Increased Activity of Some Folic Acid Enzyme Systems in Infectious Mononucleosis

By Joseph R. Bertino, Barbara M. Simmons and Dennis M. Donohue

Although infectious mononucleosis is a benign disease, many manifestations are similar to those observed in malignant transformation of lymphocytes. Among these are generalized lymphadenopathy and hepatosplenomegaly, the occurrence of morphologically abnormal cells in the peripheral blood and occasionally in the bone marrow, associated Coombs' positive hemolytic anemias, and abnormal protein production. The peripheral blood cell of infectious mononucleosis is perhaps best characterized as an atypical large lymphocyte. These “atypical cells” have been shown by Bond et al.1 and Gavosto et al.2 to incorporate tritiated H3 thymidine in vitro to a considerably greater degree than do normal circulating leukocytes. An increase in the number of cells which label with H3 thymidine has likewise been seen in leukocytes from patients with acute and chronic granulocytic leukemia, and in immature lymphocytes from certain patients with lymphatic leukemia.3 This finding indicates that infectious mononucleosis cells are actively synthesizing DNA and are comparable, in this regard, to those seen in leukemia.

The present study was undertaken to make biochemical observations on these atypical lymphocytes and to contrast them with normal cells and leukemic cells. Three enzymes involved in folic acid metabolism, and thus important in cellular replication, were studied, as well as glucose-6-phosphate dehydrogenase.

Methods and Materials

All patients with infectious mononucleosis studied were acutely ill and had fever, pharyngitis, posterior cervical adenopathy, lymphocytosis and positive heterophile agglutination tests at 1:224 or greater. Laboratory data are presented in table 1. Atypical cells numbered at least 60 per cent of the total leukocyte count in all five subjects. Less than 10 per cent of the lymphocytes in every subject were morphologically normal.

Leukocyte separation and enzyme assays. Leukocytes were isolated rapidly from heparinized blood by dextran sedimentation, differential centrifugation to remove platelets and lysis of residual erythrocytes by a brief exposure to distilled water. Lysates were prepared by high speed homogenization. The clear supernatant solution resulting from centrifuging the homogenate at 10,000 g for 15 minutes (International Centrifuge, Model PR-2, high speed attachment) was used for enzyme assays. All steps were carried out at 4 C.

The following enzymes were assayed: the formate-activating enzyme, N5,N10-methyleneFH4 dehydrogenase, FH4 reductase and glucose-6-phosphate dehydrogenase. Assays for

587

From the King County Central Blood Bank, and the Department of Medicine, University of Washington School of Medicine, Seattle, Wash.

Supported by a grant from the United States Public Health Service (CY-4252(C2)). Dr. Bertino carried out this investigation during the tenure of a Postdoctoral Fellowship from the National Heart Institute, United States Public Health Service.

Submitted Nov. 6, 1961; accepted for publication Jan. 12, 1962.

The following abbreviations will be used: FH4, dihydrofolic acid; FH4, tetrahydrofolic acid; ATP, adenosine triphosphate; TPN and TPNH, the oxidized and reduced forms of triphosphopyridine nucleotide; DNA, deoxyribonucleic acid; PyPO4, pyridoxal phosphate.

587

Blood, Vol. 19, No. 5 (May), 1962
Table 1.—Clinical Laboratory Findings

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>WBC</th>
<th>Atypical Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. R.</td>
<td>20</td>
<td>M</td>
<td>13,400</td>
<td>84</td>
</tr>
<tr>
<td>D. P.</td>
<td>20</td>
<td>M</td>
<td>10,700</td>
<td>80</td>
</tr>
<tr>
<td>W. T.</td>
<td>25</td>
<td>M</td>
<td>9,100</td>
<td>60</td>
</tr>
<tr>
<td>M. M.</td>
<td>21</td>
<td>M</td>
<td>13,200</td>
<td>89</td>
</tr>
<tr>
<td>D. M. P.</td>
<td>19</td>
<td>F</td>
<td>9,800</td>
<td>71</td>
</tr>
</tbody>
</table>

all of the enzymes concerned with folic acid metabolism depend upon acid deproteinization and measurement of $N^9,N^{10}$-methylene FH$_4$ by light absorption at 355 $\mu$ using a Beckman DU spectrophotometer. Details of these assays are described elsewhere.$^{3,16}$ Glucose-6-phosphate dehydrogenase was assayed by the method of Kornberg and Horecker.$^{18}$ Enzyme activity is expressed as $\mu$M of product formed per ml. of assay mixture per mg. of soluble protein. Protein was determined by the biuret method using crystalline bovine albumin as the standard. Results have also been calculated per 10$^9$ WBC. Hemoglobin was determined by light absorption at 540 $\mu$ using a cyanmethemoglobin standard.

Radioautography. H$^3$-thymidine, specific activity of 390 $\mu$C/$\mu$M, was obtained from New England Nuclear Corp. Labeling was done on peripheral blood samples according to the method of Bond et al.$^4$ Stripping film (Kodak AR-10) was applied$^5$ and the slides were exposed for two weeks. After exposure and development of the slides, the cells were stained with Wright's and Giemsa stains. The per cent of cells labeled among 1000 nucleated cells on each of two slides were enumerated.

Chemicals. ATP, TPN, TPNH and the sodium salt of glucose-6-P were obtained from the Sigma Chemical Co. Folic acid and FH$_4$ were purchased from Nutritional Biochemical Co. FR, was prepared by the method of Futterman.$^6$

RESULTS

The results of the enzyme assays performed on the leukocytes of the five patients studied are contrasted to levels from leukocytes of normal subjects (table 2). A high percentage of atypical lymphocytes labeled with H$^3$-thymidine; these data are similar to the results of Gavosto et al.$^2$ Formate-activating enzyme activity and $N^9,N^{10}$-methylene-$FH_4$ dehydrogenase activity are approximately twice the levels found in leukocytes from the normal subjects, while glucose-6-P dehydrogenase activity is decreased to one-third of normal. Of particular interest is the presence of FH$_2$ reductase in the leukocyte lysates of infectious mononucleosis patients; this enzyme was not detected in leukocytes from the peripheral blood of normal subjects or in most instances of chronic lymphatic leukemia leukocytes.$^{16}$ Results calculated per 10$^9$ leukocytes were essentially similar, since approximately the same amount of soluble protein (per 10$^9$ cells) was released by homogenization of normal and infectious mononucleosis leukocytes.

DISCUSSION

Our data indicate that increased levels of three enzymes which are involved in folic acid metabolism are found in leukocytes from patients with infectious mononucleosis when compared to leukocytes from normal subjects. Elevated levels of the folic acid enzyme systems described were also found in leukocytes from patients with chronic myelogenous leukemia and acute leukemia,$^7$ while decreased glucose-6-P dehydrogenase activity, as compared to normal
Table 2.—Levels of Enzyme Activity and H³ Thymidine Radioautography Data in Normal Subjects and in Patients with Infectious Mononucleosis

<table>
<thead>
<tr>
<th>Condition</th>
<th>Subject</th>
<th>H³ Thymidine</th>
<th>Formate-activating enzyme</th>
<th>N⁵,N¹⁰-methylene FH₄</th>
<th>FH₂ reductase</th>
<th>Glucose-6-P</th>
<th>dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>μM/hr./mg.</td>
<td>μM/hr./mg.</td>
<td>μM/hr./mg.</td>
<td>μM/hr./mg.</td>
<td></td>
</tr>
<tr>
<td>Infectious</td>
<td>5</td>
<td>4.3 (2.8–6.5)*</td>
<td>0.04 (0.07–1.2)</td>
<td>0.31 (0.22–0.35)</td>
<td>0.018 (0.008–0.024)</td>
<td>5.6 (3.0–8.0)</td>
<td></td>
</tr>
<tr>
<td>mononucleosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>16</td>
<td>0.06 (0.01–0.12)</td>
<td>0.40 (0.23–0.57)</td>
<td>0.16 (0.09–0.22)</td>
<td>none detected</td>
<td>15.0 (8.5–26.6)</td>
<td></td>
</tr>
</tbody>
</table>

*Numbers in parenthesis indicate range.

leukocytes, has been reported in chronic leukemia⁸ and in acute leukemia.⁷ While it may not be valid to compare enzyme activities from cell populations containing 60 per cent or more atypical lymphocytes with leukocytes from normal subjects averaging 80 per cent polymorphonuclear leukocytes and less than 20 per cent lymphocytes, the technical difficulties in obtaining large enough quantities of normal lymphocytes suitable for assay have not been adequately worked out. When compared to the leukocytes from patients with chronic lymphatic leukemia, where cell populations of 90 per cent or more of small mature lymphocytes can be obtained, the activities of the formate-activating enzyme, N⁵,N¹⁰-methylene FH₄ dehydrogenase and FH₂ reductase, are higher in the leukocytes from patients with infectious mononucleosis, whether the enzyme activity is expressed per 10⁶ leukocytes or per mg. of soluble protein.

The coenzyme form of folic acid, FH₄, acts as a carrier for “one carbon” units, at the oxidation levels of formate and formaldehyde, in various metabolic reactions.⁹,¹⁰ The involvement of FH₄ in purine and pyrimidine synthesis has been well demonstrated.¹¹-¹⁴ The relationship of the formate activating enzyme and N⁵,N¹⁰-methylene FH₄ dehydrogenase to purine and thymidylic acid synthesis are diagrammed in figure 1.

The synthesis of the coenzyme, FH₄, is carried out by the enzyme dihydrofolate reductase (also called folic reductase).

\[ TPNH + FH₂ + H⁺ \rightarrow TPN + FH₄ \] Reaction (1).

This enzyme catalyzes the reduction of FH₂ to form FH₄ and to a lesser extent the reduction of folic acid to FH₄. Recent evidence indicates that dihydrofolate reductase may function directly in thymidylic acid synthesis by continuously regenerating FH₄ from FH₂, and therefore may play a key role in nucleic acid synthesis and cellular replication.¹²-¹⁴ This enzyme is irreversibly inhibited by the folic acid antagonists, amethopterin and aminopterin, at extremely low levels (10⁻⁸M), and is probably the target enzyme for antifolic therapy.¹⁵-¹⁷

The presence of significant activity of dihydrofolate reductase in leukocytes of infectious mononucleosis patients, as well as in acute and chronic myeloctic leukemia, correlated well with the H³ thymidine data. Thus normal subjects or patients convalescent from infectious mononucleosis had low H³ thymidine labeling² and no appreciable dihydrofolate reductase activity. These findings strengthen the characterization of the atypical lymphocyte seen in infectious mononucleosis as a cell actively synthesizing DNA. Although the
Fig. 1.—Importance of some FH₄ coenzyme mediated reactions in purine and thymine synthesis. (1) Formate activating enzyme; (2) N⁵,N¹⁰-methylene FH₄ dehydrogenase; (3) serine hydroxymethylase.

etiology of this disease is obscure, the possibility exists that these cells are virus infected and are producing viral DNA.²

The symptoms and signs of infectious mononucleosis are perhaps not as closely related to the total number of proliferating cells as is true of acute leukemia. However, because of the biochemical similarities observed in these cells, it would be of interest to treat such a patient with folic acid antagonists.

**SUMMARY**

Increased levels of activity for the formate activating enzyme and N⁵,N¹⁰-methylene FH₄ dehydrogenase have been found in the leukocytes from patients with infectious mononucleosis; in addition dihydrofolic reductase, an enzyme not found in mature circulating leukocytes, has been detected in infectious mononucleosis cells. Glucose-6-phosphate dehydrogenase activity is less in infectious mononucleosis leukocytes than in normal leukocytes.

These findings are similar to the results obtained in acute and chronic myelogenous leukemia and indicate that the leukocytes seen in infectious mononucleosis have the enzymatic apparatus associated with synthesis of DNA.

**SUMMARIO IN INTERLINGUA**

Esseva trovate, in le leucocytos ab patientes con mononucleosis infectiose, augmentate nivellos de activitate del enzyma formato-activante e de dihydrogenase de acido tetrahydrofolic N⁵,N¹⁰-methylenic; reductase de acido dihydrofolic (un emzyma non trovate in matur leucocytos circulante) esseva etiam detegite in le cellulas de mononucleosis infectiose. Le activitate de dihydrogenase de glucosa-6-phosphato esseva plus basse in le leucocytos de mononucleosis infectiose que in leucocytos normal.

Iste constatationes es simile al resultatos obtenite in casos de acute e chronic leucemia myelogene; illos indica que le leucocytos trovate in mononucleosis infectiose ha le apparato enzymatic que es associate con le synthese de acido disribonucleic.
ACKNOWLEDGMENTS

The authors would like to express gratitude to Dr. Charles Bender of the University of Washington Health Center for his interest and cooperation. We would also like to thank Dr. Clement A. Finch and Dr. Frank M. Huennekens for many helpful suggestions. The able technical assistance of Mrs. Y. Betson, who did the radioautography, is also acknowledged.

REFERENCES


Joseph R. Bertino, M.D., Assistant Professor of Pharmacology and Associate in Medicine, Yale University School of Medicine, New Haven, Conn.

Dennis M. Donohue, M.D., Clinical Associate Professor of Medicine, Department of Medicine, University of Washington School of Medicine, Seattle, Wash.

Barbara M. Simmons, B.S., Department of Medicine, University of Washington School of Medicine, Seattle, Wash.
Increased Activity of Some Folic Acid Enzyme Systems in Infectious Mononucleosis

JOSEPH R. BERTINO, BARBARA M. SIMMONS and DENNIS M. DONOHUE