Isoleucine Metabolism by Leukemic and Normal Human Leukocytes in Relation to Cell Maturity and Type

By C. Patrick Burns

The metabolism of an essential amino acid, isoleucine, by human leukemic and gradient-separated normal human leukocytes of various types and maturity was studied. Blood leukocytes were isolated and incubated with \([U^{14}C]\) isoleucine. Separation of metabolic intermediates was accomplished by sequential extraction. The rate of isoleucine incorporation into protein by immature cells from untreated patients with acute leukemia (15.9 ± 2.4 nmoles/hr per \(10^8\) leukocytes) was considerably higher than the rates of incorporation by mature neutrophils (3.2 ± 0.5 nmoles/hr per \(10^8\) leukocytes), lymphocytes (7.7 ± 1.2 nmoles/hr per \(10^8\) leukocytes), and eosinophils (6.2 ± 1.3 nmoles/hr per \(10^8\) leukocytes). Those cell preparations with more blast cells had higher rates of protein synthesis. In addition, those cells with greater thymidine incorporation had higher rates of protein synthesis. The leukocytes both oxidized isoleucine and incorporated it into cell lipid. The rates of these metabolic processes were characteristic for various types and maturity of leukocytes. This study demonstrates a relationship of rate of protein synthesis to leukocyte immaturity. This relationship is maintained in neoplastic leukocytes. It suggests that the requirement of the mitotic process for newly synthesized protein is greater than that for the elaboration of the protein products of the mature leukocyte.

Human leukocytes are capable of synthesizing cellular protein from labeled exogenous amino acids in vitro and in vivo. Studies have demonstrated that leukocytes can utilize valine, leucine, alanine, cystine, cysteine, methionine, glycine, serine, aspartic acid, and isoleucine for protein synthesis. The rates of incorporation of amino acids by leukocytes from patients with chronic myelocytic leukemia, chronic lymphocytic leukemia, and acute leukemia are higher than the rate of incorporation of amino acids by leukocytes from the blood of normal persons. However, in a cell-free system, it has been demonstrated that ribosomes from chronic lymphocytic leukemia patients catalyze protein synthesis at a lower rate than ribosomes from normal lymphocytes. Radioautographic studies of the normal human marrow have demonstrated higher uptake of labeled leucine by the normal immature myeloid cells compared to the more mature cells. Phytohemagglutinin-stimulated lymphocytes incorporate leucine, lysine, and isoleucine at a rate higher than that unstimulated lymphocytes. No systematic study of protein synthesis by the cells from a wide variety of leukemic diseases has been performed.
reported. Likewise, studies of normal cells have not included data on separate types of mature leukocytes.

The present study was undertaken to (1) compare rates of protein synthesis by mature and immature leukocytes of various origin, (2) correlate rates of protein synthesis of leukemic cell preparations with percentage of blast cells and DNA and RNA synthesis, and (3) study the oxidation of and lipid synthesis from isoleucine by various types of leukocytes.

MATERIALS AND METHODS

Patients Studied

Leukocytes were isolated from the peripheral blood of 19 patients with blood dyscrasias (Table 1) and six normal subjects. All patients were studied prior to initial therapy except DS (second study) and KH who were studied prior to instituting induction therapy after a relapse. DS and LT were studied on two occasions prior to initiating therapy. In addition, three patients with acute leukemia were studied again on day 3 after therapy was initiated, but at a time when they had received no drugs for 23 hr.

Preparation of Leukocyte Suspensions

Peripheral blood from patients was anticoagulated with heparin and the erythrocytes sedimented with 5% dextran dissolved in 0.9% saline. The cells in the leukocyte-rich supernatant were washed and the majority of the platelets removed by differential centrifugation. Leukocytes were enumerated using the vital stain, Trypan blue. Normal lymphocytes and neutrophils were obtained from Ficoll-Hypaque gradients and averaged 89% and 96% purity, respectively. The erythrocytes in the neutrophil-rich pellets from the gradients were removed by dextran sedimentation followed by hypotonic lysis. The mean platelet contamination of the leukocyte preparations was 0.08 platelet per leukocyte. The preparations of normal lymphocytes contained 0.87 platelet per leukocyte. Because of the small relative size of the platelet and its low rate of amino acid incorporation, this amount of platelet contamination was considered insignificant.

Isotopes

L-[U-14C] isoleucine, [C3H3] thymidine and [5-3H] uridine were purchased from New England Nuclear Corp., Boston, Mass. The radiopurity of the preparations, determined by paper chromatography, was at least 97%.

Incubation Techniques

Incubations with isoleucine were performed in 50 ml siliconized Erlenmeyer flasks, provided with a center well, containing 30–60 x 10^6 leukocytes in 5 ml of minimum essential medium (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 0.02 M NaHCO3 and 1% glutamine, 20% newborn calf serum, and 2 μCi [U-14C] isoleucine. Incubations of normal cells in the presence of autologous serum were performed in an early experiment to compare with incubations containing newborn calf serum. The flasks were flushed with 95% air-5% CO2, stoppered, and incubated at 37°C in a shaker bath (70 strokes per min). Corrections were made for blanks obtained from incubations performed without cells. Isoleucine content of the sera was kindly determined by Bio-Science Laboratories (Van Nuys, Calif.) with an automated column chromatography system using ion-exchange resin and pH gradient buffer system.

Thymidine incorporation, as an estimate of DNA synthesis, and uridine incorporation, as an estimate of RNA synthesis, were performed as previously described. Briefly, incubations with radioactive substrates were carried out for 4 hr in 15 x 125-mm screwcap glass tubes containing 5 x 10^6 viable cells in minimum essential medium – 20% newborn calf serum.

Extraction of Metabolic Products

To terminate the isoleucine incubations, the cells were disrupted with 35% perchloric acid. The precipitate was washed four times with 1% perchloric acid. The cellular protein was contained in
### Table 1. Clinical Data on Patients Studied

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Disease</th>
<th>White Blood Count (cells/cu mm)</th>
<th>Abnormal Cells (%)</th>
<th>Differential (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF</td>
<td>48</td>
<td>F</td>
<td>AML*</td>
<td>82,500</td>
<td>78</td>
<td>Blasts 1 Pro* 4 Meta 3 Bases 0 Lymph 0 Mono 0 Other 0</td>
</tr>
<tr>
<td>RH</td>
<td>67</td>
<td>M</td>
<td>AML</td>
<td>80,000</td>
<td>60</td>
<td>Blasts 0 Pro* 7 Myel 4 Segs 25 Bases 0 Lymph 0 Mono 0 Other 0</td>
</tr>
<tr>
<td>DS†</td>
<td>21</td>
<td>F</td>
<td>AML</td>
<td>33,000</td>
<td>95</td>
<td>Blasts 0 Pro* 2 Myel 1 Bases 0 Lymph 1 Mono 0 Other 0</td>
</tr>
<tr>
<td>JJ</td>
<td>81</td>
<td>F</td>
<td>AMML</td>
<td>278,000</td>
<td>100</td>
<td>Blasts 0 Pro* 0 Myel 0 Segs 0 Lymph 0 Mono 0 Other 0</td>
</tr>
<tr>
<td>LS</td>
<td>56</td>
<td>M</td>
<td>AMML</td>
<td>159,000</td>
<td>86</td>
<td>Blasts 0 Pro* 6 Myel 3 Bases 2 Lymph 0 Mono 0 Other 0</td>
</tr>
<tr>
<td>CL</td>
<td>45</td>
<td>M</td>
<td>AMML</td>
<td>16,700</td>
<td>47</td>
<td>Blasts 0 Pro* 0 Myel 0 Segs 0 Lymph 11 Mono 0 Other 0</td>
</tr>
<tr>
<td>KH</td>
<td>46</td>
<td>M</td>
<td>AMOl</td>
<td>114,400</td>
<td>90</td>
<td>Blasts 0 Pro* 4 Myel 0 Segs 0 Lymph 3 Mono 0 Other 0</td>
</tr>
<tr>
<td>OS</td>
<td>60</td>
<td>M</td>
<td>AMOl</td>
<td>41,000</td>
<td>68</td>
<td>Blasts 0 Pro* 4 Myel 9 Lymph 11 Mono 0 Other 0</td>
</tr>
<tr>
<td>LM</td>
<td>74</td>
<td>F</td>
<td>AMOl</td>
<td>13,600</td>
<td>68</td>
<td>Blasts 0 Pro* 0 Myel 11 Lymph 1 Mono 0 Other 0</td>
</tr>
<tr>
<td>RD</td>
<td>49</td>
<td>M</td>
<td>LRE</td>
<td>26,600</td>
<td>95</td>
<td>Blasts 0 Pro* 0 Myel 3 Lymph 11 Mono 0 Other 0</td>
</tr>
<tr>
<td>RS</td>
<td>41</td>
<td>M</td>
<td>LRE</td>
<td>20,100</td>
<td>88</td>
<td>Blasts 0 Pro* 0 Myel 0 Bases 0 Lymph 0 Mono 0 Other 0</td>
</tr>
<tr>
<td>RJ</td>
<td>41</td>
<td>M</td>
<td>LRE</td>
<td>3,500</td>
<td>75</td>
<td>Blasts 0 Pro* 0 Myel 0 Bases 0 Lymph 14 Mono 0 Other 0</td>
</tr>
<tr>
<td>SW</td>
<td>69</td>
<td>F</td>
<td>CML</td>
<td>297,000</td>
<td>2</td>
<td>Blasts 0 Pro* 30 Myel 26 Lymph 16 Mono 2 Other 0</td>
</tr>
<tr>
<td>NS</td>
<td>52</td>
<td>F</td>
<td>CML</td>
<td>112,000</td>
<td>18</td>
<td>Blasts 0 Pro* 16 Myel 12 Lymph 36 Mono 4 Other 0</td>
</tr>
<tr>
<td>AC</td>
<td>73</td>
<td>F</td>
<td>CLL</td>
<td>115,000</td>
<td>97</td>
<td>Blasts 0 Pro* 0 Myel 0 Bases 0 Lymph 3 Mono 0 Other 0</td>
</tr>
<tr>
<td>AB†</td>
<td>39</td>
<td>F</td>
<td>LSCl</td>
<td>73,000</td>
<td>93</td>
<td>Blasts 0 Pro* 1 Myel 1 Lymph 2 Mono 0 Other 0</td>
</tr>
<tr>
<td>TR†</td>
<td>18</td>
<td>M</td>
<td>IM</td>
<td>13,500</td>
<td>87</td>
<td>Blasts 0 Pro* 0 Myel 0 Bases 0 Lymph 87 Mono 4 Other 0</td>
</tr>
<tr>
<td>EP</td>
<td>63</td>
<td>F</td>
<td>Eosinophilia</td>
<td>90,500</td>
<td>81</td>
<td>Blasts 0 Pro* 0 Myel 0 Bases 5 Lymph 3 Mono 0 Other 0</td>
</tr>
<tr>
<td>LT†</td>
<td>62</td>
<td>M</td>
<td>Eosinophilia</td>
<td>18,100</td>
<td>68</td>
<td>Blasts 0 Pro* 0 Myel 0 Bases 0 Lymph 11 Mono 2 Other 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20,900</td>
<td>86</td>
<td>Blasts 0 Pro* 0 Myel 0 Bases 11 Lymph 86 Mono 4 Other 0</td>
</tr>
</tbody>
</table>

*Abbreviations: AML, acute myelocytic leukemia; AMML, acute myelomonocytic leukemia; AMOl, acute monocytic leukemia; LRE, leukemic reticuloendotheliosis (hairy cell leukemia); CML, chronic myelocytic leukemia; CLL, chronic lymphocytic leukemia; LSCl, lymphosarcoma cell leukemia; IM, infectious mononucleosis; Pro, Promyelocytes; Myel, myelocytes; Meta, metamyelocytes; Bands, band neutrophils; Segs, segmented neutrophils; Eos, eosinophils; Baso, basophils; Lymph, lymphocytes; Mono, monocytes.

†Patients DS and LT had studies on two occasions separated by 5 mo and 2 wk, respectively.

‡Abnormal mononuclear cells (hairy cells).

§Mostly atypical.

¶Lymphosarcoma cells.
the precipitate after extraction of the lipids. The residue was weighed and an aliquot dissolved in NCS Solubilizer (Amersham/Searle, Arlington Heights, Ill.), and the $^{14}$C-incorporation determined in a scintillation counter.

Isolation of the protein as well as the lipids has been described previously.\textsuperscript{19} $\text{CO}_2$ was absorbed in $3\text{N} \text{NaOH}$ added to the center well through the closed system just prior to cell disruption. Labeled $\text{CO}_2$ in the NaOH was determined after conversion to BaCO$_3$ by the addition of 5\% BaCl$_2$. After sequential washes with water and acetone, the BaCl$_2$ was dispersed in a thixotropic gel powder–Toluene scintillation fluid for $^{14}$C determination. Radioactivity of all products was counted on a Packard Tri-Carb 2425 spectrometer.

At the termination of the incubations for the determination of thymidine and uridine incorporation, the cells were washed and the nucleic acids isolated by trichloroacetic acid precipitation. Radioactivity was determined in a liquid scintillation system. The details of this procedure have been described previously.\textsuperscript{18}

\textbf{Analysis of Results}

The specific activity of isotope in each flask was calculated by dividing the total radioactivity in the flask by the number of nanomoles of isoleucine present in the medium. Incorporation of the labeled substrate was computed from the number of carbon atoms which enter into each fraction of the metabolites. Statistical comparisons were made using the $t$ test for samples of unequal size. Correlations were derived using both correlation coefficients and linear regression equations.\textsuperscript{20}

\textbf{RESULTS}

\textit{Isoleucine Incorporation Into Protein}

There were only small differences in the rates of protein synthesis of normal leukocytes incubated in the presence of 20\% autologous serum compared to leukocytes incubated with 20\% newborn calf serum (Table 2). In order to keep the serum source consistent for all incubations and to avoid the effect of inhibitory or stimulatory factors that might be present in some sera, all further studies were done using newborn calf serum from the same lot.

Initial kinetic experiments determined that isoleucine incorporation into cell protein by immature leukemic cells and mature lymphocytes and neutrophils is approximately linear for 4 hr. After the kinetic experiments, studies were done using a 4-hr incubation time. Increasing the number of cells per flask between 20 and $70 \times 10^6$ increases the incorporation of labeled isoleucine proportionally. The incorporation of isoleucine into protein by various leukocytes is shown in Fig. 1. The incorporation into protein by cell suspensions varies from 2.0 to 33.4 nmoles/hr per $10^8$ leukocytes. Protein synthesis is significantly elevated in the cells of patients with acute leukemia and lowest in cell preparations of normal mature leukocytes. Mature normal lymphocytes utilize isoleucine for protein synthesis at a higher rate ($7.7 \pm 1.2$ nmoles/hr per $10^8$ leukocytes) (mean $\pm$ SE) than mature normal neutrophils ($3.2 \pm 0.5$ nmoles/hr per $10^8$ leukocytes) ($p < 0.05$). Mature eosinophils and lymphocytes from patients with lymphoproliferative diseases incorporate isoleucine into protein at intermediate

\begin{table}
\centering
\caption{Effect of Source of Serum on Protein Synthesis}
\begin{tabular}{lcc}
\hline
 & Neutrophils & Lymphocytes \\
\hline
Newborn calf serum & $2.7 \pm 0.1$ & $6.1 \pm 0.4$ \\
Autologous serum & $2.1 \pm 0.1$ & $6.5 \pm 0.2$ \\
\hline
\end{tabular}
\end{table}

Values shown are in nanomoles per hour per $10^8$ leukocytes and are the mean $\pm$ SE of triplicate incubations of gradient-separated cells from a normal subject.
rates. The abnormal mononuclear cells of leukemic reticuloendotheliosis and those of chronic myelocytic leukemia synthesize protein from isoleucine at rates higher than mature cells, but lower than the populations of blast cells from patients with acute leukemia. As a group, patients with acute leukemia undergoing therapy, but prior to remission, have rates of protein synthesis similar to those studied before therapy.

Those cell preparations containing the highest percentage of immature cells by light microscopy have the highest rates of protein synthesis. The protein synthesis is directly correlated with the percentage of blast cells present (Fig. 2). Likewise, there is a correlation of rate of protein synthesis with maturity, as assessed biochemically. Thymidine and uridine incorporation were determined on cell preparations from 20 patients. The cells utilize thymidine at rates varying from 11.0 to 310.2 pmoles/hr per $10^8$ leukocytes. There is a correlation of protein synthesis with thymidine incorporation for the entire group of 20 patients (Fig. 3A). There was an even more positive correlation of protein synthesis and thymidine incorporation for the cells from patients with acute leukemia (Fig. 3B). There is a positive correlation of uridine incorporation with protein synthesis for all cell preparations studied (Fig. 4) and especially for cell preparations from patients with acute leukemia studied prior to therapy ($r = 0.97, p < 0.001$).
Isoleucine Oxidation and Incorporation Into Cellular Lipids

The incorporation of radioactivity by immature and mature cells into CO₂ and lipid is linear for 4 hr. The radioactivity in each fraction for various cells is shown in Table 3. The rates of synthesis of both products are variable among the different cell types and are not related to protein synthesis, or thymidine or uridine incorporation. The rates of oxidation of isoleucine to CO₂ varied widely between the leukocyte types. The highest rates were observed with cells from patients with leukemic reticuloendotheliosis. The lowest rates were found with normal neutrophils, and these were about 13% that of normal lymphocytes. Contrariwise, the incorporation of isoleucine radioactivity into cellular lipid varied little between the groups. The rates of incorporation into lipid were lowest for the cells from patients with lymphoproliferative diseases.

DISCUSSION

This study has demonstrated that immature leukocytes utilize isoleucine for cell protein synthesis at a rapid rate and mature leukocytes at a lower rate. Low rates were a feature of mature leukocytes obtained from normal subjects or patients with blood dyscrasias. The magnitude of the elevation of the rate of
protein synthesis by cell preparations from patients with acute leukemia was directly related to the number of blast cells present. In addition, we have demonstrated that there is a correlation of rate of protein synthesis with rate of thymidine incorporation. Rapid thymidine incorporation into DNA is a feature of undifferentiated dividing cells; the rate decreases as the cell differentiates toward maturity. Taken together, these data confirm the direct relationship of a high rate of protein synthesis to cell immaturity, whether defined morphologically or by rate of thymidine incorporation.

Since normal immature cells were unavailable for study, it is impossible to eliminate the possibility that a higher rate of protein synthesis is merely a concomitant of the leukemic process rather than a function of cell immaturity per se. However, neoplastic disorders characterized by an accumulation of mature cells, such as chronic lymphocytic leukemia, or cells of intermediate maturity, such as leukemic reticuloendotheliosis or lymphosarcoma cell leukemia, exhibit rates directly related to their maturity.

These data also suggest that the newly synthesized protein is produced, to a large extent, in response to the requirements of mitosis, perhaps as building blocks for daughter cells. The isoleucine requirements for synthesis of the protein products of the mature leukocytes, such as immunoglobulins and transcobalamins, apparently are small compared to those of dividing cells. There was a difference in the rates of protein synthesis by the various types of mature leukocytes. This is not surprising in view of their diverse function. One might predict a higher rate of protein synthesis by lymphocytes as compared to neutrophils, since a major function of that cell is the elaboration of protein mediators of humoral immunity.

Isoleucine is an essential amino acid which is incorporated into cellular protein by a wide variety of tissues. In addition, the carbon chain, after transamination and oxidative decarboxylation, can be oxidized to CO₂ by Krebs cycle activity or utilized for lipid synthesis. Previous studies have demonstrated that leukocytes contain the enzymes for oxidative decarboxylation of isoleucine. We have shown that a wide variety of normal and abnormal leukocytes are capable of the oxidative decarboxylation of this branched-chain amino acid. The rate of oxidation of isoleucine was not correlated with the maturity of the
cells. Low rates seemed to be a characteristic of mature neutrophilic cells and those of intermediate maturity (chronic myelocytic leukemia). High rates were noted for the leukocytes from patients with leukemic reticuloendotheliosis. Dancis and co-workers demonstrated that blood leukocytes from patients with maple syrup urine disease have low rates of isoleucine oxidation in vitro when compared to normal leukocytes. They showed the usefulness of the observation in the diagnosis of this inborn error of metabolism. Other investigators have shown a correlation of rate of keto-acid oxidation by human leukocytes to the number of lymphocytes in the cell preparation. We observed that normal lymphocytes oxidize isoleucine at a rate about eight times more rapid than normal neutrophils. In vitro studies of gradient-separated lymphocytes from patients and families might offer a more sensitive test for diagnosing cases of maple syrup urine disease, detecting heterozygotes, and delineating the variants of this metabolic disease.

In addition, the present study has demonstrated the incorporation of $^{14}C$ from isoleucine into cell lipid. In contrast to the rate of incorporation into protein and CO$_2$, this metabolic rate was relatively constant among the cell types studied except for the low levels which may characterize the lymphoproliferative disorders.

The present experiments provide data that indicate that isoleucine is metabolized by a wide variety of leukocytes. It is incorporated into cellular protein and lipid and oxidized through the Krebs cycle after decarboxylation. Immature leukocytes are characterized by rapid rates of incorporation of isoleucine into protein, and this metabolic function is directly correlated with degree of immaturity as measured either morphologically or by rate of thymidine incorporation. The patterns of metabolic handling of isoleucine are characteristic for leukocytes of different ages. Also, distinctive metabolic patterns for each specific type of mature leukocyte emerged, thereby underlining their individuality. Observations on the similarities and differences of mature and immature leukocytes provide further insight into the metabolic accompaniments of the leukemic process. In addition, such studies may suggest metabolic processes susceptible to chemotherapeutic attack.

ACKNOWLEDGMENT

The author is grateful to Dr. Rune L. Stjernholm for his helpful suggestions and to Ian Welshman and Richard King for technical assistance.

REFERENCES

5. Gavosto F, Pileri A, Maraini G: Protein metabolism in bone marrow and peripheral blood cells: Evaluation of $^{3}H$-DL-leucine uptake by high resolution radioautographic tech-
Isoleucine metabolism by leukemic and normal human leukocytes in relation to cell maturity and type

CP Burns