

Differential Expression of the Globin Genes in Human Leukemia K562(S) Cells Induced to Differentiate by Hemin or Butyric Acid¹

Livia Cioe,² Alistair McNab, Howard R. Hubbell, Pacifico Meo,² Peter Curtis, and Giovanni Rovera³

Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104

ABSTRACT

Human leukemia K562(S) cells were induced to differentiate by 50 μ M hemin or 1.4 mM butyric acid, and the types of hemoglobins synthesized were compared. In both cases, embryonal hemoglobins [Portland, Gower 1, Hb X, and fetal hemoglobin (Hb F)] were detected. Butyric acid-treated K562(S) cells contained mostly Hb Gower 1 ($\zeta_2\epsilon_2$) and a hemoglobin with the electrophoretic characteristics of Portland ($\gamma_2\zeta_2$). For hemin-treated K562(S), the most abundant hemoglobin synthesized was Hb X ($\epsilon_2\gamma_2$), and the second most abundant was Bart's (γ_4). Traces of Gower 1 were observed in nontreated K562(S) cells.

The kinetics of hemoglobin induction as a result of the two treatments differed; increased hemoglobin synthesis was detected after only 24 hr of hemin treatment, whereas 4 days were required in butyric acid-treated cells. Both hemin and butyric acid were able to induce their respective patterns of hemoglobin synthesis independent of the presence of serum in the K562(S) growth medium. Analysis of the globin chains in induced K562(S) cells induced to differentiate indicated that, with both inducers, adult α - but not β -globin chains were present. Karyotype analysis of K562(S) cells revealed a nearly triploid chromosome complement with a modal number of 68 chromosomes. Three copies of chromosome 11 and four copies of chromosome 16 (coding for the β -like and α -like globin genes, respectively) were present. A large marker chromosome, involving chromosome 7, and a Philadelphia chromosome were also seen. These data characterize the K562(S) subline and also indicate that hemin and butyric acid differ in their effects on the expression of embryonal globin genes.

INTRODUCTION

A cell line (K562) was established by Lozzio and Lozzio (21) from a patient with chronic myeloid leukemia in the acute phase. These cells were thought to represent stem cell precursors of the myeloid lineage. Andersson *et al.* (5), using specific antibodies and the benzidine reaction, have since shown that these cells could be induced to synthesize hemoglobin by butyric acid treatment. They also showed that glycophorin, a protein present in cells of the erythroid lineage, could be detected on the surface membranes of uninduced K562 cells (6). Rutherford *et al.* (30, 31) have reported that hemin treatment of these cells could induce the synthesis of a variety of embryonal and fetal hemoglobins rather than adult hemoglobin. However, the possibility that hemin and butyric acid could

induce the synthesis of different types of hemoglobin in K562 cells was not considered.

There is strong evidence that, in the mouse erythroleukemia cells induced to differentiate, the patterns of synthesis of the 2 adult hemoglobins depend on the inducer used (2, 23, 26). It has been reported that hemin induction of clone 745 Friend erythroleukemia cells, unlike other inducers of these cells, results in the synthesis of sizable amounts of the β minor and α -globin chains but very little β major chain (2, 26). These variations are controlled at the pretranslational level (3, 28).

We describe in this report the differential expression of the embryonal globin genes and the absence of human adult hemoglobins after treatment of a particular cell subline [K562(S)] with hemin or butyric acid. Karyotype analysis of K562(S) cells indicated the presence of several copies of chromosomes 11 and 16 which code for the globin genes.

MATERIALS AND METHODS

Cell Line and Inducibility. Three sublines of human leukemia K562 cells obtained from different laboratories were examined for inducibility. Only the K562(S) responded with increased hemoglobin synthesis to both hemin and butyric acid induction, the other cultures being poorly inducible by butyric acid. Dimethyl sulfoxide, hypoxanthine, and hexamethylene bisacetamide did not induce increased hemoglobin synthesis in K562(S) cells. The K562(S) cells were not recloned for the experiments reported here.

Cell Culture. The K562(S) cells were grown in either Roswell Park Memorial Institute Tissue Culture Medium 1640 containing 10% fetal bovine serum (Flow Laboratories) in 5% CO₂ humidified atmosphere or in Roswell Park Memorial Institute Tissue Culture Medium 1640 containing insulin (5 μ g/ml), transferrin (5 μ g/ml), and selenium dioxide (3×10^{-8} M) (8). Cells were plated at 2×10^5 /ml and treated with 0.05 mM hemin (Sigma Chemical Co.) or 1.4 mM butyric acid (Aldrich Chemical Co.) for a period of 4 to 5 days. Cells were stained with benzidine to detect hemoglobin production. Benzidine-positive cells were detected using *o*-dianisidine (Sigma) (24, 26).

Hemoglobin Determination. After growth in the presence of inducing agents, cells were chilled on ice, pelleted by low-speed centrifugation, and washed twice with PBS.⁴ The washed cell pellets were then resuspended in an equal volume of distilled water. Cells were lysed by 3 cycles of freeze-thawing and centrifuged once at 2,000 rpm for 10 min and again at 15,000 rpm for 45 min.

The visible absorbance spectrum of the supernatant and the

¹ This work was supported by Grants CA-10815 and CA-24273 from the National Cancer Institute.

² Present address: Institute of Virology, School of Medicine, University of Rome, Rome, Italy.

³ To whom requests for reprints should be addressed.

Received July 2, 1980; accepted October 3, 1980.

⁴ The abbreviations used are: PBS, phosphate-buffered saline (130 mM NaCl; 0.37 mM CaCl₂; 2.6 mM KCl; 0.05 mM MgCl₂; 1.4 mM KH₂PO₄; 15.6 mM NaH₂PO₄); SDS, sodium dodecyl sulfate; poly(A)⁺RNA, polyadenylated RNA; cDNA, complementary DNA.

absorbance at 414 nm were determined. Readings were taken at 403 and 425 nm to detect any nonspecific absorbance due to light scattering. Absorbance at 414, 540, and 576 nm, shifting to 417, 537, and 568 nm, respectively, after carbon monoxide treatment, positively identified the material as hemoglobin (30). Hemoglobin was then quantitated at 414 nm, assuming an absorbance of 1.0 at 414 nm is equivalent to 0.094 mg/ml (17).

The total cell lysate was subjected to electrophoresis in order to separate hemoglobins. Total cellular proteins from 10^8 cells were obtained by resuspending the cell pellet in an equal volume of distilled water, lysing the cells by 3 cycles of freeze-thawing, and centrifuging them at 2,000 rpm for 10 min followed by 15,000 rpm for 45 min. The supernatant was electrophoresed on CelloGel (Chemtron) in borate buffer (pH 8.6) (34) at 5 ma for 2 hr. Hemoglobin bands were visualized by benzidine staining using *o*-dianisidine (24).

Determination of Globin Chains. Hemin- or butyric acid-induced cultures were maintained in the log phase of growth and labeled for 16 hr with ^{14}C -amino acid mixture (0.5 $\mu\text{Ci}/\text{ml}$) at the height of hemoglobin synthesis as determined by benzidine staining and absorbance at 414, 540, and 576 nm of cell lysates.

Cells were harvested, washed twice in ice-cold PBS, and lysed in an equal volume of hemoglobin lysis buffer (50 mM Tris, pH 7.0:25 mM KCl:5 mM MgCl_2 :1 mM mercaptoethanol:0.3% Triton X-100) (17). A total of 0.2 to 0.5×10^6 cpm of total cell lysate contained 2 absorbance units/ml at 280 nm. Carrier hemoglobin (cord blood lysate) in a final sample volume of 75 μl containing 4% ampholines (pH 6.0 to 8.0) was added, and the samples were subjected to isoelectric focusing on 11 \times 0.3-cm disc gels at 500 V for 4 to 5 hr at 4° (25).

The main hemoglobin bands of both adult and cord blood were cut from the isoelectric focusing gels and placed in hemoglobin lysis buffer for 16 hr at 4°. The eluted proteins were separated on 2% Triton X-100:urea gels (27, 28). The material (4 to 7×10^3 cpm) present in each adult or cord hemoglobin band were resuspended in a final volume of 50 μl of denaturation buffer (4 M urea:5% acetic acid:5% 2-mercaptoethanol:0.02% pyronin Y) (27).

Gels were electrophoresed for a period of 8 hr at 120 V (27, 28); stained with a mixture of Coomassie blue, acetic acid, and methanol (0.1% w/v, 10% v/v, 20% v/v, respectively); and fluorographed (9) for 2 to 3 weeks at -70°.

RNA Extraction. Cells were centrifuged at low speed and washed twice in ice-cold PBS. The washed cell pellet was resuspended in 1 ml of hemoglobin lysis buffer. After 15 min on ice, the pellet was centrifuged at 15,000 rpm for 10 min. SDS was added to the supernatant to a final concentration of 0.5% and then extracted at room temperature with an equal volume of phenol:chloroform (1:1, v/v). Material in the aqueous phase was separated by centrifugation at 10,000 rpm for 10 min. Extraction was repeated until no protein was visible in the interphase. The RNA was then precipitated by the addition of sodium chloride to a final concentration of 3% followed by 3 volumes of ethanol. RNA was refrigerated overnight at -20°, pelleted from ethanol by centrifugation at 10,000 rpm for 15 min, and washed in ice-cold 70% ethanol. Finally, RNA was dissolved in distilled water and stored at -20°.

Isolation of Poly(A)⁺RNA. Total cytoplasmic RNA, prepared as described above, was resuspended in an equal volume of 2

\times "binding buffer" (40 mM Tris-HCl, pH 7.4:0.3 M NaCl:2 mM EDTA:0.1% SDS) and subjected to oligodeoxythymidylic acid-cellulose chromatography (oligodeoxythymidylic acid-cellulose type 7, P-L Biochemicals) (7). Poly(A)⁺-bound RNA was eluted with a buffer of 10 mM Tris-HCl, pH 7.4:1 mM EDTA:0.1% SDS and precipitated in ethanol at -20° as described above.

Determination of Globin mRNA. The plasmid containing human γ -globin cDNA sequences, pH γ G1 (19), was grown in *Escherichia coli* HB101 under P2/EK1 conditions. The plasmid was purified by the hydroxyapatite method (11). After digestion with Pst 1, the plasmids were denatured by 0.2 N NaOH, loaded onto nitrocellulose filters (Millipore HAWP, 0.45 μm , 25-mm diameter) at approximately 50 $\mu\text{g}/\text{filter}$, and then heated at 80° for 2 hr (16).

To determine the percentage of [^3H]uridine incorporation into globin RNA, a filter containing 5 μg globin DNA was hybridized with [^3H]uridine-labeled poly(A)⁺RNA in 50% formamide:0.75 M NaCl:50 mM Tris-HCl, pH 7.5:1 mM EDTA:carrier yeast RNA (100 $\mu\text{g}/\text{ml}$) at 45° for 16 hr. The filters were washed 3 times with 0.5 M NaCl:10 mM Tris-HCl, pH 7.5, followed by treatment with 5 μg RNase A at 37° for 30 min. After further washing in the same buffer, filters were dried and counted in Aquasolve 2.

Chromosome Analysis. Log-phase cells were treated with Colcemid (0.02 $\mu\text{g}/\text{ml}$) for 15 min prior to harvesting. The cells were then centrifuged and resuspended in 0.075 M KCl hypotonic solution for 30 min. The cells were fixed in Carnoy's solution (methanol:acetic acid, 3:1), and air-dried slides were prepared. Giemsa banding was carried out by the method of Seabright (33). A test for the translocation of mouse genetic material to the K562(S) chromosomes was performed using the G-11 banding procedure of Friend *et al.* (15) under conditions in which human-mouse somatic cell hybrids consistently gave differential staining. The chromosomes from 52 metaphase spreads were counted. G-11 staining of these cells, which have been previously passaged through nude mice (20) for mouse chromatin translocated to the human chromosomes, revealed that no mouse sequences were present.

RESULTS

Hemoglobin Patterns of K562(S). K562(S) cells were treated with 50 μM hemin or with 1.4 mM butyric acid for 4 or 5 days. The cell lysates and appropriate amounts of marker cord blood were electrophoretically separated on CelloGel gels. The separation of different benzidine-positive bands is shown in Fig. 1. In both cases, the 5 bands, Gower 1, X, F, Portland, and Bart's, as described by Rutherford *et al.* (30, 31), could be detected. The 2 major bands that resulted from hemin treatment were Hb X and Hb Bart's. After butyric acid treatment, the most abundant band appeared at a position corresponding to Gower 1; the second most abundant band appeared at the position characteristic of either Portland or the adult Hb A; however, the lack of detectable β chains in induced K562(S) cells ruled out Hb A. A faint band comigrating with the Hb F marker was seen both in hemin-treated and butyric acid-treated cells. In nontreated cells, only a band with the migration characteristics of the Hb Gower 1 could be detected. Essentially, the electrophoretic pattern of hemin- and butyric acid-treated cells corresponded to the electrophoretic pattern reported by Rutherford *et al.* (30, 31). The relative amount of the different hemoglobin bands (evaluated by intensity of benzidine

staining) appeared to be different, however, when hemin induction of K562(S) cells was compared to hemin induction of K562 cells used by Rutherford *et al.* (30).

Globin Chains of K562(S) Cells. If the total radioactive cytoplasmic lysate was separated on Triton-Urea gels, many polypeptides were present which interfered with the identification of the globin chains (Fig. 2). Nontreated cells and both hemin- and butyric acid-treated K562 cell lysates contained a prominent band (corresponding to the partially unresolved ϵ and ζ chains) (4, 8).⁵ The band was present in untreated cells and appeared broad and more prominent in hemin-treated cells (Fig. 2A) than in butyric acid-treated cells (Fig. 2B).

In order to detect γ -, α -, and β -globin chains, cells were labeled with the ¹⁴C-amino acid mixture, and the hemoglobins were separated by isoelectric focusing using cord blood hemoglobin (containing Hb A and Hb F) as a carrier. The adult and fetal hemoglobin bands were cut from the gel, and the globin chains were separated by electrophoresis on Triton-Urea gels (27, 28). Radioactive bands comigrating with nonlabeled carrier β chains could not be detected after butyric acid treatment (Fig. 3). The relative migration of the G γ and A γ globin chains was identified on Triton-Urea gels as described by Alter (1).

Time Sequence of Induction. The kinetics of induction of hemoglobin by hemin or butyric acid were different. After treatment of K562(S) cells with 0.5×10^{-4} M hemin, hemoglobin could be detected after only 24 hr and reached a peak between Days 3 and 4 (Chart 1). The same type of globin chains were seen throughout (data not shown). By contrast, hemoglobin could be detected only after 4 days and reached a peak after approximately 5 days in butyric acid-treated cells (Chart 1).

A base line of 3 to 10% of cells was benzidine-positive in nontreated K562(S) cells. The percentage of benzidine-positive cells after butyric acid treatment reached a peak of 60% on Day 5 under optimal conditions; whereas, after hemin treatment, it was approximately 80% after 3 days. It is possible that benzidine positivity after hemin treatment could be due to the presence of free hemin in the cell. However, presence of hemoglobin in hemin-treated cells was confirmed by scanning at 414 nm and following the shift after carbon monoxide treatment (Chart 2) as described in "Materials and Methods."

Presence of β -like Globin mRNA in Induced K562. Several of the hemoglobins synthesized by K562 cells contained the β -like chains γ and ϵ . It was therefore of interest to determine the levels of β -like mRNA's before and after treatment. β -like mRNA's were quantitated by RNA-DNA hybridization using plasmid containing γ -cDNA. β -like mRNA's were found to be present in nontreated K562 cells (0.12% of cytoplasmic mRNA). After hemin treatment, β -like mRNA's increased to at least 0.3% of the total mRNA. After butyric acid treatment, a less marked increase was observed.

Growth and Inducibility of K562(S) in Synthetic Medium. K562(S) cells grew well in synthetic medium without serum. The composition of the medium was similar to that described by Breitman *et al.* (10) for the growth of HL60 human promyelocytic leukemia cells. The cells adapted to growth in synthetic medium without a period of adjustment, but they grew with a longer doubling time and reached a lower saturation density

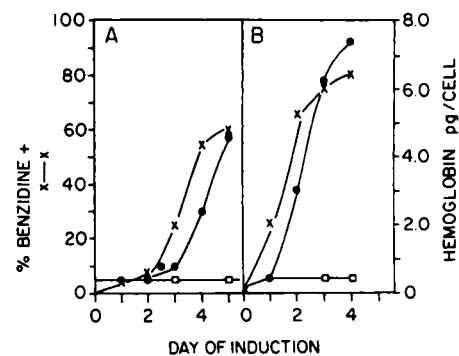


Chart 1. Kinetics of induction of hemoglobin and percentage of benzidine-positive cells in hemin- or butyric acid-treated K562(S) cells. A, butyric acid-treated; B, hemin-treated cells. Cells were washed 3 times with PBS before the assays; \square , hemoglobin in untreated cells. Each value represents the mean of 3 separate experiments.

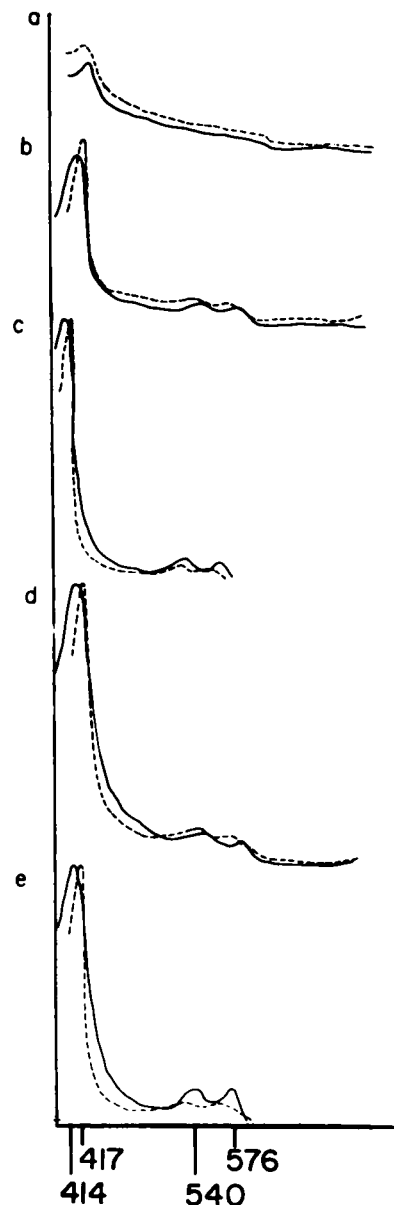


Chart 2. Absorbance spectrum of lysate (—) and of lysate treated with CO (---) of K562(S) cells. a, K562 cells; b, K562 cells treated with butyric acid for 4 days; c, K562 cells treated with butyric acid for 5 days; d, K562 cells treated with hemin for 1 day; e, K562 cells treated with hemin for 5 days.

⁵ T. Rutherford, personal communication.

Table 1

Effect of medium composition on benzidine-positive cell induction of K562(S) cells

K562(S) cells were grown in synthetic medium for 10 passages. At the time of the experiment, the cells were plated in synthetic medium alone or in complete medium containing 10% fetal bovine serum. Duplicate 10-ml cultures were untreated or treated for 4 days with 50 μ M hemin or 1.4 mM butyric acid. Benzidine-positive cells were scored after the cells were washed 3 times with PBS.

Culture condition	Treatment	% of benzidine-positive cells
Synthetic medium	None	5
	Hemin	50
	Butyric acid	31
Complete medium	None	15
	Hemin	70
	Butyric acid	50

(data not shown). We have investigated whether induction of benzidine-positive cells by hemin or butyric acid was mediated by serum factors. The results (Table 1) showed that, although both hemin and butyric acid added to synthetic medium induced an increase in the percentage of benzidine-positive cells, this was less than when added to serum-containing medium. Shifting the cultures from a serum-free to serum-containing medium caused a slight induction of hemoglobin synthesis, suggesting that serum factors could induce differentiation of K562(S) cells.

Karyology. Analysis of Giemsa-banded chromosomes revealed a near triploid karyotype and a modal number of 68 (Fig. 4). Trisomy of the chromosome 11 containing the β -like globin genes and tetrasomy of the chromosome 16 which contains the α -like globin genes (12, 13) were present. Several marker chromosomes were observed. A large marker (M1) involving a translocation of chromosome 7 was present in all cells. A small centromeric fragment which resembles the Philadelphia chromosome present in the original K562 cell line (20, 21) was also seen.

DISCUSSION

We have characterized the hemoglobin synthesis of a particular subline of K562 cells that can be induced to differentiate by hemin and butyric acid treatment. We confirm the previous reports (4, 8, 30) that hemin treatment of K562 cells induces synthesis of embryonal and fetal hemoglobins, and we report that the induction of K562(S) cells with different inducers of hemoglobin synthesis causes differential synthesis of embryonal hemoglobins. Nontreated K562(S) growing in medium with serum contained a fraction of spontaneously differentiating cells synthesizing Hb Gower 1. Butyric acid treatment of K562(S) favored the synthesis of ϵ - and ζ -globin chains (components of Hb Gower 1 and Hb Portland), whereas hemin treatment caused the preferential synthesis of the γ -globin chains. Hb X, the predominant hemoglobin in hemin-treated K562(S) cells, has in fact recently been characterized using K562(S) cells by Rutherford *et al.* (29) as a $\gamma_2\epsilon_2$ hemoglobin. After hemin treatment, an excess of the β -like chains over the α -like chains was observed. This finding has some analogies to the observation reported by Rovera *et al.* (26) in Friend erythroleukemia cells; when Friend cells were induced by hemin, an excess of β minor chain over α was observed.

The kinetics of hemoglobin synthesis after induction with hemin was different and more rapid than after induction with

butyric acid. Benzidine-positive cells after hemin treatment could be the result of hemin uptake rather than increased hemoglobin. However, we believe that, in the case of K562(S) cells treated with hemin, the increased percentage of benzidine-positive cells reflects an increase in hemoglobin synthesis because: (a) K562(S) cells treated with hemin show a bright red pellet 24 hr after treatment, while hemin alone would give a brown color; (b) as shown in Chart 2, the visible absorbance spectrum of the supernatant of K562(S) cells is shifted after carbon monoxide treatment with characteristics indicating the presence of hemoglobin; (c) there is a correlation between the increased percentage of benzidine-positive cells after hemin treatment and the amount of hemoglobin measured by the visible absorbance spectrum; and (d) separation of hemoglobin by cellulose:acetate gel electrophoresis indicates that the intensity of the various hemoglobin bands increases after 1 day of treatment with hemin. The delay in appearance of hemoglobin synthesis after butyric acid treatment may suggest that, like Friend erythroleukemia cells, a hemin-synthesizing pathway must be activated (31, 32), and this may require several days. However, when hemin is supplied exogenously, there is a rapid synthesis of hemoglobin suggesting that the rate-limiting step for hemoglobin synthesis is at the level of translation.

Evidence from hybridization studies indicates that β -like mRNA's are already abundant in nontreated K562(S) cells. At the present time, we have been unable to quantitate the relative amount of β -, γ -, and ϵ -mRNA because, under the conditions of filter hybridization used, there is some cross-hybridization between the γ -cDNA probe and the ϵ - and β -mRNA. This cross-hybridization was shown to exist by hybridizing to the filter-bound γ -cDNA probe a radioactive β -complementary RNA made by transcribing the β -cDNA plasmid with *E. coli* RNA polymerase. When a β -cDNA probe was used for hybridization of the K562 poly(A)-containing RNA, hybridized mRNA was detected. Since no β -globin chains were detected, this confirmed that the conditions of hybridization used were unable to discriminate between β - and γ -mRNA's. Translation of the hybridized mRNA's may in the future partially answer these questions but will not rule out the existence of nontranslatable mRNA in noninduced K562 cells. The studies using synthetic medium indicate that both butyric acid and hemin induce synthesis of hemoglobins by direct interaction with the target cells and without the need of serum factors. However, the fact that shifting K562(S) cells from synthetic medium to medium with serum causes an increased level of spontaneous differentiation suggests that serum factors could also be responsible for erythroid differentiation and that K562(S) cells growing in synthetic medium could be used as sensitive indicator cells in the identification of such factors.

Karyotype analysis of K562(S) cells was done to confirm their identity as human leukemic cells, inasmuch as Lozzio *et al.* (22) have suggested that some of the K562 cell lines available in different laboratories could actually be mouse cells or human cells altered by *in vivo* passage in immunodeficient mice. We were unable to detect the presence of mouse chromosomes or chromosomal fragments by G-11 staining.

The karyotype of the K562(S) subline shows further rearrangement than the lines studied by both Lozzio *et al.* (20) and Klein *et al.* (18). None of the markers reported by Klein *et al.*, including a ring Philadelphia chromosome, is present in K562(S) cells. A marker involving chromosome 7, a Philadel-

phia chromosome, and more than 3 No. 16 chromosomes is seen by Lozzio *et al.* (20), but their 15/17 translocation is not present in our line. Their rearranged chromosome 7 is also different from the one observed in K562(S) cells. Our analysis leads us to believe that K562(S) cells are related to the K562 cells of Lozzio *et al.* (20) and that they have further evolved.

Different lines of K562 apparently behave differently, probably because of variability in karyotype. We did not observe, for example, the atypical morphological patterns of erythroid differentiation reported by Andersson *et al.* (5) after butyric acid treatment. On the other hand, the K562 cells used by Rutherford *et al.* (30) were inducible by hemin but not by butyric acid.

The possibility remains that K562 cells can also differentiate along other than erythroid lineages because myeloid markers were found to be present on these cells (14), and their treatment with phorbol diesters induced partial differentiation to myelomonocytic cells.⁶

ACKNOWLEDGMENTS

We thank Dr. Rutherford for information about the identification of hemoglobin and globin chains.

REFERENCES

- Alter, B. P. The G γ :A γ composition of fetal hemoglobins in fetuses and newborns. *Blood*, 54: 1158-1163, 1979.
- Alter, B. P., and Goff, S. C. Globin synthesis *in vitro*: a switch in beta chains due to inducing agent. *Blood*, 50: 866-876, 1977.
- Alter, B. P., and Goff, S. C. Variable globin chain synthesis in mouse erythroleukemia cells. *Blood*, 52: 1047-1056, 1978.
- Alter, B. P., and Goff, S. C. Electrophoretic separation of human embryonic globin demonstrates " α -thalassemia" in human leukemia cell line K562. *Biochem. Biophys. Res. Commun.*, 94: 843-848, 1980.
- Andersson, L. C., Jokinen, M., and Gahmberg, C. G. Induction of erythroid differentiation in the human leukemia cell line K562. *Nature (Lond.)*, 278: 364-365, 1979.
- Andersson, L. C., Nilsson, K., and Gahmberg, C. G. K562—a human erythroleukemic cell line. *Int. J. Cancer*, 23: 143-147, 1979.
- Aviv, H., and Leder, P. Purification of biologically active globin messenger RNA by chromatography on oligo thymidylic acid cellulose. *Proc. Natl. Acad. Sci. U. S. A.*, 69: 1408-1412, 1972.
- Benz, E. J., Murnane, M. J., Tonkonow, B. L., Berman, B. W., Mazur, E. M., Cavallero, C., Jenko, T., Snyder, E. L., Forget, B. G., and Hoffman, R. Embryonic-fetal erythroid characteristics of a human leukemic cell line. *Proc. Natl. Acad. Sci. U. S. A.* 77: 3509-3513, 1980.
- Bonner, W. M., and Laskey, R. A. A film detection method for Tritium-labeled proteins and nucleic acids on polyacrylamide gels. *Eur. J. Biochem.*, 46: 83-88, 1974.
- Breitman, T. R., Collins, S. J., and Keene, B. R. Replacement of serum by insulin and transferrin supports growth and differentiation of the human promyelocytic cell line HL60. *Exp. Cell Res.*, 126: 494-497, 1980.
- Colman, A., Byers, M. J., Primrose, S. B., and Lyons, A. Rapid purification of plasmid DNAs by hydroxyapatite chromatography. *Eur. J. Biochem.*, 91: 303-310, 1978.
- Deisseroth, A., Nienhuis, A., Lawrence, J., Giles, R., Turner, P., and Ruddle, P. Chromosomal localization of human β globin gene on human chromosome 11 in somatic cell hybrids. *Proc. Natl. Acad. Sci. U. S. A.*, 75: 1456-1460, 1978.
- Deisseroth, A., Nienhuis, A., Turner, P., Veier, R., Anderson, F. W., Rudelle, F., Lawrence, J., Creagan, R., and Kucherlapati, R. Localization of the human β -globin structural gene to chromosome 16 in somatic cell hybrids by molecular hybridization assay. *Cell*, 12: 205-218, 1977.
- Drew, S. I., Terasaki, P. I., Bellamy, R. J., Bergh, O. J., Minowada, J., and Klein, E. Group-specific human granulocyte antigens on a chronic myelogenous leukemia cell line with Philadelphia chromosome marker. *Blood*, 49: 715-718, 1977.
- Friend, K. K., Chen, S., and Ruddle, F. H. Differential staining of interspecific chromosomes in somatic cell hybrids by alkaline Giemsa stain. *Somat. Cell Genet.*, 2: 183-188, 1976.
- Gillespie, D., and Spiegelman, S. Quantitative assay for DNA-RNA hybrids with DNA immobilized on a membrane. *J. Mol. Biol.*, 12: 829-842, 1965.
- Kabat, D., Sherton, C. C., Evans, L. H., Bigley, R., and Koler, R. D. Synthesis of erythrocyte-specific proteins in cultured Friend leukemia cells. *Cell*, 5: 331-338, 1975.
- Klein, E., Ben-Bassat, H., Newmann, H., Ralph, P., Zeuthen, J., Polliack, A., and Wanky, F. Properties of the K562 cell line, derived from a patient with chronic myeloid leukemia. *Int. J. Cancer*, 18: 421-431, 1976.
- Little, P., Curtis, P. O., Coutelle, C., Van Den Berg, J., Dalglish, R., Malcolm, S., Courtney, M., Westaway, D., and Williamson, R. Isolation and partial sequence of recombinant plasmids containing human α , β and γ globin cDNA fragments. *Nature (Lond.)*, 273: 640-643, 1978.
- Lozzio, B. B., Machado, E. A., Lozzio, C. B., and Lair, S. Hereditary asplenic-athymic mice. Transplantation of human myelogenous leukemic cells. *J. Exp. Med.*, 143: 225-231, 1976.
- Lozzio, C. B., and Lozzio, B. B. Human chronic myelogenous leukemia cell-line with positive Philadelphia chromosome. *Blood*, 45: 321-334, 1975.
- Lozzio, C. B., Lozzio, B. B., Machado, E. A., Fuhr, J. E., Lair, S. V., and Bamberger, E. G. Effects of sodium butyrate on human chronic myelogenous leukemia cell line K562. *Nature (Lond.)*, 281: 709-710, 1979.
- Nudel, U., Salmon, J. E., Terada, M., Bland, A., Rifkind, R. A., and Marks, P. A. Differential effects of chemical inducers on expression of beta globin genes in murine erythroleukemia cells. *Proc. Natl. Acad. Sci. U. S. A.*, 74: 1100-1104, 1977.
- Orkin, S. H., Harosi, F. I., and Leder, P. Differentiation in erythroleukemic cells and their somatic hybrids. *Proc. Natl. Acad. Sci. U. S. A.*, 72: 98-102, 1975.
- Righetti, P., and Drysdale, J. W. Isoelectric focusing in polyacrylamide gels. *Biochim. Biophys. Acta.*, 236: 17-28, 1971.
- Rovera, G., Abramczuk, J., and Surrey, S. The effect of hemin on the expression of globin genes in Friend cells. *FEBS Lett.*, 81: 366-370, 1977.
- Rovera, G., Magarian, C., and Borun, T. W. Resolution of hemoglobin subunits by electrophoresis in acid urea polyacrylamide gels containing Triton X-100. *Anal. Biochem.*, 85: 506-518, 1978.
- Rovera, G., Vartikar, J., Connolly, G. R., Magarian, C., and Dolby, T. W. Hemin controls the expression of beta minor globin genes in Friend erythroleukemia cells at the pretranslational level. *J. Biol. Chem.*, 253: 7588-7590, 1978.
- Rutherford, T. R., Clegg, J. B., Higgs, D. R., Jones, R. W., Thompson, J., and Weatherall, D. J. K562 human erythroleukemia cells: a model for hemoglobin switching? In: A. W. Nienhuis and G. Stamatoyannopoulos (eds.), *Second International Conference on Hemoglobin Switching*, New York: Greene and Stratton, 1981.
- Rutherford, T. R., Clegg, J. B., and Weatherall, D. J. K562 human leukaemic cells synthesise embryonic haemoglobin in response to haemin. *Nature (Lond.)*, 280: 164-165, 1979.
- Rutherford, T. R., and Weatherall, D. J. Deficient heme synthesis as the cause of noninducibility of hemoglobin synthesis in a Friend erythroleukemia cell line. *Cell*, 16: 415-423, 1979.
- Sassa, S. Sequential induction of heme pathway enzymes during erythroid differentiation of mouse Friend leukemia virus-infected cells. *J. Exp. Med.*, 143: 305-315, 1976.
- Seabright, M. A rapid banding technique for human chromosomes. *Lancet*, 2: 971-972, 1971.
- Weatherall, D. J., and Clegg, J. B. *The Thalassemia Syndromes*, Ed. 2. Oxford: Blackwell Scientific Publications, 1972.

⁶ L. Cioe and G. Rovera, unpublished results.

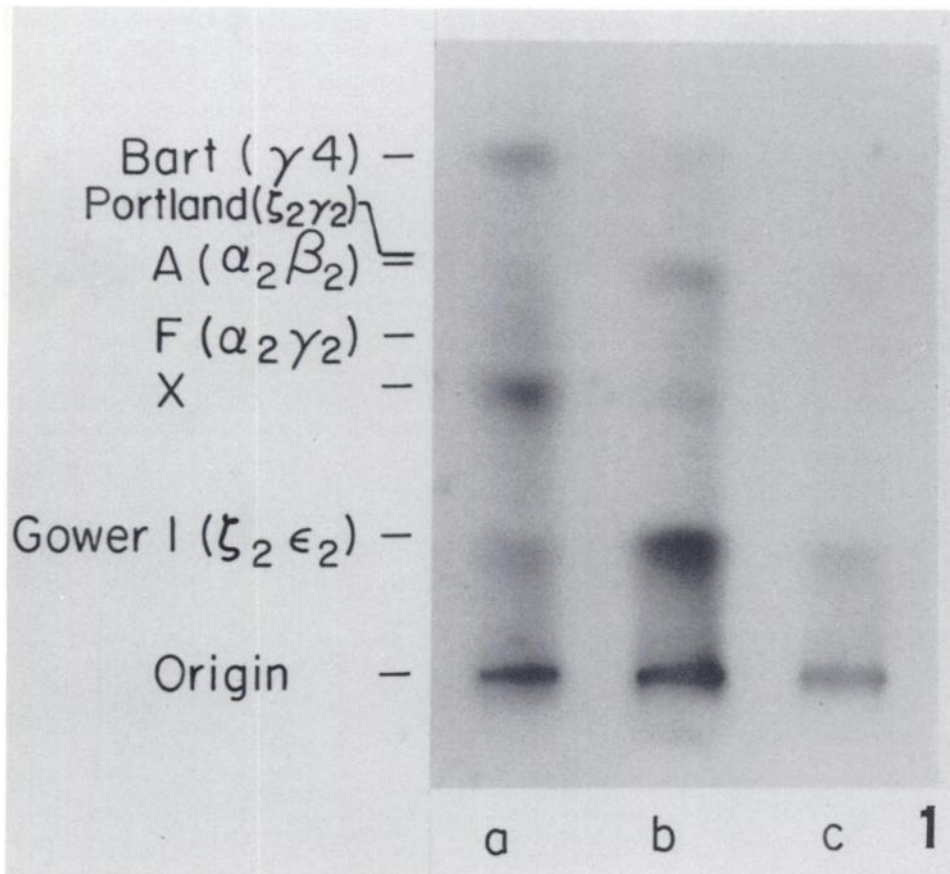
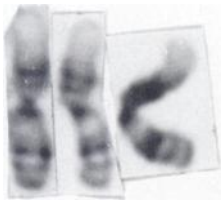
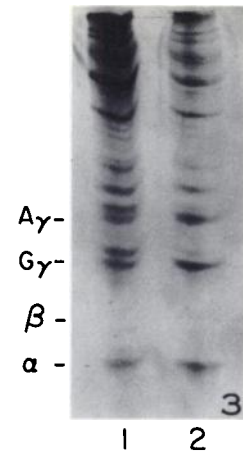
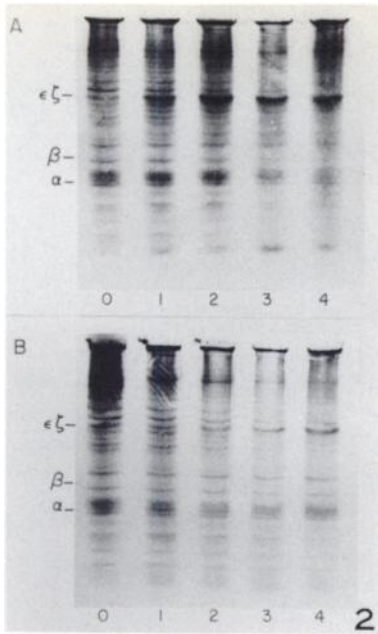


Fig. 1. Separation of hemoglobins synthesized by K562(S) cells by electrophoresis on Cellologel using Tris-borate buffer (pH 8.6). Markers for Hb F and Hb A were obtained by parallel electrophoresis of cord blood. The identification of the other hemoglobins was based on their relative migration at alkaline pH as described previously (30). a, hemin treated for 4 days; b, butyric acid treated for 5 days; c, nontreated.

Fig. 2. Separation of globin chains by electrophoresis on Triton:urea gels. Total cytoplasmic lysates of K562(S) cells labeled with ^{14}C -amino acid mixture (1 $\mu\text{Ci}/\text{ml}$) were lysed and separated by electrophoresis. Positions of α and β chains were determined by coelectrophoresis of nonlabeled human adult blood. The putative ϵ - ζ complex was tentatively identified on the basis of its relative migration rate in the 2% Triton-Urea gel system (4, 8). A, hemin-treated cells for 0, 1, 2, 3, and 4 days; B, butyric acid-induced for 0, 1, 2, 3, and 4 days.

Fig. 3. Separation of globin chains by electrophoresis on Triton gels. Total cytoplasmic lysates of K562(S) cells labeled with ^{14}C -amino acid mixture (1 $\mu\text{Ci}/\text{ml}$) were lysed and mixed with unlabeled cord blood. The cord blood was separated by isoelectric focusing on disc acrylamide gels. The proteins with the same isoelectric point of adult and fetal hemoglobin bands were excised and eluted, and the proteins were run on Triton:urea gels. The positions of α , β , $\text{A}\gamma$, and $\text{G}\gamma$ bands were identified by Coomassie blue. The radioactive peptides were identified by fluorography. 1, polypeptide pattern of proteins with the same isoelectric point as adult hemoglobin; 2, polypeptide pattern of proteins with the same isoelectric point as that of fetal hemoglobin.

Fig. 4. G-banded karyotype of K562(S) cells. Note the large marker chromosome (M1) derived by a translocation involving chromosome 7. Other marker chromosomes (M2 to M7) are present. Philadelphia chromosome (arrow) is also present.



1



2



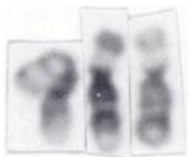
3



4



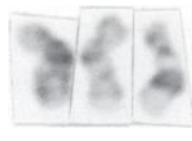
5



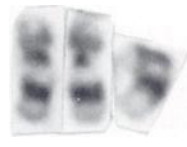
6



7



8



9



10



11



12



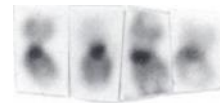
13



14



15



16



17



18



19



20



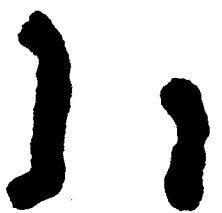
21



22



X



M1



M2



M3



M4



M5



M6



M7

4