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5-Methyltetrahydrofolate-related Enzymes and DNA Polymerase α in Normal and Malignant Hematopoietic Cells*

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Abstract—*The activities of 5-methyltetrahydrofolate (5-CH_3-THF)-related enzymes [5-CH_3-THF homocysteine methyltransferase and 5,10-methylenetetrahydrofolate (5,10-CH_2-THF) reductase] and DNA polymerase α were measured in normal and malignant hematopoietic cells. The 5-CH_3-THF homocysteine methyltransferase activity was significantly correlated with 5,10-CH_2-THF reductase activity, indicating that the hematopoietic cells with active biosynthesis of tetrahydrofolate from 5-CH_3-THF also actively synthesize 5-CH_3-THF from 5,10-CH_2-THF. The activities of 5-CH_3-THF-related enzymes had a tendency to be high in lymphoid cells and low in myeloid cells, and were not correlated with the percentage of blasts and immature cells in the samples examined. Fairly good correlations were observed among these three enzymes in non-malignant bone marrow cells. However, the activities of two of the enzymes correlated only weakly overall with DNA polymerase α activity in normal and malignant hematopoietic cells. Generally speaking, DNA polymerase α activity correlated well with the percentage of blasts and immature cells in the samples examined.*

INTRODUCTION

THERE are a number of folate coenzymes and their respective metabolic pathways, and these play important roles in cell proliferation. Among folate coenzymes, 5-methyltetrahydrofolate is the predominant form in serum and tissue and has a great influence on cellular folate metabolism [1, 2].

The intracellular level of 5-methyltetrahydrofolate is considered to be regulated by two enzymes—5,10-methylenetetrahydrofolate reductase (EC 1.1.1.68), which catalyzes the synthesis of 5-methyltetrahydrofolate [3], and 5-methyltetrahydrofolate homocysteine methyltransferase (B_{12}-methyltransferase) (EC 2.1.1.13), which catalyzes the utilization of 5-methyltetrahydrofolate [4]. However, the relationship of these enzymes and DNA synthesis has not yet been established, nor is it obvious whether these enzymes are log- or lag-phase enzymes [5–8].

DNA synthesis is catalyzed by three major polymerases, α, β and γ, all of which are present in mammalian cells. Of these, polymerase α (EC 2.7.7.7) plays an important role in DNA replication in cell growth and division [9–11]. However, 5-methyltetrahydrofolate-related enzymes and DNA polymerase α have not been sufficiently investigated in normal and malignant human hematopoietic cells [7, 12–18]. In order to elucidate the roles of 5-methyltetrahydrofolate-related enzymes in cell proliferation and DNA synthesis, we have investigated the relationship among B_{12}-methyltransferase, 5,10-methylenetetrahydrofolate reductase and DNA polymerase α in hematopoeitic cells from various kinds of hematological diseases.

MATERIALS AND METHODS

Forty-three cases were included in this study: 4 iron deficiency anemia, 3 hemolytic anemia, 2 polycythemia vera, 7 acute lymphoblastic leuk-

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emia (ALL), 3 chronic lymphocytic leukemia (CLL), 8 acute myeloblastic leukemia (AML), 7 chronic myelogenous leukemia (CML) and 9 chronic myelogenous leukemia blast crisis. Diagnoses were based on clinical and laboratory data. All the samples were obtained at the time of diagnosis, prior to therapy. Also the results of 15 normal controls were reported; 5 normal bone marrow cells, 6 normal lymphocytes and 4 granulocytes.

The bone marrow or blood samples were obtained with informed consent from patients or normal controls.

Chemicals

5-[^{14}C]-Methyltetrahydrofolate and [^{3}H]-dTTP were purchased from the Radiochemical Centre, Amersham, U.K. Other chemicals were obtained from the following commercial sources: *S*-adenosylmethionine, dithiothreitol, 5-methyltetrahydrofolate, phenylmethylsulfonylfluoride, bovine serum albumin (fraction 5), calf thymus DNA, dGTP, dCTP, dATP and dTTP from Sigma, St. Louis, MO, U.S.A.; vitamin B_{12}, EDTA, menadione, formaldehyde, dimedione, cyanogen bromide and FAD from Wako, Tokyo, Japan; and DL-homocysteine from Nakarai, Kyoto, Japan.

Enzyme preparation

Bone marrow cells and peripheral blood were obtained using heparin and EDTA as anticoagulants from patients with hematological diseases, as well as from normal volunteers. Nucleated cells were separated by a sedimentation method at unit gravity through dextra-sodium metrizoate solution or by Ficoll-Hypaque gradient centrifugation [19, 20]. Normal lymphocytes were separated from granulocytes by Ficoll-Hypaque gradient centrifugation. In leukemia cases the samples containing more than 80% leukemia cells were used for the enzyme assay. Nucleated cells were washed 3 times with PBS. They were suspended at 1×10^8 cells/ml in sucrose TKM buffer (50 mM Tris–HCl, pH 7.6, 25 mM KCl, 5 mM $MgCl_2$, 5 mM dithiothreitol, 0.15 M sucrose, 2 mM phenylmethylsulfonylfluoride and 0.1 mg/ml bovine serum albumin) for the DNA polymerase α and 5,10-methylenetetrahydrofolate reductase assays, or were suspended at $1\text{–}3 \times 10^8$ cells/ml in 50 mM potassium phosphate buffer, pH 7.4, with 1.5 mM reduced glutathione for the B_{12}-methyltransferase assay. These cell suspensions were subjected to 4 cycles of rapid freezing and thawing and were centrifuged at 100,000 *g* for 60 min. The supernatants were used for the enzyme assays.

B_{12}-Methyltransferase assay

The B_{12}-methyltransferase (holoenzyme) was determined as described previously [17]. In some instances enzyme activity was also measured without vitamin B_{12} in the reaction mixture (apoenzyme) [18]. Enzyme activity was represented as nmol of [^{14}C]-methionine formed per hr per 1×10^8 cells.

5,10-Methylenetetrahydrofolate reductase assay

5,10-Methylenetetrahydrofolate reductase was determined according to the method devised by Kutzbach and Stokstad [3], with a minor modification [18]; the standard reaction mixture contained the following to a total volume of 250 μl: 0.2 M potassium phosphate buffer, pH 6.3; 60 mM FAD; 10 mM ascorbic acid; 1.6 mM EDTA; 480 mM 5-[^{14}C]-methyltetrahydrofolate (1.8×10^4 dpm/nmol); 3.6 mM menadione (5 μl of a solution in 50% ethyl alcohol); and 100 μl enzyme solution. Following incubation at 37°C for 60 min the reaction was arrested by chilling in an ice bath and by the addition of 0.25 ml of 0.6 M sodium acetate buffer, pH 4.5, 0.1 ml of 0.1 M formaldehyde and 0.15 ml of 0.4 M dimedione, which was dissolved in 50% ethyl alcohol. The tube was tightly capped and heated at 100°C for 5 min and then cooled at 0°C for 5 min. Two milliliters of toluene were added, followed by vigorous shaking on a thermomixer. After being centrifuged for 10 min at 1000 *g* a 1-ml aliquot of toluene extract was removed for determination of radioactivity by a liquid scintillation spectrometer. A reaction mixture in which enzyme solution was added after incubation was used as the control. Enzyme activity was represented as nmol of [^{14}C]-formaldehyde formed per hr per 1×10^8 cells.

DNA polymerase α assay

DNA polymerase α activity was determined using a reaction mixture as described previously [18]. The reaction mixture contained the following to a total volume of 100 μl: 50 mM Tris–HCl, pH 7.2; 5 mM dithiothreitol; 50 mM KCl; 8 mM $MgCl_2$; 100 μg bovine serum albumin; 2.5 μg activated calf thymus DNA; 0.1 mM each of dGTP, dCTP, dATP and [^{3}H]-dTTP (1800 dpm/nmol); and 50 μl of the enzyme solution. After being incubated at 30°C for 30 min the reaction was stopped by chilling in an ice bath. The aliquot was collected on Whatmann GF/C filter discs and processed according to the Chang and Bollum method [21]. Radioactivity in the filter discs was measured in a toluene-based scintillator with a liquid scintillation spectrometer. DNA polymerase α activity was represented as nmol of

[^{3}H]-dTTP incorporated into DNA per 1×10^8 cells.

The reaction velocity was linear with time and enzyme concentration under the conditions employed. All the enzyme assays were performed in duplicate.

RESULTS

Enzyme activities in normal hematopoietic cells

DNA polymerase α, B_{12}-methyltransferase and 5,10-methylenetetrahydrofolate reductase activities in normal hematopoietic cells are summarized in Table 1. Normal bone marrow cells contained DNA polymerase α, B_{12}-methyltransferase and 5,10-methylenetetrahydrofolate reductase activities. Normal peripheral lymphocytes contained lower levels of DNA polymerase α activity but slightly higher levels of B_{12}-methyltransferase and 5,10-methylenetetrahydrofolate reductase activities than normal bone marrow cells. Normal peripheral granulocytes contained much lower levels of these three enzyme activities than normal bone marrow cells. High levels of all three enzyme activities were detected in bone marrow cells from the patients with iron deficiency anemia and hemolytic anemia.

Enzyme activities in malignant hematopoietic cells

The three enzyme activities in malignant hematopoietic cells are summarized in Table 1. Bone marrow cells from patients with ALL contained higher levels of DNA polymerase α, B_{12}-methyltransferase and 5,10-methylenetetrahydrofolate reductase activities than did normal bone marrow cells. Peripheral lymphocytes from patients with CLL contained lower levels of DNA polymerase α activity but higher levels of B_{12}-methyltransferase and 5,10-methylenetetrahydrofolate reductase activities than did normal bone marrow cells. Conversely, bone marrow cells from patients with AML contained higher levels of DNA polymerase α activity but lower levels of B_{12}-methyltransferase and 5,10-methylenetetrahydrofolate reductase activities than did normal bone marrow cells. Bone marrow cells from patients with CML in chronic phase contained lower levels of DNA polymerase α and 5,10-methylenetetrahydrofolate reductase activities, whereas bone marrow cells from patients with CML in blast crisis by and large contained higher levels of the three enzyme activities. Bone marrow cells from two patients with polycythemia vera contained somewhat lower levels of the three enzyme activities than did normal bone marrow cells.

Mutual relationship among the three enzymes

B_{12}-Methyltransferase activity (holoenzyme) was correlated significantly with 5,10-methylenetetrahydrofolate reductase activity in both normal and malignant hematopoietic cells ($n = 58$, $r = 0.62$, $P < 0.001$) (Fig. 1). By contrast, it was only weakly correlated with DNA polymerase α

*Table 1. Mean values of 5,10-methylenetetrahydrofolate reductase, 5-methyltetrahydrofolate homocysteine methyltransferase and DNA polymerase α activities in normal and malignant hematopoietic cells**

	Immature and blast cells (%)†	CH_2-THF reductase‡	B_{12}-Methyltransferase§ Holo.	Apo.	DNA polymerase α‖
Normal					
bone marrow (BM) ($n = 5$)	32.0 ± 3.5	4.49 ± 1.16	1.10 ± 0.67	0.71 ± 0.43 ($n = 3$)	2.81 ± 1.13
lymphocyte (PB) ($n = 6$)	—	6.25 ± 1.55	1.39 ± 0.80	0.90 ± 0.25 ($n = 3$)	1.56 ± 0.65
granulocyte (PB) ($n = 4$)	—	0.93 ± 0.74	0.08 ± 0.06	—	0.51 ± 0.25
IDA¶ (BM) ($n = 4$)	56.8 ± 7.0	7.41 ± 1.71	2.20 ± 0.85	—	6.15 ± 2.05
HA (BM) ($n = 3$)	65.3 ± 5.0	9.98 ± 1.46	3.97 ± 1.80	2.63 ($n = 2$)	8.54 ± 1.44
Malignant					
ALL (BM) ($n = 7$)	93.2 ± 4.7	8.80 ± 6.82	1.71 ± 0.70	1.02 ± 0.63 ($n = 4$)	8.90 ± 2.51
CLL (PB) ($n = 3$)	—	10.44 ± 2.27	3.30 ± 1.18	2.15 ($n = 1$)	2.21 ± 0.57
AML (BM) ($n = 8$)	89.5 ± 4.7	2.36 ± 1.64	0.71 ± 0.64	0.56 ± 0.40 ($n = 4$)	7.50 ± 3.77
CML (BM) ($n = 7$)	—	1.94 ± 1.84	0.23 ± 0.25	—	1.33 ± 1.35
CMLBC (BM) ($n = 9$)	91.8 ± 3.7	5.12 ± 5.54	1.91 ± 1.55	1.26 ± 1.01 ($n = 4$)	7.79 ± 2.58
PV (BM) ($n = 2$)	26	2.75	0.68	—	1.88

*Mean ± S.D.

†Immature and blast cells include blasts, promyelocytes, myelocytes, proerythroblasts, basophilic erythroblasts and polychromatophilic erythroblasts.

‡nmol of formaldehyde formed/1×10^8 cells/hr.

§nmol of methionine formed/1×10^8 cells/hr.

‖nmol of dTMP formed/1×10^8 cells/hr.

¶IDA = Iron deficiency anemia; HA = hemolytic anemia; ALL = acute lymphoblastic leukemia; CLL = chronic lymphocytic leukemia; AML = acute myeloblastic leukemia; CML = chronic myelogenous leukemia in chronic phase; CMLBC = chronic myelogenous leukemia in blast crisis; PV = polycythemia vera.

activity in both overall normal and malignant hematopoietic cells ($n=58$, $r=0.42$, $P<0.01$) (Fig. 2). A similar weak correlation was also observed between 5,10-methylenetetrahydrofolate reductase and DNA polymerase α activities ($n=58$, $r=0.33$, $P<0.01$). However, holoenzyme and DNA polymerase α had a significant correlation in non-malignant bone marrow cells ($n=12$, $r=0.81$, $P<0.01$) (Fig. 3). Also, 5,10-methylenetetrahydrofolate reductase and DNA polymerase α had a significant correlation in non-malignant hematopoietic cells ($n=12$, $r=0.83$, $P<0.01$) (Fig. 4).

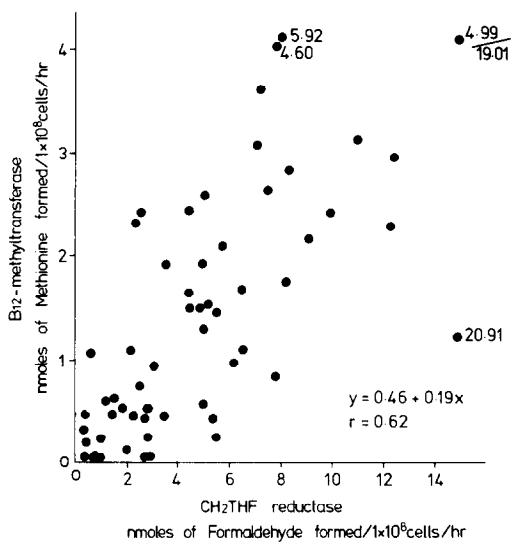

Fig. 1. Correlation between 5-methyltetrahydrofolate homocysteine methyltransferase (holoenzyme) and 5,10-methylenetetrahydrofolate reductase activities in normal and malignant hematopoietic cells.

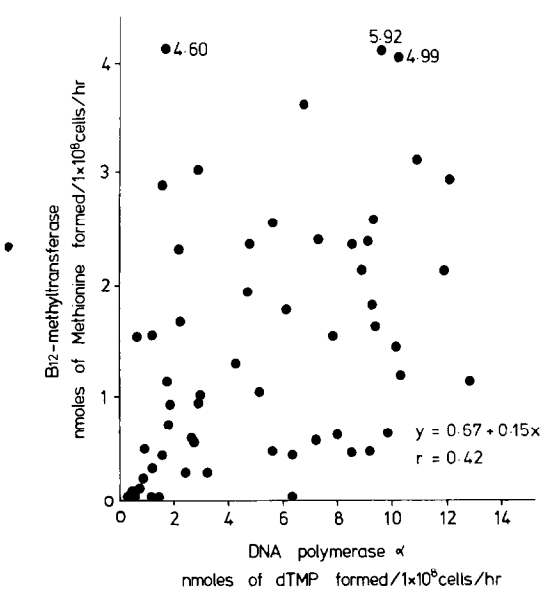

Fig. 2. Correlation between 5-methyltetrahydrofolate homocysteine methyltransferase (holoenzyme) and DNA polymerase α activities in normal and malignant hematopoietic cells.

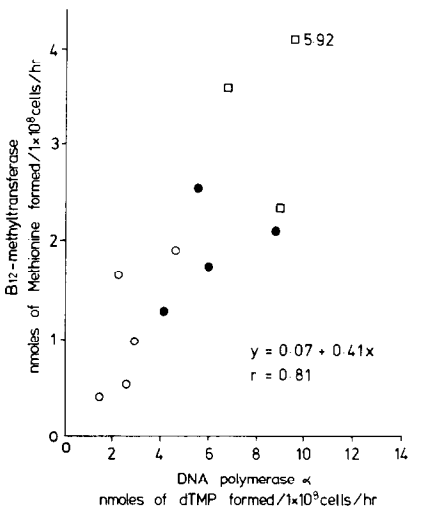

Fig. 3. Correlation between 5-methyltetrahydrofolate homocysteine methyltransferase (holoenzyme) and DNA polymerase α activities in normal bone marrow cells (○) and bone marrow cells from patients with iron deficiency anemia (●) and hemolytic anemia (□).

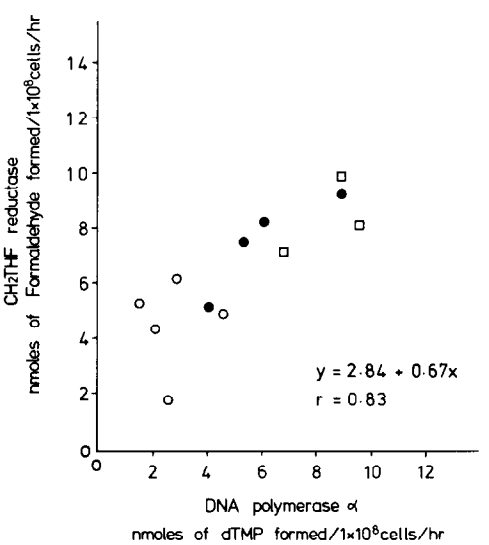

Fig. 4. Correlation between 5,10-methylenetetrahydrofolate reductase and DNA polymerase α activities in normal bone marrow cells (○) and bone marrow cells from patients with iron deficiency anemia (●) and hemolytic anemia (□).

DISCUSSION

In this study we examined the relationship between the levels of B_{12}-methyltransferase, 5,10-methylenetetrahydrofolate reductase and DNA polymerase α activities in various types of normal and malignant hematopoietic cells. Compared with normal bone marrow cells, increased B_{12}-methyltransferase activity was detected in normal lymphocytes, iron deficiency anemia, hemolytic anemia, ALL, CLL and CML blast crisis, whereas decreased activity was detected in normal granulocytes, AML and CML chronic phase. A similar tendency was observed in 5,10-methylene-

tetrahydrofolate reductase activity. This correlation between the activities of these two enzymes suggests that the cells with active biosynthesis of tetrahydrofolate from 5-methyltetrahydrofolate are also actively synthesizing 5-methyltetrahydrofolate from 5,10-methylenetetrahydrofolate.

We also demonstrated DNA polymerase α activity in various types of normal and malignant hematopoietic cells. Compared with normal bone marrow cells, the increased activity was observed in iron deficiency anemia, hemolytic anemia, ALL, AML and CML blast crisis. Decreased activity was demonstrated in normal lymphocytes and granulocytes, CLL and CML in chronic phase. Although the cell populations and degree of maturation differed in each case, these results suggest that immature cells with cell division capacity have high levels of DNA polymerase α whereas mature cells without cell division capacity have low levels of such activity. Therefore the DNA polymerase α activity in cells from patients with ALL, AML and CML blast crisis differs little from that of normal bone marrow cells and bone marrow cells from patients with iron deficiency anemia and hemolytic anemia. If the activities are corrected, and the percentage of blast cells or immature cells with a capacity to divide are taken into account, these data are in agreement with those of Coleman *et al.* [12].

The two enzymes involved in 5-methyltetrahydrofolate metabolism correlated well with DNA polymerase α activity in bone marrow cells from normal volunteers and patients with iron deficiency anemia and hemolytic anemia, suggesting the possibility that these two enzymes are, in fact, correlated with DNA synthesis. However, the activities of these two folate enzymes do not always correlate well with those of DNA polymerase α among various kinds of normal and malignant hematopoietic cells. Patients with AML have higher levels of DNA polymerase α activity but much lower levels of the two folate-related enzymes than normal bone marrow cells. On the other hand, patients with CLL and normal lymphocytes have lower levels of DNA polymerase α but much higher levels of the two folate-related enzymes. Therefore some unknown factor independent of DNA synthesis might be important in regulating these two folate-related enzymes. Recently, methylcobalamin, coenzyme of B_{12}-methyltransferase, has been reported to stimulate lymphocyte functions [22]. These data and ours may suggest that B_{12}-methyltransferase may play some role in lymphocyte functions. The role of methionine for methylation of DNA and/or lipids may be of importance in lymphocytes and/or CLL cells [23]. This would account for the high activity of B_{12}-methyltransferase. However, further investigations are necessary in this field.

Our data, which are not consistent with previous reports [5, 7], suggest that B_{12}-methyltransferase is not a good marker of DNA synthesis and cell proliferation.

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