Metabolism of Proline in a Human Leukemic Lymphoblastoid Cell Line

Georges Lorans, Claude Rosenfeld, Maurice Petitou, Françoise Phan-Dinh-Tuy, and Georges Mathé
Institut de Cancérologie et d’Immunogénétique, 14-16 Avenue Paul-Vaillant-Couturier, 94800–Villejuif, France

ABSTRACT

Amino acid analysis of the culture medium was carried out in a human leukemic lymphoblastoid cell line (REH) established from the lymphoblasts of a patient with acute lymphoid leukemia. The results are compared with those of a reference cell line (LHN₅) established from normal human lymphocytes. The most striking difference between these two cell lines concerns proline. In LHN₅ the concentration of this amino acid in the culture medium increases by 40 μg/ml/10⁶ cells during a 72-hr incubation. In REH there is a decrease under the same culture conditions. In both cell lines proline is derived from glutamic acid and from arginine, as found with the use of ¹⁴C-labeled precursors. Synthesis of proline in the REH line represents approximately 26% of the value measured in LHN₅ when the precursor is glutamic acid and 15% when the precursor is arginine. The radioisotopic assay for Δ'-pyrroline-5-carboxylate reductase showed that there is a deficiency of this enzyme in the REH cells. The defect in proline synthesis of REH was found at the establishment of this line and constitutes a metabolic marker that has persisted for more than 2 years.

INTRODUCTION

The REH line has been established in culture from the lymphoblasts of a patient with acute lymphoid microlymphoblastic-type leukemia (17).

This line has several peculiarities: (a) it does not contain the Epstein-Barr virus, as shown by the nucleic acid hybridization technique; (b) the Epstein-Barr virus nuclear antigen is not detected by the immunofluorescence test; (c) REH cells possess surface receptors for Epstein-Barr virus, but they are not susceptible to infection by this virus; (d) chromosomal abnormalities (45,XX, 2B-, 1C+) found in the lymphoblasts of the patient persist in the cells of the established line (15).

Because of these different characteristics, the REH line is considered as an exceptional line, and its antigenic properties, its membrane components, and its metabolism are under active investigation.

This paper presents the first results obtained in the study of amino acid metabolism. These results are compared with those of a reference cell line (LHN₅) established from normal, human lymphocytes (14).

MATERIALS AND METHODS

Establishment of Continuous Cell Lines. The culture of fresh cells has been made according to the technique of Moore et al. (10). The medium was Roswell Park Memorial Institute Tissue Culture Medium 1640 (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 20% fetal bovine serum, penicillin (100 units/ml), and streptomycin (5 μg/ml).

The kinetics of the establishment of human lymphoblastoid cell cultures has been described previously (16). The kinetics of REH was different from those of the other lines (15).

Cultures of the Established Cell Lines. Continuous cultures of established cell lines were carried out in Roswell Park Memorial Institute Tissue Culture Medium 1640 containing penicillin and streptomycin as mentioned above, the only difference being that the concentration of serum was 10%. The cells (0.5 × 10⁶/ml) were inoculated, and the medium was partly replaced every 72 hr. Cultures were saturated and stopped growing when the cell population reached 2 to 2.5 × 10⁶ cells/ml. Periodic controls were made for the absence of Mycoplasma contamination (18).

For the study of the uptake of amino acids, cultures were made in small Falcon flasks containing 5 ml of medium, in which 0.5 × 10⁶ cells/ml had been inoculated.

After 72-hr incubation at 37°C, the cells were counted, the cell suspension was centrifuged for 15 min at 400 × g, and the supernatant was recovered.

Amino Acid Analysis of the Culture Medium. Amino acid analysis of the medium was carried out with a Jeol-6AH automatic amino acid analyzer [Jeol (Europe) S.A., 16 Ave. de Colmar, 92 Reuil-Malmaison, France]. Basic amino acids were separated on a 15-cm column with 2 sodium citrate buffers, pH 4.25 and 5.60, respectively; the concentration of sodium was 0.40 N. Neutral and acidic amino acids were separated on a 50-cm column with 3 lithium citrate buffers; pH and lithium concentrations were, respectively, 2.70 and 0.20 N, 3.05 and 0.25 N, and 3.90 and 0.30 N (9). The culture medium was deproteinized by the addition of an equal volume of 3% sulfosalicylic acid. After centrifugation for 30 min at 10,000 × g, the supernatant was recovered, and 2 ml of a solution of norleucine were added per 4 ml supernatant. Norleucine at a final concentration of 0.1 μmol/ml was added as an internal standard. The quantities of amino acids used by the cells were calculated from the difference between the initial concentrations and those after 72 hr in culture. The values are reported per 10⁶ cells.

Some amino acids are synthesized by cells in culture more quickly than they are degraded or incorporated into proteins, so that their concentration in the medium increases. We have used minus (−) or plus (+) signs in order to indicate whether amino acid utilization (−) or synthesis (+) was predominant.

Incorporation of ¹⁴C-labeled Glutamic Acid, Arginine, and Proline. For this study, cultures were prepared in small Falcon flasks containing 5 ml of medium with the ¹⁴C-

Received March 11, 1977; accepted July 21, 1978.
labeled amino acid. The number of cells inoculated and time of culture were the same as those previously mentioned.

The 14C-labeled precursors were added to the culture medium in different experiments. Taking into account the concentration of glutamic acid (0.136 µmol/ml) and arginine (1.148 µmol/ml) in the culture medium, L-[U-14C]glutamic acid and L-[U-14C]arginine were added, respectively, at the radioactive concentrations of 0.034 and 0.344 µCi/ml. Thus, the specific activity of these precursors was 0.25 µCi/µmol for glutamic acid and 0.30 µCi/µmol for arginine. The loss of 1 carbon atom, resulting from the conversion of arginine to ornithine, gives ornithine with a specific activity of 0.25 µCi/µmol. Furthermore, when glutamic acid was used as a precursor, both 14C-labeled glutamine and glutamic acid were added to the medium.

The radioactive concentration of L-[U-14C]glutamine was 0.513 µCi/ml, so that its specific activity in the medium was 0.25 µCi/µmol. In this way, the conversion of glutamine to glutamic acid during the cell growth did not change the specific activity of glutamic acid.

Some experiments with L-[U-14C]proline added to the culture medium at a concentration of 0.5 µCi/ml were carried out so that the conversion of proline into glutamic acid and arginine could be studied. L-[U-14C]arginine monohydrochloride (specific activity, 324 mCi/mmol), L-[U-14C]glutamic acid (specific activity, 200 mCi/mmol), L-[U-14C]glutamine (specific activity, 40 mCi/mmol), and L-[U-14C]proline (specific activity, 200 mCi/mmol) were obtained from the Radiochemical Centre (Amersham, England).

Amino acid chromatography of the medium was carried out as previously described. During analysis one part of the effluent from the column was mixed with ninhydrin and was passed through the spectrophotometer for titration of the amino acids; simultaneously, the other part of the effluent was collected with the use of a fraction collector. Radioactivity of each fraction was measured with a Packard Tri-Carb scintillation counter. Comparison of chromatogram with the radioactivity profile of the collected fractions permitted accurate determination of the identity of radioactive amino acids in the culture supernatant.

Radioisotopic Assay for Δ1-Pyroline-5-carboxylate Reductase. Δ1-Pyroline-5-carboxylate reductase activity was determined according to the technique of Phang et al. (12). For both REH and LHN13 lines, the enzyme was extracted from 2 x 10^7 cells. A volume of 25 µl of enzyme extract in 0.1 M phosphate buffer (pH 6.8) containing 20 µg of protein was used for each line. Radioactive concentrations of L-[U-14C]-Δ1-pyroline-5-carboxylate, in the reaction mixtures, were 38,628 cpm for LHN13 and 38,894 cpm for REH. NADH was obtained from Merck (Darmstadt, Germany). L-[U-14C]-Δ1-Pyroline-5-carboxylic acid was generously donated by Dr. James Phang (National Cancer Institute, NIH).

RESULTS

Utilization or Production of Amino Acids. The results are given in Table 1 for REH and LHN13 lines. Some amino acids present in the medium are not mentioned in Table 1; tryptophan was not titrated because its concentration is too low; histidine, aspartic acid, and hydroxyproline concentrations did not show significant variations; and conversion of arginine, glutamine, and asparagine into ornithine, glutamic acid, and aspartic acid is due, to a great extent, to the enzymes present in the fetal calf serum and does not appear to be proportional to the cell number (4, 28).

The essential difference between REH and LHN13 concerns proline. In LHN13 cultures this amino acid is synthesized, and its concentration presents an increase in 72 hr. The increase per 10^6 cells per ml of culture medium was found to be 40.07 ± 1.46 µg/ml for 5 experiments performed at different times. In REH cultures there is a decrease in the concentration of proline, which was found to be 9.20 ± 0.18 µg/ml at 72 hr, in 8 different experiments.

Incorporation of [14C]Glutamic Acid, [14C]Arginine, and [14C]Proline. The carbon skeleton of proline, in cell cultures, is derived mainly from glutamic acid (1, 7), but for some types of cells it can be derived partly from arginine and glutamic acid (19, 30) or exclusively from arginine (21). Synthesis of proline by our cell lines has been studied with the use of [14C]glutamic acid and [14C]arginine. For each precursor, experiments were performed simultaneously with LHN13 and REH lines. The results appear in Table 2. Both precursors give labeled proline. In human lymphoblastoid cell cultures, this means that proline is synthesized from arginine and glutamic acid. When both labeled precursors are fed simultaneously into the medium with equal specific activity, one might assume that the specific activity of proline would be the sum of the values obtained with each single precursor. On this basis, it is possible to calculate the contributions of glutamic acid and arginine to the synthesis of proline. It appears from results given in Table 2 that these contributions were, respectively: (a) in the LHN13 line, 70.48 and 29.52%; and (b) in the REH line, 78.85 and 21.15%.

Radioactivity of proline in the culture medium is proportional to the amounts of labeled precursors converted into proline. This means that in REH cells total synthesis of proline and the synthesis of proline from glutamic acid and

| Table 1 |
| Incorporation (-) or synthesis (+) of amino acids by REH and LHN13 lines |
| The weights of amino acids incorporated or synthetized were calculated from the difference between the initial concentrations and those after 72 hr in culture. The values are reported per 10^6 cells/ml. |

<table>
<thead>
<tr>
<th></th>
<th>REH (µg)</th>
<th>LHN13 (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>-14.95</td>
<td>-16.54</td>
</tr>
<tr>
<td>Threonine</td>
<td>-2.48</td>
<td>-3.08</td>
</tr>
<tr>
<td>Serine</td>
<td>-15.05</td>
<td>-15.78</td>
</tr>
<tr>
<td>Proline</td>
<td>-9.20</td>
<td>40.07</td>
</tr>
<tr>
<td>Glycine</td>
<td>+18.02</td>
<td>+19.23</td>
</tr>
<tr>
<td>Alanine</td>
<td>+32.99</td>
<td>+72.32</td>
</tr>
<tr>
<td>Valine</td>
<td>-3.28</td>
<td>-7.22</td>
</tr>
<tr>
<td>Cysteine</td>
<td>-14.88</td>
<td>-10.06</td>
</tr>
<tr>
<td>Methionine</td>
<td>-3.14</td>
<td>-5.84</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>-9.14</td>
<td>-10.06</td>
</tr>
<tr>
<td>Leucine</td>
<td>-11.01</td>
<td>-12.56</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>-3.64</td>
<td>-7.00</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>-3.46</td>
<td>-6.32</td>
</tr>
</tbody>
</table>

*Mean ± S.E.*
arginine separately represent 23, 26, and 15% respectively, of the values obtained in LHN13.

Experiments with [14C]proline were carried out so that the conversion of proline into glutamic acid and arginine could be studied. When L-[U-14C]glutamic acid was added to the culture medium, neither glutamic acid nor arginine were labeled. When the precursor is L-[L-14C]arginine, no labeled glutamic acid appears in the culture medium. These experiments give proof that neither LHN13 nor REH cells oxidize glutamic acid-γ-semialdehyde to glutamic acid.

**Δ1-Pyroline-5-carboxylate Reductase Activity.** Radioactivity of [14C]proline recovered after incubation was, respectively, 28,023 cpm and 6,648 cpm for LHN13 and REH. Thus, activity of the Δ1-pyroline-5-carboxylate reductase in REH represents 23.7% of the value obtained with LHN13. This result is in agreement with those obtained with the use of L-[U-14C]glutamic acid and arginine added to the culture medium.

**DISCUSSION**

Interconversion of glutamic acid and proline was especially studied by Vogel et al. (25, 26, 29) and by Strecker et al. (11, 24). Glutamic acid is first reduced, the hydrogen donor being NADH, and gives glutamic acid-γ-semialdehyde, which spontaneously cyclizes into Δ1-pyroline-5-carboxylic acid. This is transformed into proline by Δ1-pyroline-5-carboxylate reductase (11, 20).

Proline can also be derived from arginine (5, 13, 22), which is first transformed into ornithine by arginase. Ornithine gives glutamic acid-γ-semialdehyde according to the reaction:

Ornithine + α-ketoglutarate ⇌ glutamic acid-γ-semialdehyde + glutamate

The enzyme responsible for this reaction is ornithine δ-transaminase (8, 23).

In mammalian cell cultures, which are grown in media lacking proline, the major precursor of proline is glutamate as it appears from nutritional experiments with radioactively labeled compounds (2). However, for some types of cells, such as osteoblasts (21), chick embryo legs (30), and normal rat liver cells (27), arginine and ornithine were better precursors for proline than was glutamate.

For our human lymphoblastoid cell lines, the major precursor of proline was also glutamate, but a part of the synthesized proline was derived from arginine.

In LHN13 and REH cultures, conversion of arginine to proline does not require arginase to be present in the fetal calf serum. Inasmuch as arginase cannot be inactivated by heat at 60° (4), we have selected a unique specimen of calf serum that was found to be free of arginase and glutaminase activity. In a medium supplemented with 10% of this serum, the cultures developed more slowly, but the observed conversion of arginine and glutamine, respectively, to ornithine and glutamic acid was due to the enzymes of the cells.

Whatever the labeled precursor, glutamic acid or arginine, radioactivity of proline remained very low in the REH culture medium in comparison to LHN13. This result suggested that there was probably a blockage of the reduction of Δ1-pyroline-5-carboxylate, which is a common intermediate in the 2 metabolic pathways of the proline synthesis. This hypothesis has been confirmed by the assay of Δ1-pyroline-5-carboxylate reductase. The loss of this enzymatic activity is probably connected with the chromosomal abnormalities observed in the lymphoblasts of the patient and constantly present in the cells of the established line (15). A similar mechanism was described by Kao and Puck (3) in a strain of Chinese hamster cell mutants.

Another lymphoblastoid cell line, H-SB2, derived from a lymphosarcoma, which had progressed to acute lymphoblastic leukemia, also presents a defect of an enzyme activity, but the deficient enzyme is cystathionase (6). As in the case of the REH line, H-SB2 is Epstein-Barr virus...
negative and shows a chromosomal abnormality, but in contrast with REH, this abnormality is an F-group trisomy and is found in only 50% of the cells.

The deficient synthesis of proline in REH, found at the establishment of this line and persisting for more than 2 years, constitutes a metabolic marker for this strain that could be used advantageously in future cell fusion studies.

**ACKNOWLEDGMENTS**

The authors wish to express their gratitude to Dr. James M. Phang, National Cancer Institute, for the generous supply of A'-pyrroline-5-carboxylic acid and to Dr. Michael Inbar, Weizman Institute of Science, Rehovot, Israel, for his suggestions and critical evaluation of the manuscript.

**REFERENCES**

Metabolism of Proline in a Human Leukemic Lymphoblastoid Cell Line

Georges Lorans, Claude Rosenfeld, Maurice Petitou, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/38/11_Part_1/3950

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.