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Expression of Erythroid Acetylcholinesterase in the K-562 Leukemia Cell Line[1]

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ABSTRACT

Differentiation-dependent expression of enzyme loci was evaluated in two human leukemic cell lines, the pluripotent leukemia cell line K-562 and the promyelocytic-like cell line HL-60. Acetylcholinesterase, a marker of erythroid differentiation, was present in K-562 cells and absent in HL-60 cells. This difference between the two lines was apparently unrelated to dosage effect; other enzymes carried on trisomic chromosomes in K-562 cells did not show dosage effect. Acetylcholinesterase activity was higher in subclone K-562 (S), which shows higher expression of hemoglobin. Electrophoretic mobility of acetylcholinesterase from K-562 (S) was of fetal type.

INTRODUCTION

Cell lines derived from leukemic patients are useful tools to investigate the normal and abnormal differentiation of hemopoietic stem cells. In particular, K-562, derived from a chronic myeloid leukemia patient (20), is a pluripotent cell line studied extensively as a model of erythroid differentiation because of the constitutive expression of hemoglobin, increased hemoglobin synthesis on addition of inducers, and differential expression of hemoglobin genes (14); other markers of erythroid differentiation, like glycophorin and i-and-I antigen, are also expressed (18, 25).

The purpose of the present work was to evaluate another marker of erythroid expression, ACHE.[3] This enzyme was found to be a constitutive marker of Friend erythroleukemia cells and to be inducible in parallel with hemoglobin by dimethyl sulfoxide (10). Previous data from our laboratory also suggest a coordinate expression of hemoglobin and of membrane-bound ACHE in erythrocytes (16).

The expression of commitment toward a differential cell lineage in K-562 cells can be evaluated by comparison with another cell line, HL-60, also derived from a leukemic patient (9). This line can be induced to differentiation toward mature granulocytes with dimethyl sulfoxide or retinoic acid (8).

In the present work, the expression of ACHE was measured in terms of catalytic activity and electrophoretic mobility in K-562 and HL-60 cell lines. Since the 2 lines differ consistently in the number of chromosomes, K-562 being near triploid and HL-60 hypodiploid, dosage effect was also measured to evaluate the influence of the different number of each chromosome in the expression of different genes.

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[3] The abbreviations used are: ACHE, acetylcholinesterase (EC 3.1.1.7); PGD, 6-phosphogluconate dehydrogenase (EC 1.1.1.44); PGM, phosphoglucomutase (EC 2.7.5.1); PK, pyruvate kinase (EC 2.7.1.40).

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MATERIALS AND METHODS

Cell Lines. Line K-562 was kindly provided by Dr. M. Gianni (Institute of Medical Clinics, University of Milan, Italy). Subclone K-562 (S), kindly provided by Dr. L. Pegoraro (Institute of Medical Clinics, University of Torino, Italy), was also used as indicated. Line HL-60 was kindly provided by Dr. A. Donelli (Institute of Medical Clinics, University of Modena, Italy). Cells were grown in suspension in Roswell Park Memorial Institute Medium 1640 supplemented with 10% fetal calf serum in a humidified atmosphere with 5% CO_2. Cells, collected either in the log phase or at plateau, were washed with 0.15 M NaCl and then utilized for enzyme analysis.

Fibroblasts. Fibroblasts, obtained from skin biopsy of normal volunteers, were grown in Roswell Park Memorial Institute Medium 1640 supplemented with 10% fetal calf serum and then collected with a rubber policeman and washed with 0.15 M NaCl.

Peripheral Blood Cell Populations. Adult blood samples from normal volunteers or from polycythemic patients and cord blood samples, obtained at the time of delivery, were collected in anticoagulant acid-citrate-dextrose solution (Becton Dickinson, Rutherford, N. J.) and used within 24 hr. Homogeneous populations of different cells were prepared as follows. Platelets were isolated with successive low-speed centrifugations (1) and then suspended in NH_4Cl solution (0.15 M, containing 2.5 mM D-glucose) to eliminate RBC contamination. No WBC were detectable in this preparation. After separation of platelets, remaining blood was layered on Ficoll metrizoate (Lymphoprep; Nyegaard Co., A/S, Oslo, Norway) according to the method of Boyaum (4). The cells at the interface were collected, washed, and utilized for preparation of lymphocytes or monocytes, while the RBC-rich fraction was used for preparation of granulocytes. Cells at the Lymphoprep interface were incubated in Petri dishes to remove monocytes (22); lymphocytes were then collected, washed, and suspended in NH_4Cl solution to remove RBC contamination. Monocyte contamination was less than 10%, as demonstrated by microscopic observation of slides after cytocentrifugation and May-Grünwald-Giemsa staining. For monocyte separation, cells at the Lymphoprep interface resuspended in a Percoll solution (Pharmacia Fine Chemicals, Uppsala, Sweden; density, 1.080 g/ml) were underlayered below the following Percoll step gradient: density, from bottom to top, 1.070 g/ml; 1.0605 g/ml; and 1.045 g/ml. The gradient was centrifuged at 400 × *g* for 30 min, and monocytes were collected at the interface between densities of 1.0605 and 1.0450 g/ml. Contamination by lymphocytes was less than 10%. To isolate granulocytes, the RBC-rich Lymphoprep fraction was sedimented on Dextran solution, 3% in 0.15 M NaCl (Macrodex; Baxter, Trieste, Italy), and granulocytes were isolated from the supernatant. Cells were suspended in NH_4Cl solution to eliminate RBC contamination. Purity of cell preparation was tested by microscopic observation of slides as described above. RBC were washed with 0.15 M NaCl and then treated as described below.

Analytical Procedure. Assay and electrophoresis of cytoplasmic enzymes were performed after diluting cells (1:4, v/v) in lysis buffer (2.7 mM EDTA:0.7 mM 2-mercaptoethanol, pH 7.0), lysing them by 3 cycles of freezing-thawing, and centrifuging them at 40,000 × *g* for 30 min at 4°. Enzymes were assayed according to the method of Beutler (3); electrophoresis was performed according to the method of Harris and Hopkinson (17).

For ACHE preparation, cells were solubilized (1:4, v/v) with lysis buffer

(10 mM cytrate phosphate buffer, pH 7.0: 1% Triton X-100) and were then centrifuged at 40,000 × *g* for 30 min at 4°, and the supernatant was used for ACHE assay according to the method of Ellman *et al.* (12). In order to exclude substances which react with 5,5-dithiobis-2-nitrobenzoic acid, a blank was set for cell lysate omitting the substrate in the spectrophotometric assay. A specific inhibitor, 1,5-bis(4-allyldimethylammoniumphenyl)pentane-3-one dibromide, 20 μM (Sigma Chemical Co., St. Louis, Mo.) was used to distinguish true ACHE. ACHE activity, measured by the radiometric method (26), gave the same results as did the spectrophotometric method.

Electrophoretic analysis was performed as described previously by Garrè *et al.* (15). Proteins were assayed according to the method of Lowry *et al.* (19), as modified by Dulley and Grieve (11) for samples containing Triton X-100.

Chromosome analysis was performed according to the method of Caspersson *et al.* (5).

Table 1

Distribution of ACHE in peripheral blood cells, cell lines, and fibroblasts in humans

Sources	ACHE activity		
	Units[a]/10^{11} cells	Units/g of protein	Hemoglobin (pg/cell)
Cell lines			
K-562	28	1.4	0.3
K-562 (S)	106	5.9	0.8
HL-60	<1		
Fibroblasts	<1		
RBC			
Adult	63	16.7	30
Cord	51	8.7	30
WBC			
Unfractionated (cord, adult); granulocytes, monocytes, lymphocytes (adult)	<1		

[a] Units are defined as μmol of the substrate utilized at 25° per min.

RESULTS

ACHE Activity. The activities of ACHE from K-562 cells, HL-60 cells, RBC, WBC, platelets, and fibroblasts are compared in Table 1. RBC and K-562 cells had significant ACHE activity, which was not detectable in HL-60 cells, WBC, platelets, and fibroblasts. Clone K-562 (S), with higher cellular content of Hb than K-562, also had a higher level of ACHE.

Gene Dosage Effect. To determine if the level of ACHE in K-562 cells was differentiation dependent or was due to gene dosage effect, other enzyme activities were compared in K-562 and HL-60 cells and were related to difference in the number of chromosomes carrying the relative structural genes. For this purpose, a detailed karyotype analysis was also performed on the K-562 line, especially in view of its extensive clonal evolution. The line examined is near triploid, with 65 to 72 chromosomes and a modal number of 69 chromosomes found in 29 of 77 metaphases examined. A representative karyotype is shown in Fig. 1. Clone K-562 (S), although different from the K-562 cell line essentially because of a more stable modal number of 67 chromosomes and because of the presence of 7q+, M_1, according to Cioe *et al.* (7), is comparable to K-562 in being trisomic for chromosome 2.

The results of the enzyme activities, as a function of the number of chromosomes present, are shown in Table 2. No significant dosage effect was found for the following enzymes: glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49); PGD; glutathione reductase (E.C. 1.6.42); lactate dehydrogenase (E.C. 1.1.1.27); adenylate kinase (E.C. 2.7.4.3); and enolase (E.C. 4.2.1.11). PGM and PK showed a significantly lower activity in K-562, in spite of prevalent trisomy for chromosomes carrying their respective structural genes. Cytogenetic analysis did not reveal obvious deletions in chromosome 1 where the PGM_1 gene maps. As for chromosome 15, where the structural gene for PK_3

Table 2

Comparative analysis of enzyme activities in cell lines K-562 and HL-60 as a function of the number of chromosomes present

Enzyme activity was measured at cell concentrations of 5 × 10^5/ml (log phase) and 1 × 10^6/ml (plateau) for the K-562 line and 6 × 10^5/ml (log phase) and 1.2 × 10^6/ml (plateau) for the HL-60 line. No significant difference was found in the levels and in the ratio of activity of the enzyme tested, and the values were averaged. Chromosome assignment is according to the systems of McKusik (21) and Berg *et al.* (2).

Enzyme	Chromosome assignment	Chromosomes present in cell line		Enzyme units[c]				Ratio (K-562:HL-60)
		K-562[a]	HL-60[b]	K-562	HL-60	p		
PGD	1	3	2	103.25 ± 68.44[d]	99.50 ± 14.25	NS[e]		1.030
Enolase 1	1	3	2	320.12 ± 97.62	324.0 ± 37.66	NS		0.988
Enolase 2	12	3	2					
PGM 1	1	3	2	10.46 ± 2.98	158.0 ± 12.49	<0.01		0.066
PGM 2	4	3	2					
PGM 3	6	3	2					
Adenylate kinase 2	1	3	2	450.00 ± 152.28	488.75 ± 128.57	NS		0.921
Adenylate kinase 1	9	3–2	2					
Adenylate kinase 3	9							
Glutathione reductase	8	3–4	1–2	62.75 ± 20.22	44.75 ± 14.43	NS		1.400
Lactate dehydrogenase A	11	3	2	2075.71 ± 1333.70	2787.73 ± 810.43	NS		0.971
Lactate dehydrogenase B	12	3	2					
PK 3	15	3–2 Marker t(15; 18)	2	819.75 ± 300.51	3743.75 ± 1109.00	<0.01		0.219
Glucose-6-phosphate dehydrogenase	X	2	1	266.75 ± 84.02	171.75 ± 38.10	NS		1.550

[a] Chromosome 9 was trisomic in about 50% of the observed metaphases. Chromosome 8 was tetrasomic in 50% and trisomic in 50% of the metaphases. Chromosome 15 was trisomic in 60% of the metaphases.

[b] Chromosome 8 was prevailingly monosomic.

[c] Enzyme units are defined as μmol of substrate utilized per min per g of protein at 25°.

[d] Mean ± S.D. of 8 and 4 experiments for K-562 and HL-60 cell lines, respectively.

[e] NS, not significant.

is assigned, 1 of 3 chromosomes was constantly involved in a translocation [marker t(15; 18)], while the other 2 chromosomes 15 were normal.

Electrophoretic Pattern. The electrophoretic pattern of some of the enzymes, the activity of which had been tested previously, was also examined to detect possible differential expression between erythroid and granulocytic lineages. Glucose-6-phosphate dehydrogenase, PGD, and glutathione reductase were uninformative and identical on both lines; lactate dehydrogenase confirmed previous results of Pantazis *et al.* (23). PGM confirmed the absence of a PGM_1 band and increased intensity of PGM_3 as described previously (24).

ACHE electrophoresis demonstrated a band of activity in K-562 (S), with migration equal to ACHE from fetal RBC and slightly faster than adult RBC (Chart 1). No band was visible in WBC, platelets, fibroblasts, or HL-60 cells.

DISCUSSION

The pluripotent leukemia cell line K-562 expresses several erythroid markers: Hb (14); glycophorin (18); and i-and -I antigen (25). Our results demonstrate also the presence of membrane-bound ACHE in this cell line. This enzyme can be considered a differentiative marker, its activity being found only in neural tube, muscles, and erythrocytes (6). Indirect support of ACHE specificity as an erythroid marker stems from its constitutive presence in Friend erythroleukemia cells, where it is also inducible together with Hb (10). Furthermore, carefully isolated blood fractions, as reported in the present work, did not reveal any ACHE activity in platelets or in various leukocyte components, thus confirming the presence of the enzyme only in the erythrocytes. A promyelocytic-like leukemic cell line, HL-60, taken as a control, also failed to show ACHE activity.

Subclone K-562 (S), with a higher base-line concentration of Hb, also has a higher level of ACHE activity, thus suggesting a coordinate expression of these 2 erythroid markers. The increased level of ACHE activity in this clone also allows electrophoretic detection of the enzyme, the demonstration of which on polyacrylamide gel is otherwise difficult. The electrophoretic migration of the enzyme is comparable to that from fetal erythrocytes, *i.e.,* slightly faster than adult RBC, as demonstrated previously (16). Expression of this immature form of ACHE may be correlated with the immature type of Hb present in line K-562.

Aside from differentiated tissues, exceptionally elevated ACHE activity was described in cultured fibroblasts which were trisomic for chromosome 2, thus suggesting, on the basis of gene dosage, a location of the gene for ACHE on chromosome 2 (6). Since line K-562 is quasitriploid, chromosome 2 being trisomic, the possibility exists that higher activity is related to this karyotype abnormality. The increased level of ACHE in fibroblasts which are trisomic for chromosome 2 is not, however, a simple gene dosage effect, since normal diploid fibroblasts have undetectable activity, while activity is instead increased 28-fold in the trisomic line (6).

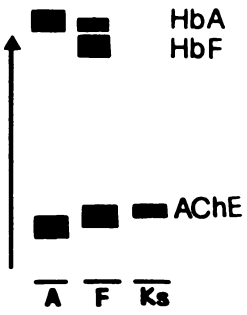

Chart 1. Schematic drawing of the electrophoretic pattern of ACHE. *A*, adult RBC; *F*, fetal RBC (cord); *Ks*, K-562 (S).

The significantly higher level found in a subclone K-562 (S), with higher hemoglobin level, may suggest a coordinate regulation of expression of 2 differentiative markers. The level of other enzymes, the genes of which map on trisomic chromosomes, does not demonstrate dosage effect as compared to a diploid leukemic line, HL-60. Two enzymes have paradoxically lower activity in the K-562 line, in spite of trisomy of the chromosomes carrying their structural genes. The first one is PGM, the lower activity of which in K-562 cells is traceable to the absence of the major component, PGM_1, as described previously (24) and confirmed on our electrophoretic assay. No large deletion is cytologically detectable in region p22, and other enzymes in the adjacent region of chromosome 1 (PGD, enolase) are present and fully expressed. A silent allele for PGM_1 was described (13), thus allowing for the possibility that the line originated from a patient heterozygous for a silent allele and that the chromosome 1 carrying this allele had replicated twice. This unlikely hypothesis is not testable. However, the increased activity of PGM_3 in K-562 cells suggests also a regulatory mechanism in this multiloci system. As for the decreased activity of PK, the only detectable abnormality is a rearrangement marker t(15; 18) of 1 of the 3 chromosomes 15. How this may be related to a decreased activity is not known. From our data, therefore, it appears that, in spite of the complexity in the regulation of enzyme activity in cell lines, ACHE can be considered a marker of erythroid commitment of the blast line K-562.

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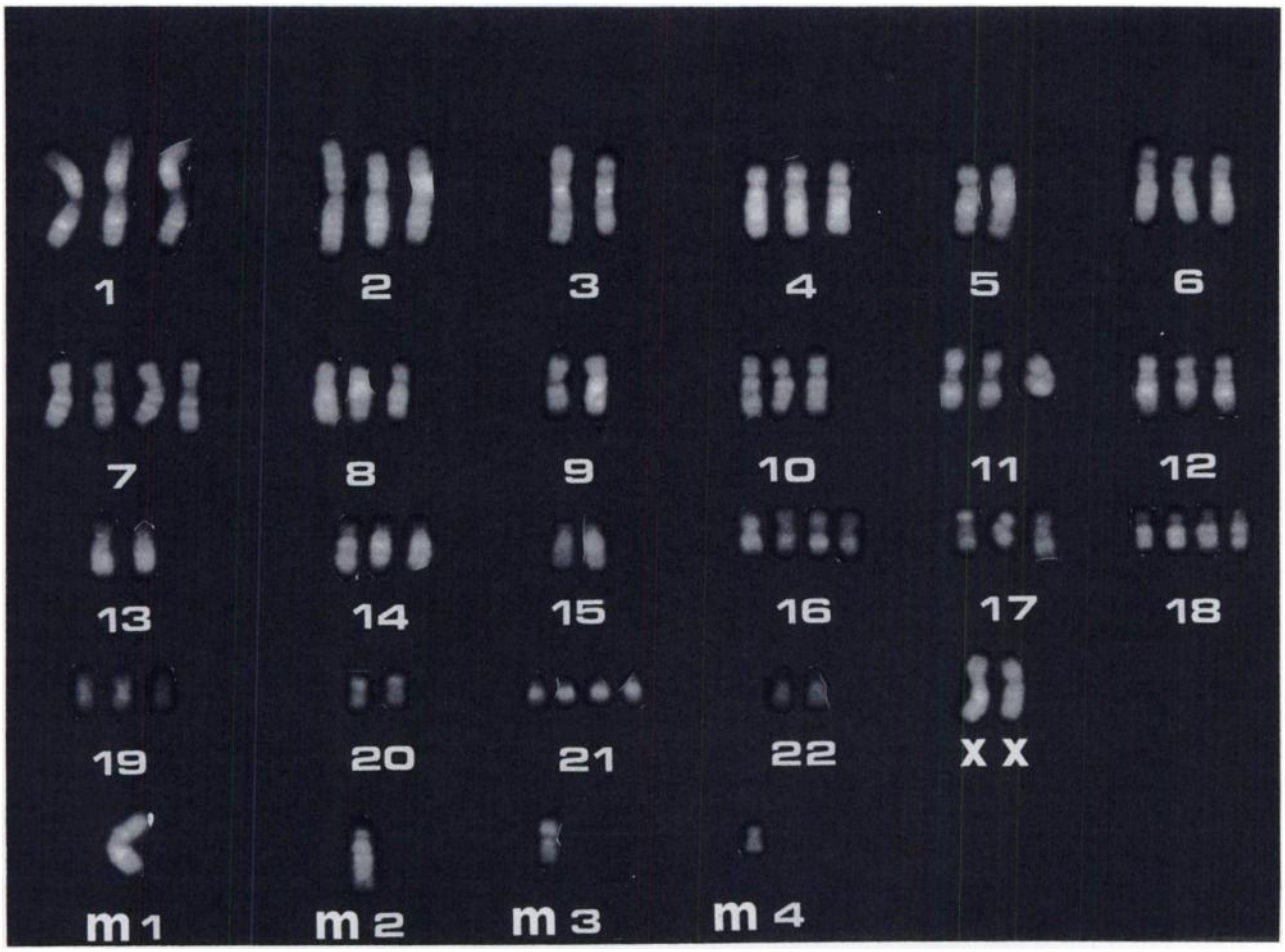

Fig. 1. Q-banding karyotype of the K-562 metaphase. The first chromosome 6 shows additional material on the short arms (6p+). No Ph^1 chromosome is present. M2, a typical marker of the K-562 cell line, is interpreted as t(15; 18)(q 21; q 23). This metaphase also shows a structural rearrangement, present only in a few mitoses, in the short arms of the first chromosome 2 (2p+).