.</td>
<td>1.87</td>
<td>0.43</td>
<td>0.13</td>
<td>0.01</td>
</tr>
<tr>
<td>Normal lymphocytes</td>
<td>9</td>
<td>Mean ± S.D.</td>
<td>46.88 ± 3.34</td>
<td>Traces</td>
<td>2.45 ± 0.51</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S.E.</td>
<td>0.37</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Molecules/10⁶ cells/hr.

Fig. 1. Segment of radiograms from amino acid analyzer. No difference between normal lymphocytes and lymphocytes from CLL. GLU, glutamic acid; ASP(NH₂), asparagine; ASP, aspartic acid.

LySa and the other groups. A partial hydrolysis of the protein fraction did not reveal any qualitative difference.

The distribution of the amino acids shown in Table 2 indicates that asparagine is poorly converted to glutamic acid by normal lymphocytes and lymphocytes from patients with CLL. The synthesis of this acid by lymphocytes from AcLL and LySa should be considered to be a result of a transamination reaction with α-ketoglutaric acid. Conversion of asparagine to arginine could be achieved only via the urea cycle, during which arginine is synthesized. Alanine most probably is the result of transamination reaction via pyruvate. This amino acid is poorly synthesized by normal lymphocytes.

It is difficult to determine from the overall metabolic pattern of asparagine which pathway is preferential for the various types of lymphoproliferative disorders. The overall contribution of the transamination reaction between asparagine and α-ketosuccinamic acid to the total synthesis of aspartic acid was not determined in our study. The lack of precise information about the incorporation of asparagine into the proteins (difficult hydrolysis) leaves unanswered questions about the extent of participation of this amino acid in protein synthesis in normal and leukemic cells.

Many hematologists have emphasized the peculiar abnormalities in the cytoplasm and nucleus of the lymphoproliferative disorders.
Fig. 2. Segment of radiograms from amino acid analyzer. Glutamic acid identified in AcLL and LySa. Greater conversion of L-asparagine to aspartic acid; ASP (NH₂), asparagine; ASP, aspartic acid.

Chart 3. Serial studies of total utilization of asparagine in a case of terminal stage CLL. Normal range and range for lymphosarcoma cell leukemia indicated by stippled areas.

ACUTE LYMPHOCYTIC LEUKEMIA

LYMPHOSARCOMA CELL LEUKEMIA

Chart 4. Major metabolic pathways of asparagine and aspartic acid.

We have no explanation for the 2 different types of leukemic lymphocytes in childhood leukemia. In these groups, the origin of the lymphocyte may play an important role. Thus, cells originating from the thymus could have different metabolism as compared to those originating from lymphoid tissue in the bone marrow. More studies are required to support such a speculation.

It is generally accepted that blastic transformation in CLL is unusual (5). Our observations in 3 cases with CLL sarcoma cell and accept its existence (1, 5, 15, 17, 26).

In this study with asparagine, as in previous studies with propionic acid, acute leukemia in children could be metabolically subdivided into 2 groups. The metabolic differences correlated with the clinical presentation, and the pattern of the more aggressive group was similar to that of lymphosarcoma in adults. This finding gave us reason to present the results as 1 group designated "lymphosarcoma" with leukemic blood picture. These studies emphasize the importance of metabolic determination of the lymphoproliferative disorders even when the morphological picture is debatable and subject to various interpretations.
showed alteration in asparagine metabolism with progression of the disease. Even in the absence of dramatic morphological changes of the lymphocytes in the terminal stages of CLL, the metabolic pattern of asparagine was similar to that of LySa. Similar observations were reported by Vereschagina (24), who demonstrated an increase in glycogen of the lymphocytes with progression of CLL. Our results indicate that CLL can terminate in a more malignant form and that transitional forms of this disease exist. Our data further suggest that a significant change in the metabolism of lymphocytes during the course of the lymphoproliferative disorders should be interpreted as a poor prognostic sign.

The differences found in asparagine metabolism might correlate with the effectiveness of L-asparaginase therapy in some lymphoproliferative disorders. If the therapeutic use of L-asparaginase is shown to be of value, then in vitro metabolic studies may elucidate the relationship between the metabolic changes in the lymphocytes and the effectiveness of the enzyme.

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