PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 166, 546–550 (1981)

A Multipotential Leukemia Cell Line (K-562) of Human Origin (41106)[1]

BISMARCK B. LOZZIO, CARMEN B. LOZZIO, ELENA G. BAMBERGER, AND AURORA S. FELIU

University of Tennessee College of Medicine Department of Medical Biology/Memorial Research Center, Center for the Health Sciences, Knoxville, Tennessee 37920

Abstract. The K-562 leukemia cell line, originally established in our laboratory, has been characterized as an early precursor of the granulocytic series with a block for differentiation. Since K-562 blasts did not differentiate when cultured for 7–8 days in liquid media or 14–16 days in agar-gel an attempt was made to stimulate their potential for spontaneous differentiation by prolonging the time in culture. Inducers of differentiation were not added to the cultures and the cells were studied when they reached the steady state rather than during exponential growth. The cultivation of K-562 cells for 10 to 11 days in media gradually depleted of the essential nutrients needed for cell division induced their differentiation into early precursors of the monocytic, granulocytic, and erythrocytic series. Thus, the peroxidase reaction for hemoglobin demonstrates benzidine-positive material limited to the region of the Golgi apparatus. Analysis of the hemoglobin by isoelectric focusing indicated major bands in the region of embryonic hemoglobin. Most cells (80–90%) give a strong reaction for α-naphthyl acetate esterase typical of monocytes and as many as 30 to 40% of the cells have abundant red cytoplasmic granules of naphthol AS-D chloroacetate esterase characteristic of granulocytic precursors. Myeloperoxidase activity was found in 5 to 10% of the cells. Polyploid cells (5–8%) and early myelomonocytic precursors have PAS-positive material, were stained with Sudan black, and possessed abundant acid phosphatase. The data support the conclusion that K-562 is, indeed, a multipotential leukemia cell line of human origin.

The lack of a human multipotential leukemia cell line has been a major limitation for the study of the differentiation of hematopoietic cells (1). Inasmuch as existing *in vitro* systems to study the differentiation of human leukemia stem cells are restricted to short-term cultures (1, 2), there is an obvious need for long-term cultures of well-characterized leukemia blasts that may be induced to differentiate into one or more hematopoietic cell series. We report herein that K-562 leukemia blasts have the potential for spontaneous differentiation into very early progenitor cells usually seen in the bone marrow and peripheral blood of individuals with chronic myelogenous leukemia (CML).

Material and Methods. The K-562 leukemia cell line was originally established in our laboratory nearly 10 years ago and it has been characterized as a very early precursor of the granulocytic series with a block for differentiation into more mature cells (3–5). Serial culture passage 237 was used. Each bottle, containing an initial inoculum of 150,000 cells in 10 ml of EM 15A (3, 4) medium was cultured for 10 to 11 days. No inducers of differentiation were added to cultures. Cells were harvested, counted, and adjusted to 10^6/ml of spent media. Smears, made by use of a cytocentrifuge, were processed as follows: stained with May–Grünwald–Giemsa for general morphology, Lepehne peroxidase reaction for hemoglobin, and Graham–Knoll peroxidase reaction for general differentiation as described in the Sandoz Atlas of Hematology. For comparison, the benzidine-peroxidase-H_2O_2 methods for the detection of hemoglobin in tissue sections (6) was also used because it was very sensitive to detect small amounts of intracellular hemoglobin. In addition, the histozyme and histochrome methods (Sigma Chemical Co., St. Louis, Mo.) were also used. This included the following kits: leukocyte

[1] This investigation was supported by Grant CA 18185-06 awarded by the National Cancer Institute, DHHS.

0037-9727/81/040546-05$01.00/0

peroxidase with *p*-phenylenediamine-catechol H_2O_2 (bulletin 390); naphthol AS-D chloroacetate and α-naphthyl acetate esterases (90); alkaline (85) and acid (386) phosphatases; Sudan black B II (380), and periodic acid–Schiff (PAS) system (395). Cell viability was determined by the trypan blue dye exclusion technique as described previously (7). The nature of the hemoglobins synthesized by K-562 cells was analyzed by isoelectric focusing (8). Lysozyme was determined in the spent media and cell pellets, disrupted by sonic vibrations, by a radial diffusion assay (Quantiplate lysozyme test kit, Kallestad Inc., Chaska, Minn.).

Results. The number of cells increased between 65 and 72 times in 10 to 11 days continuous culture and nearly all (95–98%) cells were viable. There were 9.75 and 10.8 $\times 10^6$ cells on Days 10 and 11 of culture, respectively. Cells were in a stationary phase of growth by the 9th day in culture and no differentiation was observed prior to the 10th day in culture. The great majority of cells have similar structural features. Examinations of smears stained with May–Grünwald–Giemsa revealed two well-defined elements: intact cells and very few cells in degeneration. Intact mononucleated cells (70–80%) alternated with binucleated cells (8–10%) and highly polyploid cells (5–8%). All intact cells had unstained vacuoles in the region corresponding to the Golgi apparatus and from two to six bizarre nucleoli. Typical and atypical mitoses diminished with the time in culture. The peroxidase reaction for hemoglobin demonstrated benzidine-positive material limited to the region of the Golgi apparatus (Table I; Fig. 1A) of the large blasts with undifferentiated nuclei containing two to six nucleoli. However, there were no intermediate forms such as normoblasts or terminal differentiation into reticulocytes and/or erythrocytes. A band could be identified by isoelectric focusing in the region corresponding to hemoglobin Bart.

The majority of the cells gave a strong reaction for α-naphthyl acetate esterase typical (fluoride sensitive) of the monocytic series while other cells have abundant red cytoplasmic granules characteristic of naphthol AS-D chloroacetate esterase in granulocytic precursors. When the combined method for both α-naphthyl acetate and naphthol AS-D chloroacetate esterases was applied to the same smears, a relatively large number of cells had coarse black and fine bright red granules, thus confirming the presence of both esterases (Fig. 1B) in progenitors common to both monocytes and granulocytes. The differentiation into early precursors of the monocytic–granulocytic series was further assessed by myeloperoxidase activity. (Table I; Fig. 1C). Occasionally, an almost typical neu-

TABLE I. SPECIFIC MARKERS DETECTED IN THE PLURIPOTENTIAL LEUKEMIA CELL LINE, K-562

Reaction	Marker	Expected cell series	Observed (Range)
Lepehne peroxidase benzidine	Hemoglobin	Erythrocytic	6–14
α-Naphthylacetate[a]	Esterase[a]	Monocytic	80–90
Naphthol AS-D chloroacetate	Esterase	Granulocytic	30–40
α-Naphthylacetate and Naphthol AS-D chloroacetate	Esterases	Monocytic–granulocytic	35–44
Graham–Knoll Peroxidase–Benzidine	Myeloperoxidase	Granulocytic (strong) Monocytes (weak)	5–12
Naphthol AS-B1 phosphoric acid	Acid phosphatase (A)	Monocytic and T-derived cells	90–100
plus tartrate	Acid phosphatase (B)	T-derived cells (ALL) B-derived cells (HCL)	16–20
Sudan black B	Phospholipids	Paralleled Graham–Knoll Peroxidase reaction	80–90

Note. Percentages are ranges rounded to the nearest unit obtained by counting 10^4 cells from five cultures. ALL, acute lymphoblastic leukemia; HCL, hairy cell leukemia.

[a] Fluoride sensitive.

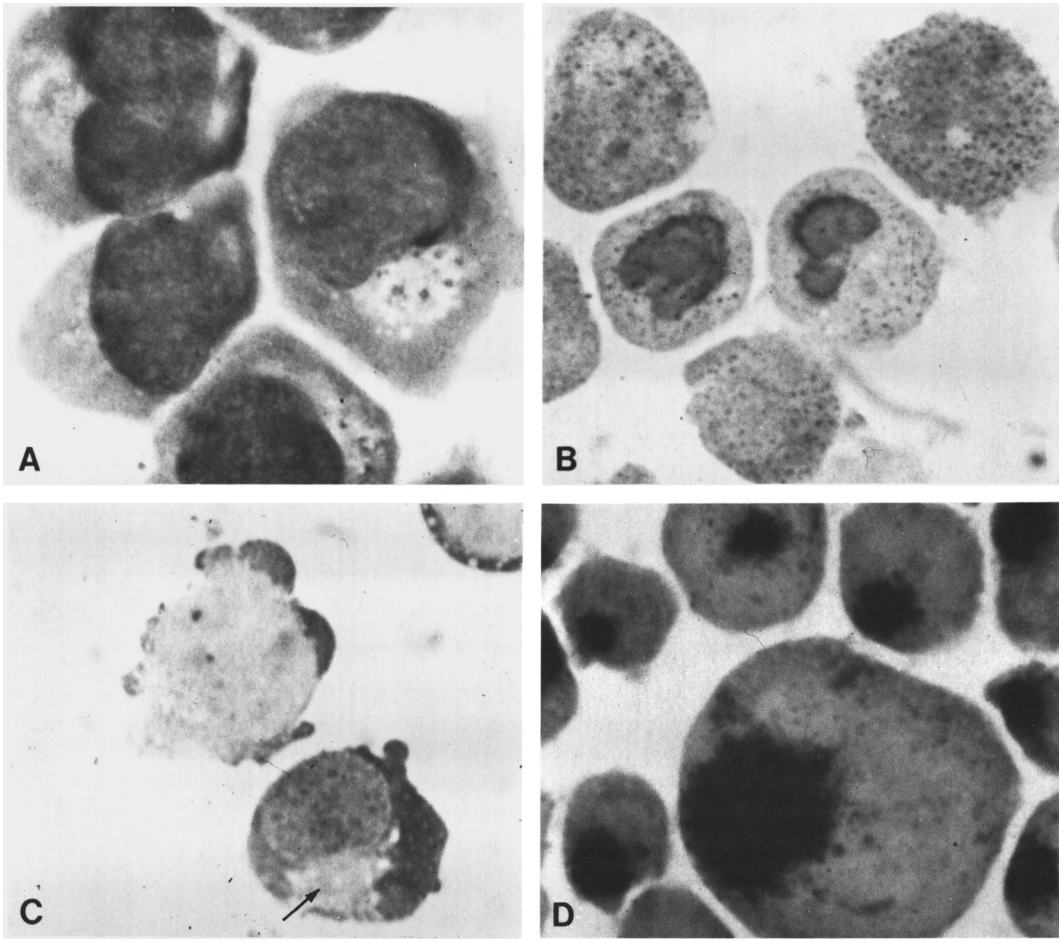

FIG. 1. Composite photograph of K-562 cells cultured for 11 days. (A) The benzidine-positive hemoglobin appeared as aggregates in the region of the Golgi apparatus of undifferentiated erythroid precursors. (B) Combined reactions for α-naphthyl (coarse granules) and naphthol AS-D chloroacetate (fine granules) esterases usually found in cells of monocytic and granulocytic series, respectively. (C) Blasts with tightly packed granules of myeloperoxidase and very small vacuoles demonstrating early progenitors of the granulomonocytic series. There is no myeloperoxidase in the region of the Golgi apparatus (arrow). (D) Strong acid phosphatase reactions in a large polyploid and small myelomonocytic cells. The enzyme is largely concentrated in the Golgi zone even though scattered spots appear throughout the cytoplasms. Original magnification was 1000×. For more details see text.

trophil or eosinophil with coarse peroxidase-positive granulation was seen. As may be expected, monocytic precursors had coarse black granulation when stained with Sudan black B II. The strong acid phosphatase activity (Fig. 1D) markedly diminished when sodium tartrate was added to the reagents. No alkaline phosphatase could be demonstrated. While few cells display a faint PAS reaction (monocytic precursors) in the form of small granules, the majority of the cells (granulocytic precursors) have a deeply red positive reaction as demonstrated by the accumulation of coarse PAS-positive material.

Positive controls made up of peripheral blood cells from a normal individual were run simultaneously. Neutrophils were strongly positive for AS-D chloroacetate esterase, myeloperoxidase, and alkaline

phosphatase. Eosinophils contained high myeloperoxidase activity and a low concentration of AS-D chloroacetate esterase. The Sudan black reaction paralleled the myeloperoxidase activity. Monocytes were particularly rich in α-naphthyl esterase which was not detectable in granulocytes. Lymphocytes displayed a weakly positive alkaline phosphatase activity. The PAS reaction was strongly positive in granulocytes and platelets and weakly positive in some monocytes and few lymphocytes.

As described earlier, the myelomonocytic series were predominant in prolonged cultures of K-562 cells even though terminal differentiation rarely occurred. Thus, atypical neutrophils, eosinophils, and monocytes were occasionally seen. Accordingly, the search for lysozyme was negative in cell pellets and in the spent media.

Discussion. As reported elsewhere (2–4) K-562 cells did not differentiate in semisolid media although, the plating efficiency was as high as 70%. Thus, cells obtained from agar colonies have been consistently found to be more undifferentiated than K-562 cells proliferating in suspension cultures (3–5). In view of the finding described above, we decided to investigate the nature of K-562 blast population in liquid media. To this end, we began to prolong the duration of the cultures of K-562 cells beyond the seventh day to have most cells at the steady state rather than during exponential growth. The cultivation of K-562 cells for 10 to 11 days in media gradually depleted of the essential nutrients needed for cell division induced their differentiation into early cell precursors usually seen in the course of CML.

Although the positive reactions described above were consistently seen in K-562 cells maintained in suspension cultures for 10 to 11 days, there were slight variations in the proportional number of positive cells and the intensity (degree) of the reactions. In any event, the cytochemical data indicate that numerous K-562 blasts, in a stationary phase of growth, undergo spontaneous differentiation to committed and even "recognizable" precursors of the granulocytic, monocytic, and erythrocytic series. In this regard, we believe that this is the first time that the intracellular site of embryonic and fetal hemoglobin synthesis has been seen in very early precursors of human leukemia cells. Previous analysis by isoelectric focusing indicated major bands in the region of hemoglobin F and Bart's when K-562 cells were incubated for 8 days in the presence of 0.05 m*M* hemin (8).

The presence of acid phosphatase is not surprising since myelomonocytic leukemia cells often react strongly (9). Moreover, other leukemia cells such as T lymphoblasts, that may give a moderately positive reaction, can be excluded because T-cell markers have not yet been found in the K-562 cell line (3–5). The presence of PAS-positive material is in agreement with the glycogen seen in many K-562 cells by electron microscopy (10, 11).

The effect of inducers, namely, hemin on sublines derived from the original K-562 leukemia cell line have recently been reviewed by Koeffler and Golde (12). Thus, some K-562 blasts are rich in glycophorin and may be induced to synthesize embryonic and fetal hemoglobin when incubated with hemin. The results of our most recent experiments indicated that by diminishing the amount of hemin added to cultures of K-562 cells to 0.05 m*M* and prolonging the time of incubation to 8 days, two cell populations emerged: one synthesized embryonic and fetal hemoglobins, whereas the other did not. The great majority of K-562 cells producing hemoglobin were highly undifferentiated and did not resemble erythroblasts or more differentiated erythroid cells (8). In no instance, however, was spontaneous differentiation observed. The results reported herein are the first evidence that the original K-562 cells can spontaneously differentiate depending on the time in culture in the absence of inducers.

Other findings indicate that sublines of K-562 cells express fetal (i) antigen and that the cell-surface glycoprotein profile of these cells is distinctly different from that of mature erythrocytes (13). The presence of group specific granulocyte antigens (4, 14), the isolation of a specific myelogenous leukemia antigen (15), and the development

of a monospecific antiserum for K-562 cells and for cells from patients with CML (7) are evidence that lend further support to the myeloid characteristics of these cells. On the other hand, the absence of T or B lymphocyte markers (4, 5) is an indication that K-562 cells are probably not precursors of lymphopoietic cells.

In conclusion, the original K-562 blasts are multipotential hematopoietic malignant cells that spontaneously differentiate into recognizable progenitors of the erythrocytic, granulocytic, and monocytic series.

1. Messmer, H. A., and Fauser, A. A., Blut **41**, 327 (1980).
2. Metcalf, D. "Hemopoietic Colonies" Springer-Verlag, New York (1977).
3. Lozzio, C. B., and Lozzio, B. B., Blood **45**, 321 (1975).
4. Lozzio, C. B., Lozzio, B. B., Yang, W. K., Ichiki, A., and Bamberger, E. G. Cancer Res. **36**, 4657 (1976).
5. Lozzio, B. B., and Lozzio, C. B., Leukemia Res. **3**, 363 (1979).
6. Humason, G. "Animal Tissue Techniques." W. H. Freeman, San Francisco (1972).
7. Lozzio, B. B., Lozzio, C. B., Krauss, S., Wust, C. J., and Girardi, A., Blood **50**, 115 (1977).
8. Fuhr, J. E., Bamberger, E. G., Lozzio, C. B., and Lozzio, B. B., Blood Cells, in press.
9. Dacie, J. V., and Lewis, S. M., "Practical Haematology." Churchill Livingston Medical Div. of Longman Group Ltd. Edinburgh, Great Britain (1977).
10. Machado, E. A., Maxwell, P., Lozzio, C. B., and Lozzio, B. B., Blood **50** (Suppl. 1) 226 (1977).
11. Maxwell, P. A., Machado, E. A., Lozzio, C. B., and Lozzio, B. B., Proc. Elec. Micros. Soc. An. **31**, 232 (1979).
12. Koeffler, H. P., and Golde, D. W., Blood **56**, 344 (1980).
13. Fukuda, M., Nature (London) **285**, 405 (1980).
14. Drew, S. I., Terasaki, P. I., Billing, R. J., Bergh, O. J., Minowada, F., and Klein, E., Blood **49**, 715 (1977).
15. Collins, J. L., Wust, C. J., Lozzio, B. B., and Lozzio, C. B., J. Nat. Cancer Inst. **59**, 1667 (1977).

Received November 10, 1980. P.S.E.B.M. 1981, Vol. 166.