at a rate approximately proportional to the density of the material traversed. Beam penetration is shallower in bone than in muscle; in air and air-filled tissue penetration is increased by an amount about equal to the length of the air path traversed.

3. Attributed to C. A. Tobias in (*6*).
4. M. C. Taylor, G. C. Phillips, R. C. Young, *Science* **169**, 377 (1970).
5. G. W. Bennett and J. O. Archambeau, *Nucl. Instrum. Methods* **125**, 333 (1975).
6. H. D. Maccabee, U. Madhvarath, M. R. Raju, *Phys. Med. Biol.* **14**, 212 (1969).
7. Supported by grant CA-188347 from The National Institutes of Health and by contract EY-76-C-02-0016 with the Department of Energy.

5 December 1977; revised 28 February 1978

Acute Myelogenous Leukemia: A Human Cell Line Responsive to Colony-Stimulating Activity

Abstract. *A permanent human cell line that maintains the granulocytic characteristics of acute myelogenous leukemia cells has been established. The cells of this line form myeloid colonies in soft gel culture in the presence of human colony-stimulating activity. The cell line may be useful for studying human acute myelogenous leukemia and the mechanism of response to colony-stimulating activity.*

Research on acute myelogenous leukemia in humans has been impeded by the lack of an adequate model system. Leukemia in rodents does not parallel the human disease, and continuous cell lines from patients with acute myelogenous leukemia usually lose their myeloid characteristics in vitro (*1*).

We describe here a cell line (KG-1) which was derived from a 59-year-old man with erythroleukemia that developed into acute myelogenous leukemia. This cell line retains its granulocytic nature in vitro and forms myeloid colonies in soft gel culture in response to colony-stimulating activity (CSA). The patient's bone marrow contained 30 percent myeloblasts and 10 percent erythroblasts with marked megaloblastic and dysplastic changes. The patient died of sepsis 2 months after study. Cells obtained by bone marrow aspiration were placed in T flasks with alpha medium (Flow) containing 20 percent fetal calf serum, penicillin, streptomycin, and $10^{-4}M$ α-thioglycerol; the cultures were incubated at 37°C in an atmosphere of 5 percent CO_2 in air. After 24 days in culture, the cells were actively proliferating. They have since been passaged 52 times in an 8-month period. The cells grow in suspension and do not form clumps. Whenever the cells were transferred in the liquid culture, there was an initial 1-day lag phase followed by growth with a mean doubling time of 70 to 80 hours, until cell densities of 2.5×10^6 to 3.5×10^6 cells per milliliter were reached.

The cultured cells morphologically resemble acute myelogenous leukemia cells, with 98 percent myeloblasts and promyelocytes, 2 percent intermediate and late stage granulocytes, and rare macrophages (Fig. 1). More than 85 percent of the cells stain positively for naphthol AS-D chloroacetate esterase (Sigma); 98 percent stain positively for acid phosphatase; 52 percent give a positive reaction with periodic acid–Schiff reagent; 10 percent are peroxidase positive; and a rare macrophage-like cell has a positive reaction with α-naphthyl butyrase (Fig. 1). These cytochemical characteristics are typical of moderately well-differentiated acute myelogenous leukemia. Medium from 5-day cultures that had been initiated with 3×10^6 cells contained lysoenzymes at 5.5 ± 1.2 μg/ml.

Chromosomal abnormalities were consistently observed upon cytogenetic examination with standard methodology and banding technique (*2*). Metaphase studies of 19 consecutive cells showed 45 chromosomes, and banding of five cells showed 45,XY,(5p$^+$9q$^-$),−12,−17, +MAR,+fragment. Epstein-Barr virus (EBV) nuclear antigen and the EBV capsid antigen were not present. The leukemic cells had HLA-A30 and HLA-B35 histocompatibility antigens, assayed by microcytotoxicity (*3*). A human granulocyte-specific antigen was detected by microcytotoxicity on KG-1 cells by 8 of 40 human granulocyte-reactive antiserums (*4*). A B lymphocyte–associated or Ia-like antigen was detected on 20 percent of the cultured cells (*5*). This antigen was previously found in leukemic cells from 70 percent of patients with acute myelogenous leukemia (*5*).

Surface membrane immunoglobulin was not demonstrable by immunofluorescence. No rosette formation occurred with sheep erythrocytes, but 7 ± 3 percent of the cells formed rosettes with antibody-coated sheep red blood cells. The cells did not respond to phytohemagglutinin (PHA) by an increase in [^{3}H]thymidine incorporation. The cultured cells used had a minimal (3 percent) ability to lyse chromium-labeled murine lymphoblasts (EL-4) by antibody-dependent cell-mediated cytotoxicity (*6*). The cultured leukemic cells did not phagocytose opsonized *Candida albicans* (*7*), but 29 ± 5 percent ingested one or more 1-μm latex particles. The

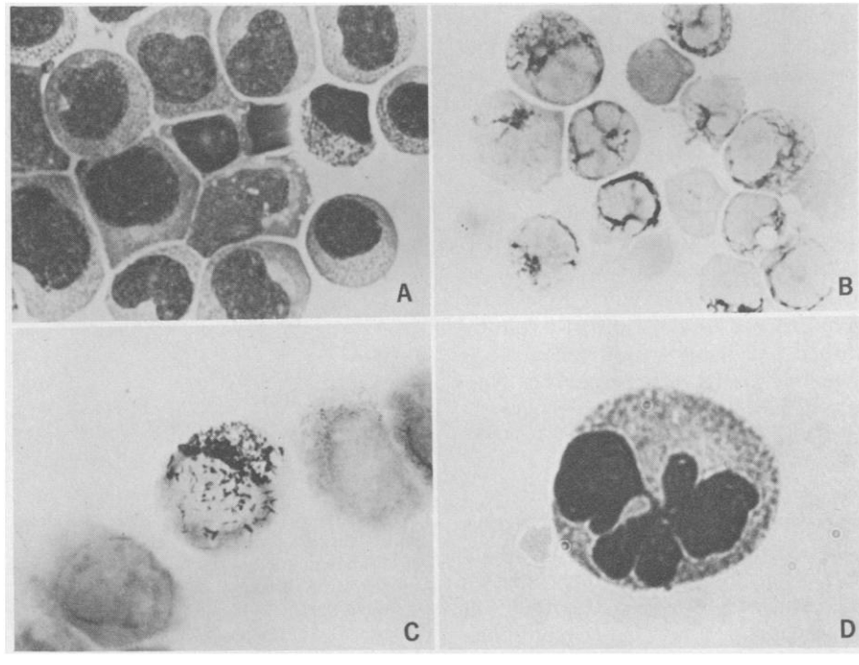

Fig. 1. Morphological characteristics of KG-1 cells in liquid culture. (A) Early and intermediate stage granulocytes stained with Giemsa (×400). (B) Numerous naphthol AS-D chloroacetate esterase-positive cells (×256). (C) Prominent peroxidase-positive cell (×400). (D) Multilobed polymorphonuclear granulocyte (×640).

0036-8075/78/0609-1153$00.50/0

leukemic cells did not exhibit chemotaxis in a Boyden chamber when either zymosan-activated or normal human type AB serum was used as the chemoattractant (*7*).

Cultured KG-1 cells are dependent on CSA for colony formation in vitro. Myeloid colonies develop from cells that are plated in 0.3 percent agar overlying 1×10^6 normal human peripheral white blood cells in 0.5 percent agar as the source of CSA (*8*). Colonies containing more than 50 cells were enumerated with an inverted microscope on day 14 of culture. A linear relation exists between the number of myeloid colonies formed and the number of cells plated (Fig. 2). The cloning efficiency was 2.5 to 3.0 percent, and there was no colony formation in the absence of CSA. When random colonies were aspirated from the cultures, smeared on slides, and stained with Wright-Giemsa or chloroacetate esterase, they revealed predominantly myeloblasts and promyelocytes. However, 30 percent of the colonies contained a small number of intermediate and mature granulocytes.

Several sources of CSA effective in stimulating colony formation of normal myeloid progenitors were tested for their ability to induce colony formation in KG-1 cells (Fig. 3). The supernatants from PHA-stimulated human peripheral blood lymphocytes, or conditioned medium from either human peripheral blood leukocytes (*9*) or continuous culture of T lymphocytes derived from the spleen of a patient with hairy-cell leukemia (*10*), stimulated colony formation when added directly to the agar cultures. A sigmoid dose-response curve was seen for each CSA tested; the ability of CSA to stimulate colonies reached a plateau at about 50 μl/ml. A similarly shaped sigmoid CSA dose-response curve has been noted when monkey lung-conditioned medium was the source of CSA and normal human bone marrow was the target cell population (*11*). When KG-1 cells were exposed to CSA in liquid culture, washed, and plated in agar without CSA, no colony formation occurred (*12*). Normal human myeloid precursors also require the continuous presence of CSA for colony formation (*13*).

Murine CSA derived from pregnant mouse uterus (*14*), when tested over a wide range of concentrations, did not induce colony formation by the leukemia cells. Murine CSA is also a poor inducer of normal human myeloid colony formation. Conditioned medium (25 to 100 μl) from liquid cultures of KG-1 growing at a stationary density of 3.0×10^6 cells per milliliter was tested for possible effects

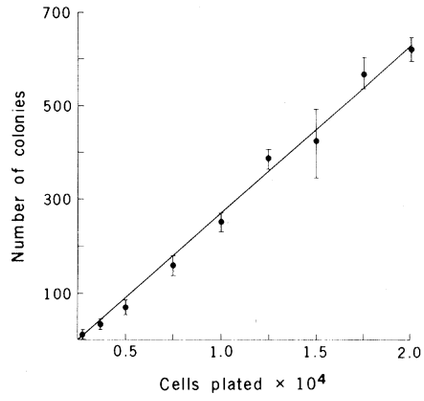

Fig. 2. Myeloid colony formation in agar is linearly related to the number of KG-1 cells plated. Each point represents the mean ± the standard error of four experiments done in triplicate.

on normal human myeloid colony formation. When the conditioned medium was added to duplicate cultures of 1×10^5 nucleated bone marrow cells from three normal volunteers, colony formation did not differ from the control. When conditioned medium was added to agar cultures containing 1×10^5 normal bone marrow cells in the presence of a suboptimal concentration of CSA (25 μl of leukocyte-conditioned medium), colony formation did not differ significantly from the control.

Acute myelogenous leukemia cells from humans are difficult to grow in long-term culture, although some cell lines have been established (*1*). These lines are generally composed of poorly differentiated lymphoblastoid-like cells, containing EBV-associated antigens and lymphocyte cell markers. Two well-characterized myeloid cell lines are the murine M-1 cell line (*15*) and a human chronic myelogenous leukemia cell line

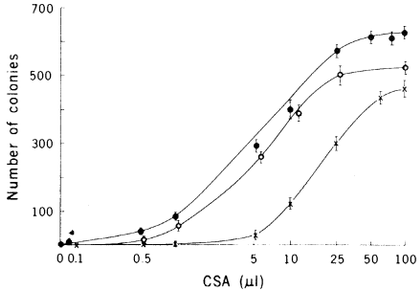

Fig. 3. Colony-stimulating activity dose-response curves. Colony formation by KG-1 cells is shown as a function of CSA concentration for three different CSA sources; X, human peripheral blood leukocyte-conditioned medium; ○, PHA-stimulated human peripheral blood lymphocyte supernatant; ●, conditioned medium from continuous line of leukemic T lymphoblasts. Each point represents the mean ± the standard error of three experiments done in triplicate.

that contains a Philadelphia chromosome, but shows little evidence of differentiation and no response to CSA (*16*). Gallagher and co-workers reported continuous growth and some differentiation of acute human myelogenous leukemia cells with conditioned medium from human embryonic cells (*17*), and Collins *et al.* have derived a line similar to the one that we describe (*18*).

We have established an acute myeloid-leukemia cell line from humans that retains its myeloid characteristics and forms granulocytic colonies in response to human CSA. This cell line should provide a valuable tool for studying the pathophysiology of acute myelogenous leukemia and the interaction of CSA with myeloid cells.

H. PHILLIP KOEFFLER
DAVID W. GOLDE
Division of Hematology-Oncology, Department of Medicine, School of Medicine, University of California, Los Angeles 90024

References and Notes

1. B. Clarkson, *Cancer Res.* **26**, 2483 (1967); ——, A. Strife, E. de Harven, *Cancer* **20**, 926 (1967); D. Belpomme, J. Minowada, G. E. Moore, *ibid.* **30**, 282 (1972); A Karpas, F. G. J. Hayhoe, J. S. Greenberger, C. R. Barker, J. C. Cawley, R. M. Lowenthal, W. C. Moloney, *Leukemia Res.* **1**, 35 (1977).
2. A. T. Sumner, H. J. Evans, R. A. Buckland, *Nature (London) New Biol.* **232**, 31 (1971).
3. K. K. Mittal, M. R. Mickey, D. P. Singal, P. I. Terasaki, *Transplantation* **6**, 913 (1968).
4. S. I. Drew, P. I. Terasaki, R. J. Billing, O. J. Bergh, J. Minowada, E. Klein, *Blood* **49**, 715 (1977).
5. R. Billing, B. Rafizadeh, I. Drew, G. Hartman, R. Gale, P. Terasaki, *J. Exp. Med.* **144**, 167 (1976).
6. G. Berke and D. B. Amos, *Nature (London) New Biol.* **242**, 237 (1973).
7. M. C. Territo and M. J. Cline, *J. Immunol.* **118**, 187 (1977).
8. B. L. Pike and W. A. Robinson, *J. Cell. Physiol.* **76**, 77 (1970); D. W. Golde and M. J. Cline, *J. Clin. Invest.* **51**, 2981 (1972).
9. P. A. Chervenick and D. R. Boggs, *Science* **169**, 691 (19 → P. A. Chervenick and A. F. LoBuglio, *ibid.* **178**, 164 (1972); M. J. Cline and D. W. Golde, *Nature (London)* **248**, 703 (1974).
10. A. Saxon, R. H. Stevens, D. W. Golde, *Ann. Intern. Med.* **88**, 323 (1978); A. Saxon, R. H. Stevens, S. G. Quan, D. W. Golde, *J. Immunol.* **120**, 777 (1978).
11. D. Metcalf, M. A. S. Moore, J. W. Sheridan, G. Spitzer, *Blood* **43**, 847 (1974).
12. The PHA-stimulated human peripheral blood leukocyte-conditioned medium, the source of CSA, was added to liquid cultures containing 1×10^6 cells per milliliter for either 1, 12, or 24 hours at a concentration of 50, 100, or 200 μl/ml with alpha medium, 20 percent fetal calf serum, and $10^{-4}M$ α-thioglycerol.
13. M. Paran and L. Sachs, *J. Cell. Physiol.* **72**, 247 (1968).
14. T. R. Bradley, E. R. Stanley, M. A. Sumner, *Aust. J. Exp. Biol. Med. Sci.* **49**, 595 (1971).
15. Y. Ichikawa, *J. Cell. Physiol.* **74**, 223 (1969).
16. C. B. Lozzio and B. B. Lozzio, *Blood* **45**, 321 (1975).
17. R. E. Gallagher, S. Z. Salahuddin, W. T. Hall, K. B. McCredie, R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4137 (1975).
18. S. J. Collins, R. C. Gallo, R. E. Gallagher, *Nature (London)* **270**, 347 (1977).
19. We thank Drs. Ian Drew, P. Terasaki, Robert Sparkes, Ronald Como, and Shirley Quan. Supported in part by PHS grants CA 15688, CA 15619, and RR 00865.

17 November 1977; revised 24 January 1978