Vol. 89, No. 1, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 12, 1979 Pages 103-107

EXPRESSION OF FETAL THYMIDINE KINASE IN HUMAN COBALAMIN OR FOLATE DEFICIENT LYMPHOCYTES

Peter H. Ellims, Ronald J. Hayman and Martin B. Van der Weyden*

Monash University Department of Medicine,
Alfred Hospital, Prahran, 3181, Victoria,
Australia

Received May 29,1979

SUMMARY

In extracts of peripheral blood lymphocytes of cobalamin or folate deficient patients thymidine kinase activity is increased three fold and exhibits properties of the fetal isoenzyme. Appropriate vitamin therapy results in reduction of this activity to normal levels and change from fetal to adult isoenzyme. The occurrence in cobalamin or folate deficiency of fetal thymidine kinase activity in non proliferating human lymphocytes is unique and may reflect events in the deficient marrow lymphoid progenitor cells.

Two unique thymidine kinase (TK) activities which differ in chromatographic, electrophoretic and kinetic properties occur in human tissue (1-4). In proliferating cells this activity termed fetal exhibits properties similar to that of human fetal tissue, whereas that termed adult predominates in normal dormant cells (1,3). Folate or cobalamin deficiency occurring in human hemopoietic tissue results in characteristic morphological nuclear and cytoplasmic abnormalities, the molecular basis for which is obscure but current hypotheses imply an interference in DNA but not RNA metabolism (5). In our biochemical studies on the perturbed DNA metabolism in these deficiency states (6) we found an unexpected expression of fetal TK activity in extracts of peripheral blood lymphocytes of individuals with anemia and cobalamin or folate deficiency. The details of this finding is the subject of the present communication.

MATERIALS AND METHODS

Lymphocytes were isolated by the Isopaque-Ficoll Gradient Centrifugation Technique (7) from heparinized blood of normal volunteers (Group I) and

* To whom to address correspondence.

0006-291X/79/130103-05$01.00/0

patients with anemia associated with either normal (Group II) or reduced folate or cobalamin status (Group III) as defined by previously described criteria (6). The lymphocytes were suspended, at a concentration of 10^7 cells/ml, in 10 mM Tris HCl and 5 mM EDTA pH 7.4, and lyzed by rapid freeze thawing in liquid nitrogen. Extracts were centrifuged at 10,000g for 15 minutes at 4° and after dialysis for 2 hours against 1000 vol of 10 mM Tris HCl pH 7.4, the supernatant were assayed for enzyme activities as described below.

Thymidine kinase was assayed using a total volume of 0.1 ml containing the following components: 100 mM Tris-HCl pH 7.4, 5 mM ATP, 10 mM $MgCl_2$, 10 mM NaF, 5 µM [6-^{3}H] thymidine at a specific activity of 5 Ci/mM. Reagents, thymidine and nucleoside or deoxynucleoside triphosphates were obtained from Sigma. [6-^{3}H] thymidine was obtained from Amersham-Searle Inc. The reaction mixture was incubated at 37° for 15 minutes and terminated by placing in a boiling water bath for one minute. After centrifugation at 10,000g for 10 minutes, aliquots (50 µl) were spotted onto Whatman DE-81 paper squares (1.5 x 1.5 cm) which were washed four times in 1 mM ammonium formate and once in methanol. The paper squares after drying were placed in scintillation vials and spotted with aliquots (50 µl) of 0.2 M KCl in 1 M HCl. Radioactivity was measured in 5 mls of PCS (Amersham-Searle) in a liquid scintillation counter at an efficiency of 32% and enzyme activity expressed as picomoles of product formed/hr/mg protein. Protein was determined by the method of Lowry *et al* (8).

Adult and fetal thymidine kinase activity were differentiated on the basis of the ability of cytidine triphosphate (CTP) to serve as a phosphate donor; inhibition of activity by deoxycytidine triphosphate and activity at pH 5.0 compared to that at 7.4 (1,3). For these experimental conditions the following alterations were made in the standard assay. The concentration of ATP 2mM, $MgCl_2$ 2 mM, (a) substitution of 2 mM CTP for ATP (b) addition of 2 mM dCTP (c) substitution of 50 mM sodium acetate pH 5.0 for Tris HCl pH 7.4.

RESULTS

The thymidine kinase activities in lymphocyte extracts of controls (Group I) and patients with anemia and either normal (Group II) or reduced (Group III) cobalamin or folate status are presented in Table I. No significant differences exist between the mean activities of thymidine kinase of preparations from controls and patients in Group II whereas that of extracts of patients with cobalamin or folate deficiency were increased three fold that of controls. The degree of utilization of CTP as a phosphate donor, activity at acid pH and its inhibition by dCTP are among useful properties to distinguish fetal from adult TK (1,3). Table II summarizes these comparative properties of TK in lymphocyte extracts of controls and patients with reduced cobalamin or folate status before and after appropriate vitamin therapy. The thymidine kinase activity in

TABLE I

TK Activity in Lymphocyte Extracts of Patients with Cobalamin or Folate Deficiency and Controls

	TK activities pmoles/hr/mg protein	Ratio TK activity with CTP or ATP as phosphate donor
Group I		
n = 15[a]	169 ± 60[b]	0.9 ± 0.1 [b]
Group II		
n = 10	142 ± 45	0.8 ± 0.1
Group III		
n = 6	485 ± 200	0.2 ± 0.1

a. number of patients studied. b. mean ± 1SD.
Group I, normal controls; Group II, patients with anemia and normal serum vitamin B_{12} and folate level; Group III, patients with anemia and reduced serum vitamin B_{12} or folate levels.

TABLE II

Characteristics of TK in Lymphocyte Extracts of Controls and Patients with Cobalamin or Folate Deficiency

Source of Enzyme	% activity with CTP as phosphate donor	% inhibition with dCTP	% activity at pH 5.0
Group I	80[a]	70[a]	85[a]
Group III			
- before therapy	16	35	50
- after therapy	95	75	90

Mean percentage of two separate determinations. For experimental assays the following alteration was made in the standard assay. (a) Substitution 2 mM CTP for 5 mM ATP; activity expressed as percentage of activity with 2 mM ATP (b) Addition of 1 mM dCTP activity expressed as percentage of inhibition compared with control without dCTP (c) Substitution of 50 mM sodium acetate for the buffer, percentage activity at pH 7.4.
Group I: two normal controls. Group III: two patients with anemia and cobalamin and folate deficiency.

control and Group III patients following cobalamin or folate therapy exhibit properties consistent with adult TK, namely an ability to utilize CTP as a substrate, considerable inhibition by dCTP and activity at pH 5.0 (1). In

contrast, during the deficient state, the characteristics of activity are dissimilar and suggest that the predominant TK activity is fetal. In agreement with this Table I shows that the ratio of TK activities with either CTP or ATP as phosphate donor in lymphocyte extracts of Group I and Group II patients are similar but are distinctly different to that observed in patients with cobalamin or folate deficiency. In four vitamin deficient patients, appropriate replacement therapy resulted in a decrease of lymphoid TK activity from a mean of 460 ± 200 (1SD) to 165 ± 30 (1SD) pmoles/hr/mg with a concomitant change and the ratio of CTP to ATP TK activities from 0.3 ± 0.1 to 0.75 ± 0.1. This apparent change of fetal to adult TK activity occurred at the earliest 14 days after commencing vitamin B_{12} and folate treatment.

DISCUSSION

The occurrence of fetal TK in tumour or proliferating tissue (1,9) and the adult form in normal tissue (1,3) has been related either to the re-expression of fetal antigens in transformed cells (2) or a normal re-expression of that variant of TK when cells are stimulated to enter the S phase of the cell cycle (3,10). In view of this our finding of elevated TK activity which is fetal in non-dividing human peripheral blood lymphocytes of individuals with either cobalamin or folate deficiency is of some interest. The basis for the diverse cellular aberrations observed in human folate or cobalamin deficiency is unclear. Of prime importance is thought to be interference at one or another step in DNA synthesis consequent to impaired *de novo* thymidylate synthetase activity which in turn reflects decreased cellular N5,10 methylene tetrahydrofolate levels (11-13).

In keeping with this postulate are the observed haematological responses, in cobalamin deficient individuals, to thymidine therapy (14) and the markedly elevated TK activities reported in human cobalamin deficient marrow extracts (15,16). This increased TK activity is not

simply due to an increased number of primitive or proliferating cells but possibly reflects enzyme induction due to thymidylate deficiency (16-18). Whether our observation of elevated fetal TK activity in non dividing lymphocyte cells reflects persistence of elevated TK activity in marrow deficient lymphoid progenitor cells or its induction in the non dividing cell is unclear. Data on the intracellular deoxynucleotide pools of peripheral blood lymphocytes under these conditions is lacking and although the observed response of lymphocyte TK activity following appropriate replacement therapy may be explained by either hypothesis the time interval involved would favour the former of these postulates. Clearly, further studies on *in vitro* models are required to resolve these questions.

ACKNOWLEDGEMENTS

This work was supported in part by the Anti-Cancer Council of Victoria and the National Health and Medical Research Council of Australia.

REFERENCES

1. Taylor, A.T., Stafford, M.A. and Jones O.W. (1972) J. Biol. Chem. 247, 1930-1935.
2. Bull, D.L., Taylor, A.T., Austin, D.M. and Jones O.W. (1974) Virology 57, 279-284.
3. Adler, R. and McAuslan, B.R. (1974) Cell 2, 113-117.
4. Kit, S. (1976) Mol. & Cell Bioch. 11, 161-182.
5. Hoffbrand, A.V., Ganeshaguru, K., Lavoie, A., Tattersall, M.H.N. and Tripp, E. (1974) Nature 248, 602-604.
6. Van Der Weyden, M.B., Cooper, M. and Firkin, B.G. (1973) Blood 41, 299-308.
7. Boyun, A. (1968) Scand. J. Clin Lab Invest. 97, 1-109.
8. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol Chem 193, 265-275.
9. Stafford, M.A. and Jones, O.W. (1972) Biochim. Biophys. Acta 277, 439-442.
10. Munch-Petersen, B. and Tyrsted, G. (1972) Biochim.Biophys. Acta 478, 364-375.
11. Lavoie, A., Trippe, E. and Hoffbrand, A.V. (1974) Clin. Sci Mol. Med. 47, 617-630.
12. Taylor, R.T., Hanna, M.L. and Hutton, J.J. (1974) Arch. Biochem Biophys. 165, 787-795.
13. Shane, B., Watson, J.E. and Stokstad, E.L.R. (1977) Biochim. Biophys. Acta 407, 241-252.
14. Killman, S.A. (1964) Acta Med. Scand. 175, 489-497.
15. Bock, H.E., Hartje, J., Muller, D. and Willman, S.W. (1967) Klinische Wochenschrift 45, 176-188.
16. Nakao, K. and Fujioka, S. (1968) Life Sciences 7, 395-399.
17. Ekker, P. (1966) J Biol Chem. 241, 659-662.
18. Stubblefield, E. and Murphee, S. (1967) Exp. Cell Res. 48, 652-656.