

Characterization of the Continuous, Differentiating Myeloid Cell Line (HL-60) From a Patient With Acute Promyelocytic Leukemia

By R. Gallagher, S. Collins, J. Trujillo, K. McCredie, M. Ahearn, S. Tsai,
R. Merzgar, G. Aulakh, R. Ting, F. Ruscetti, and R. Gallo

In a preliminary communication,¹ we described the establishment of a continuous human myeloid cell line (HL-60). Here we report the detailed properties of this cell line and document its derivation from the peripheral blood leukocytes of a patient with acute promyelocytic leukemia. As characterized by light and electron microscopy, the predominant cell type in both the fresh and cultured sources is a neutrophilic promyelocyte with prominent nuclear/cytoplasmic asynchrony. Up to 10% of the cultured cells spontaneously differentiate beyond the promyelocyte stage, and the proportion of terminally differentiated cells is markedly enhanced by compounds known to stimulate differentiation of mouse [Friend] erythroleukemia cells.² The HL-60 cells lack specific markers for lymphoid cells, but express surface receptors for Fc fragment and complement (C3), which have been associated with differentiated granulocytes. They exhibit phagocytic activity and responsiveness to a chemotactic stimulus commensurate with the proportion of mature cells. As characteristic of transformed cells, the HL-60 cells form colonies in semisolid medium

and produce subcutaneous myeloid tumors (chloromas) in nude mice. A source of colony-stimulating activity stimulated the cloning efficiency in soft agar 5-30-fold. Despite adaptations to culture, the morphological phenotype and responsiveness to chemical induction of differentiation is essentially unchanged through at least 85 passages. Cytogenetic studies reveal aneuploidy. Metaphases with 44 chromosomes predominated in vivo and in early culture passages; however, clones with 45 or 46 chromosomes became predominant with continued passaging. The most consistent karyotypic abnormalities were the deletion of chromosomes 5, 8, and X and the addition of a marker resembling a D-group acrocentric and of a submetacentric marker, most likely an abnormal E-group chromosome. No DNA herpesvirus or RNA retrovirus was isolated in the fresh or cultured cells. The HL-60 cultured cell line provides a continuous source of human cells for studying the molecular events of myeloid differentiation and the effects of physiologic, pharmacologic, and virologic elements on this process.

IN MICE, the availability of sustained cultures of erythroid¹ and myeloid^{2,3} cells has fostered experiments to determine regulatory mechanisms involved in cell replication and differentiation and to study external factors that may affect these processes.¹ In man, in vitro culturing of myeloid cells has primarily been restricted

From the Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Md.; the Departments of Developmental Therapeutics and Laboratory Medicine, M. D. Anderson Hospital, Houston, Texas; Biotech Research Laboratories, Rockville, Md; the Department of Microbiology and Immunology, Duke University, Durham, N. C.; and the Division of Virology, Bureau of Biologics, Bethesda, Md.

Supported in part by National Cancer Institute Contracts N01-CM-93719 and N01-CM-02271 and NIH Grant CA-12687.

Submitted January 23, 1979; accepted May 25, 1979.

Address reprint requests to Robert C. Gallo, M.D., National Institutes of Health, 9000 Rockville Pike, Building 37, Room 6B04, Bethesda, Md. 20014.

© 1979 by Grunr & Stratton, Inc. 0006-4971/79/5403-0018\$02.00/0

Blood, Vol. 54, No. 3 (September), 1979

This material supplied
by the Interlibrary Loan Section⁷¹³
from the collection of the
Archib R. Dykes Library of the Health Sciences
University of Kansas Medical Center

NOTICE: This material may be protected by
copyright law (Title 17 United States Code)

to growth of small colonies for short periods of time in semisolid medium,⁶ which imposes severe experimental limitations. We previously reported that human leukemic myeloid cells can be carried in an actively replicating and differentiating state for periods up to several months when cultured in the presence of conditioned medium from certain human embryo cells.⁷⁻⁹ However, there have been difficulties obtaining adequate quantities of active conditioned medium for extensive studies.¹ In one case, after initiation with conditioned medium from a human embryonic lung culture, the leukemic myeloid cells continued to grow and differentiate in the absence of any exogenous growth stimulator. The basic characteristics of this cell line (HL-60) and its ability to respond to pharmacologic inducers of differentiation have been reported elsewhere.^{10,11} Here we report more detailed information regarding the HL-60 line and the patient with acute promyelocytic leukemia from whom it was derived. Recently, Koefler and Golde¹² also established a differentiating myeloid cell line from a patient with erythroleukemia. Prior to these reports, there had been numerous successes in establishing human lymphoblast cell lines, mostly B cell lines engendered by Epstein Barr virus (EBV).¹³ However, there had been only two successes with the extended propagation of human myeloid cells, and in neither case was differentiation observed in suspension culture.^{14,15} Recently, Karpas et al.¹⁶ have also reported the establishment of human leukocyte cell lines with a few myeloid features, but these lines primarily have the properties of B-lymphoblast cells, including the presence of EBV and surface immunoglobulins.

MATERIALS AND METHODS

Case Report

S.G., a 36-yr-old white female, was referred to the M. D. Anderson Hospital and Tumor Institute with a diagnosis of acute promyelocytic leukemia. She had been well until 2 mo previously when following an upper respiratory infection, she developed easy bruisability, swollen gums, and bone pain. On the admission physical examination, she had multiple small nodules, 1 cm or less in diameter, over her scalp, cervical lymphadenopathy, and a 6 x 8 cm nodule in the left lateral vaginal wall. Numerous ecchymotic spots and petechiae were present over the arms, trunk, and legs. The liver extended 10 cm below the right costal margin, the spleen 3 cm below the left costal margin. Admission peripheral blood counts were as follows: hemoglobin 9.3 g/dl, hematocrit 27.4%, platelets 80,000/cu mm, white blood cells 35,700/cu mm with 70% myeloblasts and promyelocytes, 14% myelocytes, 2% bands, and 14% lymphocytes. The bone marrow showed 4.06 blasts, 85% promyelocytes, 2.8% myelocytes, 0.8% metamyelocytes, and 1% normoblasts. The cells were heavily granulated, and special stains showed peroxidase positivity and PAS negativity. The Serum lactic dehydrogenase (LDH) activity was elevated to 650 μ U/ml (normal 30-110 U); other values of the SMA 12 autoanalyzer were within normal limits. Coagulation parameters, including prothrombin time, partial thromboplastin time, fibrinogen, and fibrin split products were within normal limits. Treatment was initiated with intravenous C-parvum, systemic prophylactic antibiotics, and chemotherapy with adriamycin, vincristine, prednisone, and cytosine arabinoside. There was a partial response to two initial courses of this therapy, with a reduction in the WBC to less than 2000/cu mm and a decrease in the marrow leukemic infiltration and in size of the subcutaneous nodules. However, disease progression was noted during a third course of chemotherapy. On the 64th hospital day, the patient complained of the sudden onset of abdominal pain and succumbed to intraabdominal hemorrhage and shock. The coagulation profile, which up to this time had been normal, showed evidence of a consumptive coagulopathy with marked prolongation of the prothrombin time, an increase in the partial thromboplastin time, and a reduction of the serum fibrinogen level to 36 mg/100 ml. At autopsy, there was evidence of leukemic infiltrates in the mediastinum, lymph nodes, uterus, vagina, and ovaries, and there was a massive hemoperitoneum, splenomegaly, and multiple petechial hemorrhages. The source of bleeding was the spleen, which had a subcapsular infarct and capsular tears.

Establishment of the Promyelocytic HL-60 Cell Line in Suspension Culture

Prior to treatment of the patient, peripheral blood leukocytes were obtained by leukopheresis, using a blood cell separator. The cells were shipped on wet ice to the NCI and on arrival were 98% viable, as determined by trypan blue dye exclusion. As previously detailed,¹⁷ the leukocytes were cultured in the presence and absence of conditioned medium from human embryonic lung cultures. After a 3-wk lag period, active growth of cells in suspension was observed in flasks supplemented with conditioned medium from one human embryonic lung culture. Subsequently, it was found that continued growth of the leukocytes did not require conditioned medium supplements.

Cytogenetic Studies

Cytogenetic examinations were done on the following samples: (1) fresh bone marrow prior to chemotherapy; (2) cultured HL-60 cells from passages 6, 8, 15, 28, 35, 45, and 55, grown on RPMI 1640 medium with 10% heat-inactivated fetal calf serum, or from passages 21 and 41, grown for the last 6 passages on Ham's F-10 medium with 20% fetal calf serum; and (3) after passage through nude mice. For analyses, the cells were incubated in the presence of colcemid, 0.02 μ g/ml, for 1 hr at 37°C, then treated with 0.075 M KCl for 20–30 min at room temperature, and fixed in three changes of methanol-acetic acid (3:1). Chromosome preparations were made using air-drying or flame-drying techniques. Chromosomes were banded by the trypsin-Giemsa method; slides were treated with 0.01%–0.03% trypsin in phosphate-buffered saline (Ca^{++} and Mg^{++} free) for 3–6 min at room temperature and stained with 4% Giemsa stain in Sorenson's buffer at pH 6.8.

Histochemical Staining Procedures

Published procedures were followed for Naphthol ASD chloroacetate and α -methyl acetate esterases.¹⁸ Sudan black B,¹⁹ myeloperoxidase,²⁰ alkaline and acid phosphatases,^{19,21} and the periodic acid Schiff (PAS) reaction.²²

Electron Microscopic Preparation

The initial bone marrow and subsequent cultured HL-60 cells were mixed in 2.5% glutaraldehyde, pH 7.2, in Sorenson's phosphate buffer, 300 mosmol, for 1 hr at 25°C. Dianiobenzidine tetrahydrochloride was utilized to demarcate endogenous peroxidase activity. The tissue was post-fixed in 1% osmium tetroxide, pH 7.2, for 1 hr. Following dehydration in graded acetones, the cell pellets were embedded in Epon 812. Sections were cut on an LKB ultramicrotome and examined with a Siemens Elmiskop 102 at 80kV.

Cell Surface Studies

Erythrocyte rosette tests were performed by slight modifications of established techniques, as detailed elsewhere.²³ The E-rosette assay utilized sheep erythrocytes. The EA and EAC-rosette tests were performed with both sheep and bovine erythrocytes. Tests for surface immunoglobins IgG, IgM, and IgA utilized immunobeads (Bio-Rad Laboratories), as detailed elsewhere.²³ Direct testing of HLA antigens was done at Duke University by the standard two-stage microcytotoxicity method of Amos et al.²⁴ and by the method of Mittal et al.²⁵ with a panel of sera used to define HLA-A, B, and C locus antigens at past HLA Histocompatibility Testing Workshops. Direct testing for human Ia-like antigens was done by complement dependent microcytotoxicity²⁵ using rabbit and monkey antisera known to specifically detect Ia antigens by cytotoxicity and by radioimmunoprecipitation and polyacrylamide gel electrophoresis analysis.²⁶

Chemotaxis Studies

Cells were added to the upper well of a blind well chamber (model F013 WLB 00101 Neuro Probes, Inc., Bethesda, Md.) at a concentration of 2.5×10^4 ml of Gey's balanced salt solution containing 2% bovine serum albumin and 0.01 M Herpes.²⁷ The dipeptide N-formylmethionyl-leucine (Sigma Corp., St. Louis, Mo.), a potent chemoattractant,²⁷ was added to the lower well at a concentration of 10^{-7} M. A 13-mm chemotactic membrane (Nucleopore Corp., Pleasanton, Ca.), containing an average pore diameter of 5.0 μ , separated the two compartments. The chamber was then incubated at 37°C in a humidified 5% CO_2 atmosphere for 1 hr. The filters were then removed, stained with Wright-Giemsa,

and examined microscopically for cells that had migrated to the underside. Results were expressed as the number of cells counted in four successive low power fields. All experiments were performed in triplicate and the results averaged.

Phagocytosis Studies

Cells were suspended at 10^6 cells/ml in RPMI 1640 medium supplemented with 10% FCS. *Candida albicans*, grown overnight in Sabouraud's medium (Difco, Detroit, Mich.), were washed twice with saline and added to the above HL-60 cell suspension at 4×10^6 organisms/ml. The suspension was then incubated for 30 min at 37°C, and the percent of cells that had phagocytosed yeast particles was determined by light microscopy on Wright-Giemsa stained slides.

Lysozyme Studies

Cells, at a concentration of 10^7 /ml, were lysed by incubating with 1% NP-40 in PBS, pH 6.2, for 10 min at room temperature. The cell lysate was then spun at 3900 rpm at 4°C for 10 min, and the lysozyme assay was performed on 1 ml of the supernate with egg white lysozyme (Sigma) used as the standard. In addition, lysozyme assays were performed on 1-ml aliquot samples of the cell-free supernate of actively proliferating cells harvested at a time when cells were at a concentration of 1.5×10^6 /ml.

Growth and Cloning in Semisolid Medium

To assay for colony formation, various concentrations of HL-60 cells were suspended in 1.2% methylcellulose (Dow Chemical Corp.) or 0.3% agar (Difco) in McCoy's 5A medium containing 10% fetal calf serum (FCS) and 5% horse serum. One milliliter of this mixture was plated in 35-mm culture dishes (Falcon) and incubated in a humidified 5% CO₂ chamber at 37°C. Colony counts (≥ 30 cells) were performed after 7, 14, and 21 days of incubation. For cloning studies, the HL-60 cells were suspended at a concentration of 10^5 cells/ml in 1.2% methylcellulose in McCoy's 5A medium containing 10% FCS and cultured as above. After 5-6 days, visible colonies containing 20-30 cells were individually transferred to wells of a 16-mm multiwell dish (Linbro Chemical Co., New Haven, Conn.) containing 0.5 ml of RPMI 1640 medium supplemented with 10% FCS. The cells were then recloned using the same procedure. As cell growth continued, cells were transferred to larger wells and ultimately to Falcon T/60 flasks.

Growth in Nude Mice

Athymic nude mice (BALB/c nu/nu) were obtained from ARS/Sprague Dawley (Madison, Wisc.). HL-60 cells at different passage numbers were injected subcutaneously into the mice. Tumors that developed were dissected, stained with Wright-Giemsa and myeloperoxidase, and analyzed for karyotype. A separate cell line (designated HL-60 Nu.) was established by mincing a tumor obtained from injection of HL-60 at passage 45, incubating the tumor mince with 0.25% trypsin for 30 min at 37°C, resuspending the resultant cell suspension in RPMI 1640 plus 15% heat-inactivated FCS, and continuing the leukocyte suspension cultures under standard conditions.

Test for Herpesviruses

The Epstein-Barr virus nuclear antigen (EBNA) test was performed by a modification of the Reedman-Klein technique.^{21,22}

Herpes simplex type I and cytomegalovirus (Town strain) probes were prepared by labeling the DNA with tritium in vitro by "nick translation," using all four ³H-labeled nucleoside triphosphates to a specific activity of 1.5×10^7 cpm/ μ g as detailed by Maniatis et al.²⁰ These probes were hybridized to excess cellular DNA up to a final Ecot of 8000, and the hybrids were processed by hydroxyapatite chromatography.

Tests for Retroviruses

To test for possible extracellular virus, up to 100-ml samples of HL-60 cell culture fluid were concentrated and assayed for RNA-directed DNA polymerase (reverse transcriptase) activity, as previously reported.²³ In addition, cultures were tested for release of RNA-containing particles by

HL-60

pulse
ultra
Att
5'-iod
testes
Th
exam
searc
mic n
by se
retrov
deter
weigh
adeno
retrov
indire
acids
Final
with

Gro
A
pres
cult
leuk
supp
3.0
pass
shov
pres
with

Fi
on ti
and
perfr
and
Coul
the
of c
cult
half
were
RPM
50 μ
fetal
A.A.
seru
were
seru

pulse-labeling up to 50-ml culture samples with ^3H -uridine, concentrating the labeled particles by ultracentrifugation, and centrifuging them to equilibrium on a 20%–60% sucrose gradient."

Attempts to induce virus production followed published procedures, including treatments with 5'-iododeoxyuridine,³⁴ dimethylsulfoxide,¹⁰ and arginine-deprived medium." 5-Iododeoxyuridine was tested with and without the addition of hydrocortisone, 10^{-6}M .

The possible presence of subviral components within fresh and cultured HL-60 leukocytes was examined by a variety of tests. From 2 to 25 g of viable, nonfrozen cells were used per test. Methods to search for intracellular reverse transcriptase, included the preparation and purification of the cytoplasmic-microsomal-membrane fraction³⁵ and detergent extraction of whole cytoplasmic extracts followed by sequential column chromatography.³³ Tests for cellular antigens related to p30 proteins from known retroviruses were performed on whole cell extracts using a competition radioimmunoassay capable of detecting 1 ng of competing virus-related antigen." Attempts were made to demonstrate high molecular weight RNA associated with intracytoplasmic virus-like particles by direct pulse-labeling with ^3H -adenosine.³⁶ Also, cytoplasmic RNA was tested for possible relatedness to RNA from known retroviruses by two methods: (1) direct hybridization assays with ^3H -cDNA viral probes" and (2) indirect competition assays in which cytoplasmic RNA is tested for possible relatedness to viral nucleic acids by its ability to interfere with the hybridization of homologous viral RNA to proviral DNA." Finally, the HL-60 cellular DNA was tested for viral-related proviral sequences by direct hybridization with tritium-labeled type-C viral cDNAs³⁷ and with ^{32}P -labeled whole viral RNAs."

RESULTS

Growth Kinetics in Suspension Culture

As previously reported," the HL-60 blood leukocytes initially grew only in the presence of culture fluid supplemented with conditioned medium harvested from cultured human embryonic lung cells. However, after a few weeks, the cultured leukocytes grew as well in the absence as in the presence of conditioned medium supplements. At passage 9, the saturation density of the cultured cells was $2.3\text{--}3.0 \times 10^6$ cells/ml, and the cell-doubling time was 55–60 hr.¹⁰ With continued passage, the HL-60 cells have continued to adapt to in vitro culture conditions. As shown in Fig. 1, the growth of the cells has remained absolutely dependent on the presence of FCS through 68 passages. Maximum growth stimulation was achieved with 10%–20% FCS. However, at a given serum concentration, the saturation

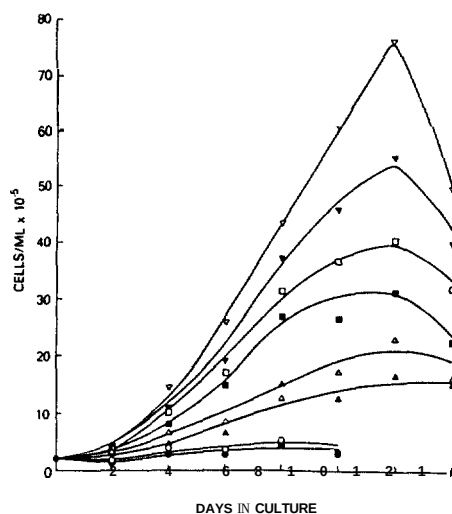


Fig. 1. Effect of serum concentration on the growth of HL-60 leukocytes at low and high passage levels. Cell counts were performed at passage 17 (closed symbols) and passage 68 (open symbols), using a Coulter counter. Plotted points represent the average of triplicate counts from each of duplicate 10-ml culture flasks. The cultures were refed on day 6 by replacing half of the culture fluid volume. Cultures were carried, as previously detailed," in RPMI-1640 medium containing gentamicin, 50 $\mu\text{g}/\text{ml}$, and varying concentrations of fetal calf serum as follows: $\circ, \square$, no serum; $\triangle, \Delta$, 1% serum; $\blacksquare, \square$ 5% serum; $\nabla, \triangledown$ 10% serum. Curves observed using 20% serum were superimposable on those with 10% serum.

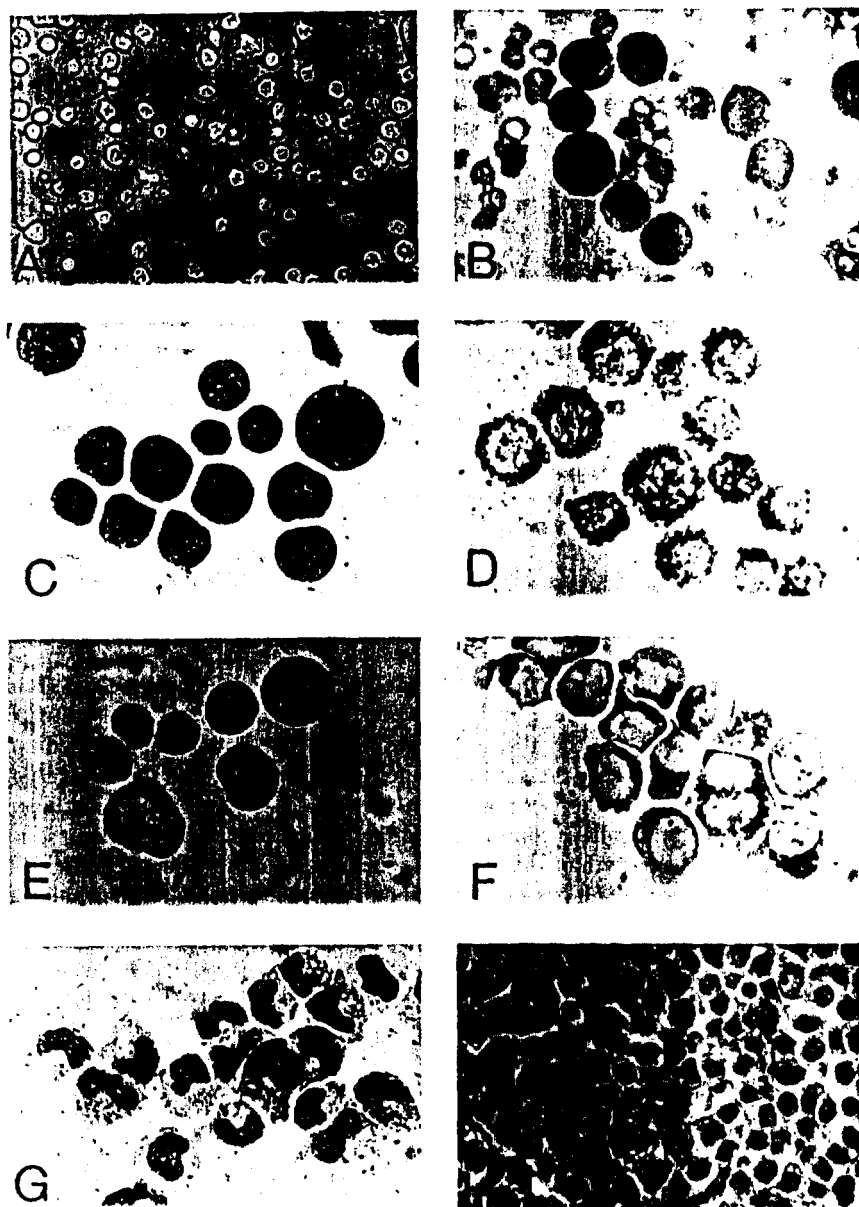


Fig. 2. Light microscopic appearance of fresh and cultured HL-60 leukocytes. (A) Cells from passage 38 growing in single-cell suspension as viewed in an inverted microscope (Leitz Diavert, Germany; $\times 160$). (B) Smear of fresh, uncultured bone marrow from patient S.G. (HL-60) prior to chemotherapy, illustrating predominant leukemic promyelocytes. (Wright-Giemsa stain). (C) Cyto-centrifuge preparation of cultured HL-60 leukocytes, illustrating predominant promyelocytes. (Wright-Giemsa stain.) (D) Strongly positive myeloperoxidase stain of cultured cells. (E) Positive ASD chloroacetate esterase stain of cultured cells. (F) Positive Sudan black B stain of cultured cells. (G) Wright-Giemsa stain of cultured HL-60 cells after treatment with DMSO for 6 days, showing differentiation of cells to myelocytes and predominant metamyelocytes. (H) Hematoxylin and eosin stained section of nude mouse tumor produced by subcutaneous inoculation of cultured HL-60 ($\times 160$). Nuclei are predominantly undifferentiated but eosinophilic cytoplasmic granules are present, which stain heavily with myeloperoxidase. Photomicrographs of cultured cells were taken with a LEITZ Ortholux II, Germany, at $\times 1000$.

HL-60 IN A

density h
level. At
cell-doub
these val
was also
the cultu

Cytologic

The ce
adhere to
occasiona
there is co
larger cel

Figure:
Giemsa-st
cultured
promyeloc
distinct n
basophilic
intensive
specimens
including
Sudan bla
tase, a sta
promyeloc
acid-Schil
The more
negative f
esterase w
cells.³⁷

As sumi
tiate into
banded an
size, decre
tation, dec

Passage
Number

Fresh*

0†

9

18

35

63

*From diffe

†From diffe

density has increased and the doubling time has decreased as a function of passage level. At passage 17, the saturation density was 5.5×10^6 cells/ml, with a cell-doubling time of 36 hr during the logarithmic phase of growth; at passage 68, these values were, respectively, 7.6×10^6 cells/ml and 30 hr. Continued passage was also accompanied by some shift in the cytologic and karyotypic properties of the cultured cells (see below).

Cytologic Characteristics of Fresh and Cultured Cells

The cells grow in single-cell suspension without any tendency to clump or to adhere to plastic or glass (Fig. 2A). The cells are generally round or ovoid, and occasional cells have blunt pseudopods. The modal cell diameter is 13μ ; however, there is considerable variation in cell size, ranging from 9μ to 25μ in diameter. The larger cells are frequently binucleate.

Figures 2B and 2C illustrate typical leukemic myeloid cells observed in Wright-Giemsa-stained preparations from the patient's fresh blood specimen and from the cultured HL-60 cells. In both instances, the predominant cell is an abnormal promyelocyte. This cell typically contains a large round nucleus with regular, distinct margins, fine chromatin, and 2-4 nucleoli. The cytoplasm is deeply basophilic and contains multiple prominent azurophilic granules. Despite an intensive search, Auer rods were not observed in either the fresh or cultured specimens. These cells stained heavily with stains specific for myeloid cells, including myeloperoxidase (Fig. 2D), ASD chloroacetate esterase (Fig. 2E), and Sudan black B (Fig. 2F). However, no positivity was noted with alkaline phosphatase, a stain characteristically positive in normal neutrophilic granulocytes. The promyelocytes showed slight diffuse staining of the cytoplasm with the periodic acid-Schiff reagent, and occasional cells stained positively for acid phosphatase. The more mature granulocytes stained strongly positive with PAS and were negative for acid phosphatase. Stains for α -naphthol AS-D acetate (nonspecific) esterase were uniformly negative. This staining pattern is characteristic of myeloid cells.³⁷

As summarized in Table 1, 5%-10% of the myeloid cells spontaneously differentiate into more mature granulocytes, including myelocytes, metamyelocytes, and banded and segmented neutrophils. This process is accompanied by diminished cell size, decreased nuclear-cytoplasmic ratio, increased nuclear pycnosis and segmentation, decreased cytoplasmic basophilia, and replacement of the coarse azurophilic

Table 1. Differentiation of HL-60 Cells as a Function of Culture Passage

Passage Number	Percent of Myeloid Cells			Pawnt Inducible by 1.25% DMSO
	Myeloblasts to Promyelocytes	Myelocytes	Metamyelocytes to Segmented Neutrophils	
Fresh*	81	16	2	Not tested
0†	95	4	1	Not tested
9	89	7	4	70-90
18	92	4	4	70-90
35	97	1	2	70-90
63	96	2	2	70-90

*From differential WBC on fresh blood specimen at M. D. Anderson Hospital.

†From differential WBC on leukopheresis specimen prior to culture at NCI.

s from
lavert,
rior to
Cyto-
cytes.
e ASD
ls. (G)
owing
eosin
HC-60
asent,
with a

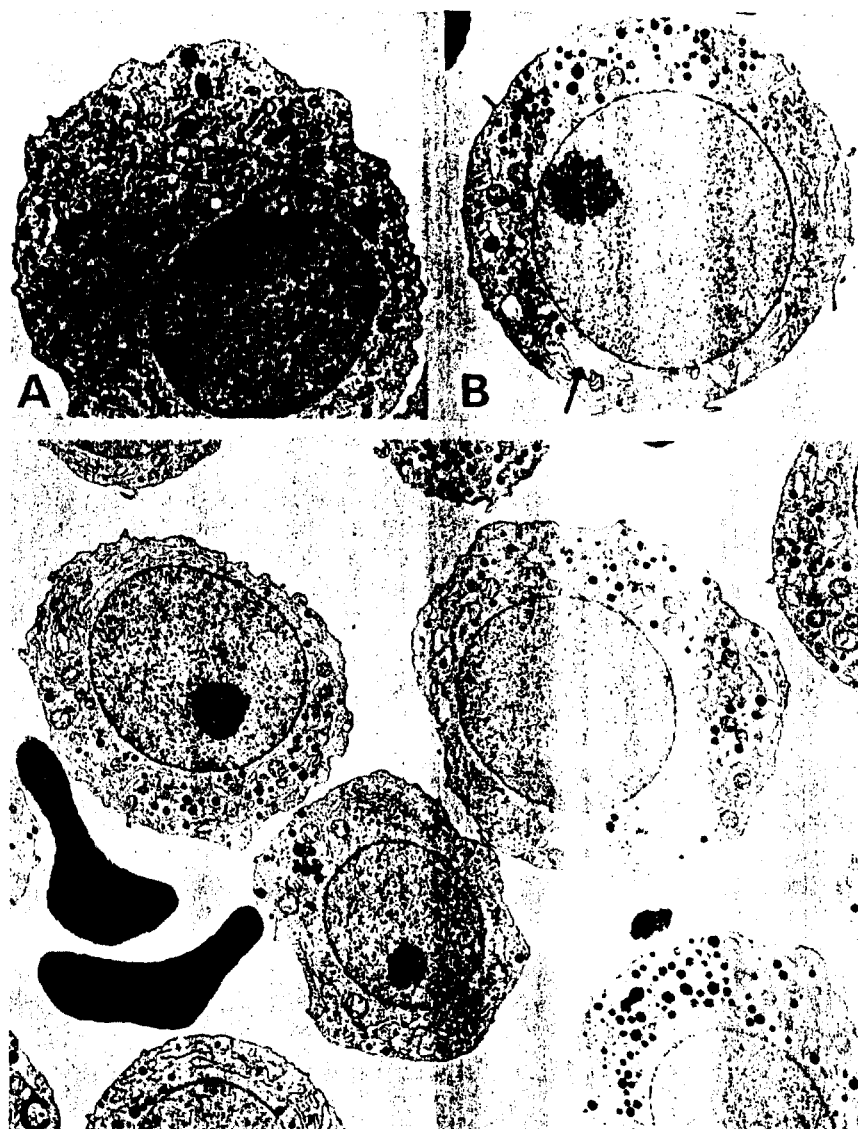


Fig. 3. Electronmicroscopic appearance of fresh and cultured HL-60 cells. (A) This promyelocytic HL-60 cell displays peroxidase-positive cytoplasmic granulation. The granule morphology and enzymatic content is, in general, more variable than that observed in similarly staged cells *in vivo*. The nuclear development is asynchronous, and the structure bears a nucleolus and a nuclear bleb (arrow) on its surface. This latter morphological abnormality is associated in high frequency with aneuploid acute leukemias²⁸ ($\times 9600$). (B) Asynchronous development in the nuclear/cytoplasmic areas is apparent in this promyelocyte from the initial bone marrow specimen. The cytoplasm demonstrates evidence of both peroxidase-positive azurophilic and peroxidase-negative specific granules. Bundles of fibrils, a feature associated with leukemic cells,²⁸ are noted (arrow) in the juxtannuclear area of the cytoplasm. Some dilated cisternae of the endoplasmic reticulum are also evident ($\times 6400$). (C) Low magnification micrograph illustrating the overall morphology observed in



the initial bone marrow specimen of this patient. The developing nuclei are primarily blastic in appearance, whereas the cytoplasmic areas are promyelocytic ($\times 5000$). (D) This "band" or "stab" HL-60 cell displays evidence of nuclear and cytoplasmic differentiation. Although the granules are malformed, both peroxidase-positive azurophilic (large arrow) and peroxidase-negative specific (small arrow) forms are present ($\times 20,000$). (E) Following dimethyl formamide (50 mM) incubation for 8 days, the nuclei of HL-60 display dramatic nuclear segmentation and increased definition of heterochromatic areas. Cytoplasmic granules are of both the azurophilic (large arrow) and specific (small arrow) type ($\times 39,000$).

promyelo-
logy and
s in vivo.
lear bleb
ncy with
oplasmic
toplasm
specific
i) in the
are also
erved in

granules by smaller specific granules. As reported in detail elsewhere,¹¹ the proportion of differentiating promyelocytes could be greatly enhanced by adding certain compounds known to induce differentiation of mouse (Friend) erythroleukemia cells, e.g., **DMSO**, to the cultured **HL-60** cells (Fig. 2G). Differentiation was limited to the neutrophilic series in suspension culture, since no eosinophilic or basophilic granules were detected in **HL-60** cells after either spontaneous or induced differentiation. In addition to the neutrophilic granulocytes, a minor population of cells resembled monocytes with folded nuclei, pale-staining blue cytoplasm, and less distinct and regular cytoplasmic margins. No cells resembling immature erythroid cells were observed, and benzidine stains for hemoglobin were negative. With continued passaging of the **HL-60** cells, the percentage of myeloid cells showing spontaneous terminal differentiation has diminished slightly. This did not substantially affect the induction of differentiation by **DMSO** (Table 1).

Electron Microscopy of Fresh and Cultured Cells

Ultrastructurally, the **HL-60** line consists of a heterogeneous population of cells with the predominant morphology varying from myeloblastic to promyelocytic. Nuclear/cytoplasmic asynchrony is present, the cytoplasm representing the more mature element. The nuclear chromatin is primarily euchromatic, and well developed nucleoli are situated adjacent to the nuclear membrane. Cytoplasmic granulation in the cultured cells is mostly of the azurophilic type. The size, shape, and density of these peroxidase-positive granules (Fig. 3A) is more diverse than those observed in the patient's original bone marrow (Fig. 3B). In this initial specimen, 85% of the cells are characterized by blast-like nuclei and progranulocytic cytoplasm (Fig. 3C).

In addition to the nuclear/cytoplasmic asynchrony, nuclear blebs and bundles of cytoplasmic fibrils are present in leukemic cells both in vivo and in vitro. Under routine culture conditions, a small percentage of these cells demonstrate "stab" or "band-like" nuclei and evidence of both azurophilic and specific granulation of the cytoplasm (Fig. 3D). The addition of the differentiating agent, dimethyl formamide, to the **HL-60** culture medium induces further segmentation of the nuclei and increased specific granule formation in the cytoplasm (Fig. 3E).

Cytogenetic Analysis of Fresh Bone Marrow

A total of 40 metaphases were analyzed from the initial bone marrow sample. The analysis showed 31 cells with 44 chromosomes (approximately 75%), 8 with 45 (20%) and 1 with 46 (5%) (Table 2). Examination of these clones, with the help of Giemsa-banding techniques, indicated that the common alterations in all of the

Table 2. Distribution of Chromosome Counts of **HL-60** Cells as a Function of Culture Passage

Passage Number	Metaphases Counted	Chromosome Number					
		≤43	44	45	46	47	≥48
0 (Fresh)	40	—	31	8	1	—	—
6 and 8	145	6	87	18	26	6	2
15 and 28	105	2	1	47	51	—	4
35	50	3	7	29	8	3	—
45	90	2	5	64	8	9	2
55	71	2	4	47	7	8	3

HL-60 11

cells st
resem
metap
D-gro
karyo

1

1

1

1

A

1

1

1

Fi
kary
sam
bone
(44,)
som

cells studied were as follows: $-5, -8,$ and $+$ a submetacentric marker chromosome resembling an E-group chromosome (M3) (Fig. 4A). Additional changes in one metaphase included the presence of a marker acrocentric chromosome resembling a D-group chromosome (M2) and an extra chromosome 18. The pseudodiploid karyotype shown in Fig. 4B illustrates these additional changes. Other marker

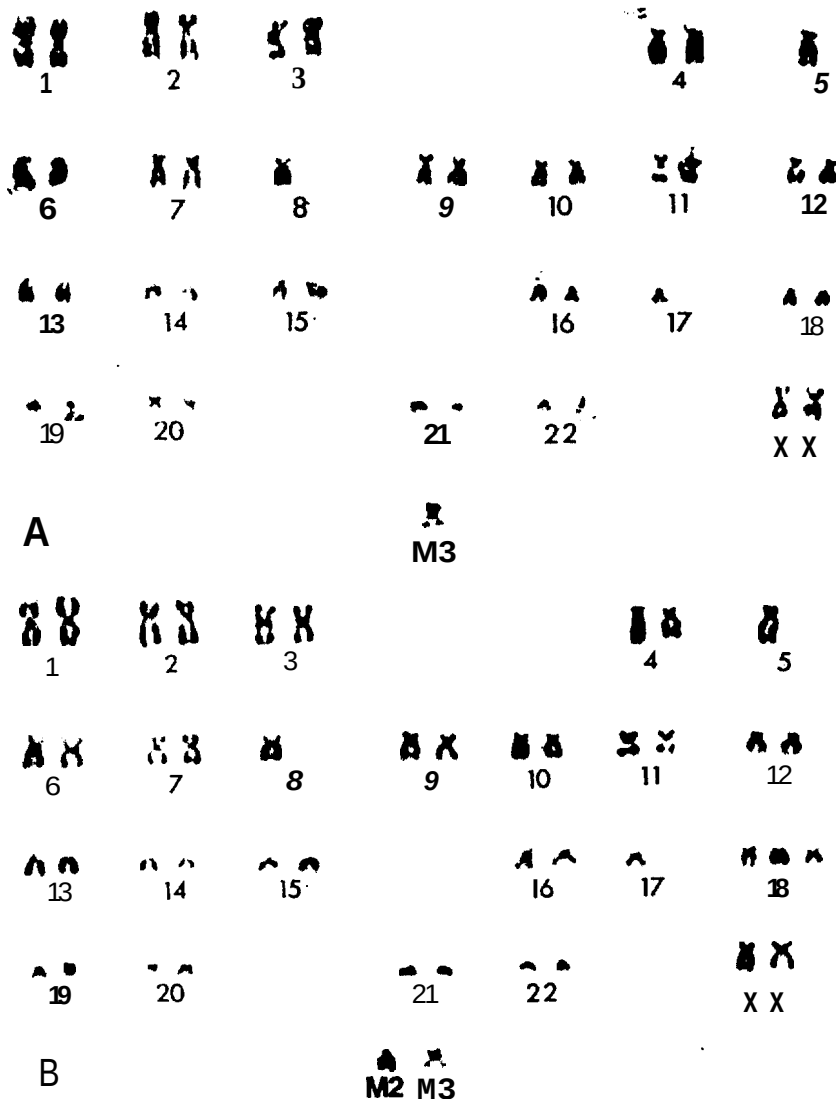


Fig. 4. Karyotype analysis of fresh, uncultured HL-60 bone marrow specimen. (A) Hypodiploid karyotype with 44 chromosomes, representing the main clone (75%) found in the initial bone marrow sample of the patient ($44, XX, -5, -8, -17, +M3$). (B) Pseudodiploid karyotype found in the initial bone marrow sample of the patient (5%) in the in vitro leukemic cell line, passage 28 (50%) ($44, XX, -5, -8, -17, +18, +M2, +M3$). The two markers most likely represent a D-group chromosome (M2) and a 17p+ (M3).

chromosomes, double minute chromosomes, and structural alterations involving other C-group chromosomes (numbers 7, 9, 10) were also occasionally observed in a few hypodiploid metaphases. These variant karyotypes differed from cell to cell and were difficult to categorize even with the use of Giemsa-banding techniques.

Cytogenetic Analysis of Cultured HL-60 Cells

During the early passages in vitro, the modal chromosome number was 44, as in the fresh leukemic bone marrow (Table 2). However, with continued passaging, the modal chromosome number shifted to a mixed population with 45 or 46 chromosomes. Using the Giemsa-banding technique, considerable variation was noted with respect to individual chromosome composition. However, as in the fresh marrow, the following karyotypic alterations were commonly found: -5 , -8 , $+$ an abnormal E-group chromosome, $+18$, and $+$ an acrocentric D-group marker. Although inconsistent, other alterations included missing C-group chromosomes 9 and 10, loss of chromosome 16, loss of chromosome X, the presence of a variable number of double minute chromosomes, and translocation of additional chromatin material to chromosome 7.

Figure 5 illustrates a frequently observed karyotype with 45 chromosomes that emerged after passage 35. It includes 7 recognizable abnormalities as follows: (1) -5 ; (2) -8 ; (3) -16 ; (4) $-X$; (5) $+$ an A-group marker (M1); (6) $+$ an

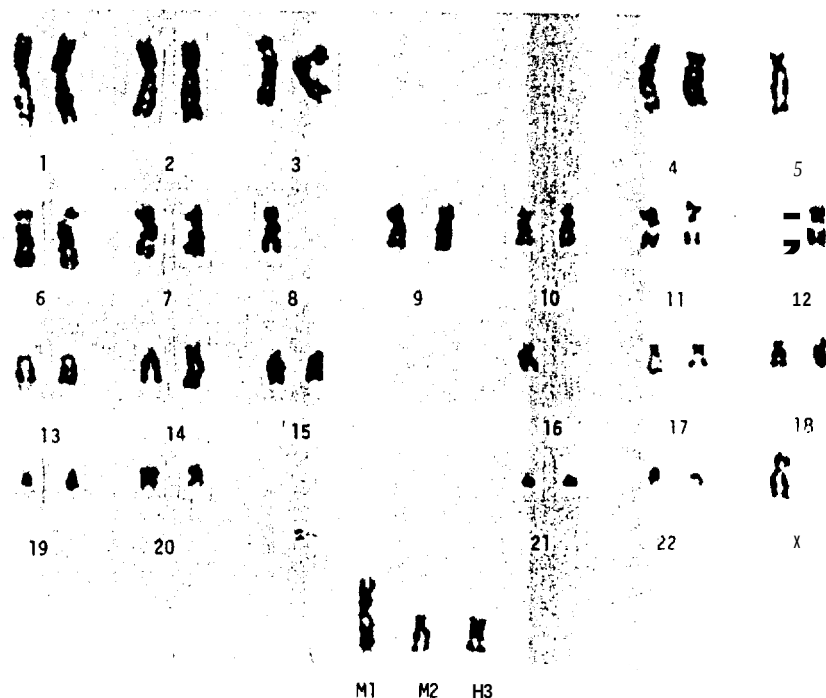


Fig. 6. Karyotype analysis of the predominant tissue culture clone of HL-60 cells after passage 35 (45; $-X$, -5 , -8 , -16 , $+M1$, $+M2$, $+M3$).

acrocent
(M3). A
passages
bone ma
chromos

Cell Sur

The re
ize huma
character
lin, char.
the HL-
cytes, bu
forming
bovine er
erythrocy
increased
cultured
usually fo

Micro
antisera
could be
membran
membran
zee anti
preparati

HLA t
HLA-A1
quantitat
some indi

Cellular

In add
HL-60 c
transfera

E rosette
EA rosette

EAC rose?

Surface im
(IgG, IgM)
la-like anti

*Some 2'
lymphocytes

involving
ved in a
cell and

44, as in
ging, the
chromo-
ted with
narrow,
abnor-
lthough
and 10,
mber of
terial to

nes that
ows: (1)
) + an

5

5

12

12

18

18

6

X

assage 36

acrocentric D-group marker (M2); and (7) + a submetacentric E-group marker (M3). All alterations except no. 5 were also noted commonly in the earlier passages. The M1 marker was only rarely observed in metaphases from the fresh bone marrow. The M3 marker appears to correspond to the abnormal E-group chromosome, but its identity remains to be determined with certainty.

Cell Surface Markers

The results of surface marker tests, which have been generally used to characterize human leukocyte cell lines, are summarized in Table 3. The E-rosette test, characteristically positive with T lymphocytes, and tests for surface immunoglobulin, characteristically positive with B lymphocytes, were uniformly negative with the HL-60 cells. The EA test for Fc receptors was negative using sheep erythrocytes, but was quite positive using bovine erythrocytes, with over 50% of the cells forming rosettes. Conversely, in the EAC test, no rosetting was observed using bovine erythrocytes, but 2-9% of the cells formed EAC rosettes using the sheep erythrocyte assay. The percentage of sheep cell EAC-rosette-forming HL-60 cells increased up to sixfold after induction of differentiation by DMSO. Control cultured T lymphocytes (Molt-4) and undifferentiated myeloid cells (K-562) usually formed no EAC rosettes using sheep erythrocytes.

Microcytotoxicity testing of HL-60 cells with high-titered rabbit and monkey antisera to human Ia-like antigens was negative. Moreover, no Ia-like antigens could be immunoprecipitated from ¹²⁵I-labeled, deoxycholate-solubilized HL-60 membranes with these antisera. More detailed studies on the nature of other membrane antigens of HL-60 cells and the characterization of rabbit and chimpanzee antisera to the cell line will be published separately (Metzgar et al., in preparation).

HLA typing revealed that HLA-Bw17 was a major marker at all passage levels. HLA-A1 (predominant) and HLA-A2 were also detectable but varied somewhat quantitatively at different passage levels. In addition, there were reactions with some individual typing sera defining other minor HLA-A, B and C antigens.

Cellular Enzymes

In addition to enzyme assays by histochemical staining procedures, extracts of HL-60 cells were tested for lysozyme (muramidase), terminal deoxynucleotidyl transferase, and reverse transcriptase.

Table 3. Surface Marker Studies Performed With Cultured HL-60 Leukocytes

Test	HL-60 Cell Results	Reported Leukocyte-Receptor Association
E rosette	--	T lymphocytes**
EA rosette	+	Fc receptor lymphocytes and differentiated granulocytes ^{40,41}
EAC rosette	± *	Complement (C3) receptor: B lymphocytes, ⁴² differentiated granulocytes**
Surface immunoglobulins (IgG, IgM, IgA)	--	B lymphocytes ⁴³
Ia-like antigen	--	B lymphocytes, ⁴⁶ immature granulocytes ^{46,47}

*Some 2%-9% of HL-60 cells formed EAC rosettes with sheep cells versus 0%-2% of control T lymphocytes (Molt-4) or undifferentiated myeloid cells (K562).

Lysozyme, an enzyme marker for leukocytes of the neutrophilic-monocytic cell lineage,^{48,49} was detected at a concentration of $4.7 \mu\text{g}/10^7$ HL-60 cells. It was also detected at a concentration of $2.3 \mu\text{g}/\text{ml}$ in the cell culture fluid after growth of HL-60 cells to a density of 1.5×10^6 cells/ml. No intra- or extracellular lysozyme was found in a myeloid blast cell line (K562) or in a B-lymphoblast cell line (NC37). The values obtained with the HL-60 cultured cells are similar to those reported for a differentiating mouse myelogenous leukemic cell line⁴⁴ and for fresh and cultured myelogenous leukemia cells;⁴⁸ they are substantially less than values reported for fresh or cultured leukemic leukocytes with prominent mononocytic

Terminal deoxynucleotidyl transferase is an enzyme that may be specific for immature T lymphocytes but which has been detected in some cases of human myelogenous leukemia lacking a prominent lymphocyte cell population.⁵⁰⁻⁵² This enzyme was not detectable in extracts of fresh or cultured HL-60 cells, i.e., incorporation of ^3H -dGMP was less than $0.1 \text{ nmole/hr}/10^9$ cells using a primer of oligo(dA)₁₂.⁵⁰

Reverse transcriptase, an enzyme specific for RNA retroviruses, has been partially purified from the blood leukocytes of a few patients with acute myelogenous leukemia⁵³ or, in one case, from the spleen of a patient with myelofibrosis prior to conversion to acute myelogenous leukemia.⁵⁴ To date, this enzyme has not been identified in any human culture cell line. In the present studies, up to 10 g of fresh and cultured HL-60 cells were extracted and analyzed for reverse transcriptase-like activity. A cytoplasmic DNA polymerase activity was identified that transcribed a synthetic RNA template better than a synthetic DNA template, i.e., incorporation of ^3H -TMP was $152 \text{ pmole/hr}/10^9$ cells with oligo(dT)₁₂₋₁₈.poly(A) and was $43 \text{ pmole/hr}/10^9$ cells with oligo(dT)₁₂₋₁₈.poly(dA). However, this polymerase failed to transcribe more specific primer templates for RNA-directed DNA polymerase, oligo(dG)₁₂₋₁₈.poly(C) or oligo(dG)₁₂₋₁₈.poly(MeC), and it was not at all inhibited by antisera that inhibit ($>90\%$) reverse transcriptase from the woolly monkey sarcoma-gibbon ape leukemia virus group or from the baboon endogenous virus group. This pattern of results is most consistent with human cellular DNA polymerase γ activity rather than reverse transcriptase.^{52,55,56}

Cell Functional Studies

Cultured HL-60 cells were tested for their ability to phagocytize yeast particles and to respond to a chemotactic stimulus, as described in Materials and Methods. On repeated testing, 5% – 10% of the cultured cells were capable of ingesting one or more particles of *Candida albicans*. This corresponds to the percentage of terminally differentiated granulocytes in the culture (Table 1).

As presented in Table 4, the ability of the cultured HL-60 cells to migrate through a 5μ pore size membrane was stimulated about fourfold by the powerful chemoattractant dipeptide N-formylmethionyl-leucine. In the absence of chemoattractant, the number of migrating HL-60 cells was the same as cultured leukocytes that fail to respond to chemotactic stimuli. This corresponds to the anticipated properties of HL-60 cells as partially differentiated neutrophils vis-à-vis K-562 cells (undifferentiated myeloid cells), NC37 cells (B lymphoblasts), and Molt-4 cell (T lymphoblasts).

c
ti
K
N
N
*4LPF, 4
†Number

Both t
enhance
ties of t
presente

Growth

The t
passage
efficienc
sources
5–30-fol
In addit
clones, l
have no
counts c
uncclone

Tumor

The r
HL-60 c
no insta
distant
the turn
which v
tion of t
one suc
(3/3) z
Unfort

*3-5
†Nu.
of passag

Table 4. Chemotaxis Assays of Cultured Leukocytes

Con Culture	Migrating Cells/4LPF*	
	- Chemoattractant	+ Chemoattractant
HL-60	3 ± 2†	17 ± 7
K 562	0	2 ± 1
NC-37	5 ± 2	3 ± 2
Molt-4	0	0

*4LPF, 4 low-power microscope fields (160×).

†Numbers equal the average number of migrating cells ± the observed range from triplicate experiments.

Both the phagocytic and chemotactic functions of the **HL-60** cells were greatly enhanced by induction of further differentiation with **DMSO**. The detailed properties of the induced **HL-60** in comparison to normal mature neutrophils will be presented in another report (S. Collins et al. in preparation).

Growth and Cloning in Semisolid Medium

The **HL-60** cells formed colonies in both methylcellulose and agar. At culture passage 16, the plating efficiency was $0.5\% \pm 0.3\%$, while at passage 85, the plating efficiency was $8.0\% \pm 3.2\%$. When the **HL-60** cells were stimulated with various sources of colony-stimulating activity (**CSA**), the plating efficiency was increased 5–30-fold, as will be presented in detail elsewhere (Ruscetti et al., in preparation). In addition, over 150 clones of **HL-60** cells, presumably representing single-cell clones, have been picked and propagated from methylcellulose. Although these have not as yet been analyzed in detail, only minor variations in differential cell counts of cultures derived from the clones have been observed in comparison to the uncloned culture.

Tumor Formation in Nude Mice

The number of mice developing tumors after injection with different passages of **HL-60** cells is shown in Table 5. All tumors developed at the site of injection and in no instance did postmortem examination show any evidence of metastases to a distant site. Macroscopically, all the tumors had a greenish hue. Microscopically, the tumor consisted predominantly of promyelocytes and myeloblasts (Fig. 2H), which were strongly peroxidase positive. Karyotypic markers confirmed the derivation of the tumor from **HL-60** cells. The cell culture **HL-60** Nu, established from one such tumor was most tumorigenic, with all mice injected developing tumors (3/3) and with as few as 10^7 cells required to produce tumors (Table 5). Unfortunately, it has not been possible so far to serially transplant the nude mouse

Table 5. Formation of Tumors by HL-60 Cells in Athymic Nude Mice

Cell History	Tumors/Mice Injected*
Fresh	Not done
Passage 15	1/8
Passage 45	3/8
Nu†	3/3

*3–5 × 10⁶ cells inoculated subcutaneously.†Nu, suspension culture derived from an explant of a subcutaneous tumor originally formed by inoculation of passage 15 **HL-60** cells.

Table 6. Tests of Cultured HL-60 Cell DNA for Herpesvirus-Related Nucleic Acid Sequences

Source of Unlabeled DNA	DNA Probe Hybridized (%)	
	HBV-1*	CMV†
HL-60	4.0	2.9
Uninfected human cells	3.9	2.6
Infected human Cells	85.0	76.3

*Herpes simplex virus type I (courtesy of Dr. Alvaro Puga).

†Cytomegalovirus (Townstrain).

tumors for more than three generations either by direct in vivo passaging or by reestablishment of the HL-60 Nu₁ cells in tissue culture between inoculations. Also, no success has been encountered in attempts to develop an ascites tumor by intraperitoneal injection. The HL-60 Nu₁ culture has not been productively infected by a mouse retrovirus, as determined by reverse transcriptase assays or electron microscopic observation.

Tests for DNA Herpesvirus Information

The HL-60 cultured cells were repeatedly demonstrated to be negative for Epstein-Barr virus (EBV) by the nuclear antigen (EBNA) test. This assay has been reported to be 100% effective in detecting intracellular EBV DNA.⁵⁷ Nucleic acid hybridization analyses were performed to test for two other herpesviruses that have been associated with transformation of human cells, cytomegalovirus, and herpes simplex virus. Tests for both viruses were negative at a sensitivity capable of detecting less than 0.1 virus genome copy per cell (Table 6).

Tests for RNA Retrovirus Information

Extensive tests were performed on the fresh and cultured HL-60 leukocytes in attempts to detect retrovirus information according to procedures described or referenced in Materials and Methods. No evidence for the release of extracellular virus-like particles was observed from the cultured leukocytes either spontaneously or after procedures known to induce or stimulate retrovirus production in animal culture systems, including treatment with DMSO, 5'-iododeoxyuridine, corticosteroid, and arginine deprivation. An intracellular cytoplasmic DNA polymerase activity was observed that banded on a sucrose equilibrium density gradient at 1.16 g/ml, the characteristic density of type-C retroviruses. However, as described in the section on cellular enzymes, this polymerase activity had properties of DNA γ , not reverse transcriptase.⁵⁸ No other evidence of intracytoplasmic virus-like particles was observed by electron microscopy or by ³H-adenosine-labeling of cellular RNA. Finally, in tests for possible relatedness to specific retrovirus molecules, including p30-related protein and nucleic acids, no positive results were observed in systems designed to detect homology to probes from woolly monkey (simian) sarcoma virus, baboon endogenous virus, murine leukemia virus, and feline leukemia virus.

DISCUSSION

This article documents the derivation of the myeloid cell line HL-60 from the leukemic blood cell population of a 36-yr-old female with acute promyelocytic

HL-

leu
cul
and
I
my
rep
con
the
the
nor
mo
for
lulo
stim
mye
sour
size
HL-
as t
char
of th
Ti
asso
asyn
3).⁵⁸
have
leuko
sive
ciste
parti
disse
turn,
lcuke
intra;
essen
thron
are re
lcuko
In
comm
chron
eviden
E-gro
certifi
arm.
15. Pc
long a

leukemia (APL) by demonstrating virtual cytologic identity of the fresh and cultured leukocytes and by defining distinctive chromosomal markers in the fresh and cultured cells.

In contrast to cultures of peripheral blood leukocytes from other cases of myelogenous leukemia that we have studied,⁷⁻⁹ the HL-60 cells were able to sustain replication in suspension culture in the absence of a continuous added source of conditioned medium (i.e., after culture initiation). This does not appear to be due to the secretion of an endogenous growth stimulator, since conditioned medium from the cultured HL-60 cells did not stimulate the growth of leukemic leukocytes or normal bone marrow in liquid suspension or semisolid medium. Similar autonomous growth was noted with all clones of HL-60 cells derived from colonies picked from methylcellulose. Further, the HL-60 cells formed colonies in either methylcellulose or agar in the absence of any added source of supplemental growth stimulator. Nevertheless, like fresh myeloid progenitors from some cases of acute myelogenous leukemia,⁶ the cultured HL-60 cells can respond to an exogenous source of colony-stimulating activity (CSA), since the plating efficiency and colony size are greatly enhanced by CSA (Ruscetti et al., in preparation). Finally, the HL-60 cells formed localized subcutaneous tumors in athymic nude mice as early as the 15th in vitro passage (when first tested; Table 5). These properties are characteristic of progressed malignant leukocytes" and establish the oncogenicity of the HL-60 cells in vivo.

The cultured HL-60 cells have certain cytologic properties that have been associated with leukemic promyelocytes. These include nuclear/cytoplasmic asynchrony, nuclear bleb formation, and heavy azurophilic granulation (Figs. 2 and 3).^{58,59} However, both the fresh and cultured HL-60 cells lack other features that have been described for promyelocytes from some cases of acute promyelocytic leukemia, including morphologically abnormal granules with Auer rod and extensive intracytoplasmic fibril formation and deformation and dilatation of the cisternae of the endoplasmic reticulum.^{58,59} The latter cytologic features, have particularly been associated with cases of APL in which hemorrhage due to disseminated intravascular coagulation (DIC) has been prominent.^{58,59} This, in turn, has been related to the presence of high amounts of tissue thromboplastin in leukemic promyelocytes.⁶⁰ Although the present patient ultimately died from an intraabdominal hemorrhage associated with DIC, her coagulation parameters were essentially normal until terminally. Our preliminary studies indicate that some thromboplastin activity is present in the HL-60 leukocytes; however, further studies are required to quantitatively relate this thromboplastin activity to that present in leukocytes from other patients with APL and DIC.

In addition to specific cytologic alterations, APL has been associated with a common karyotypic abnormality involving a reciprocal translocation between chromosomes 15 and 17.⁶¹ In both the fresh bone marrow and cultured HL-60 cells, evidence was obtained in most metaphases for the presence of a submetacentric E-group-like chromosome. Although the identity of this chromosome could not be certified, it could be a chromosome 17 with extra chromosomal material on its short arm. This chromatin did not appear to arise by a translocation from chromosome 15. Possibly, it could have originated from one of the heavy bands of the short or long arms of the missing chromosome 5. The loss of chromosome 8 in the fresh and

cultured HL-60 cells is also interesting, since abnormalities involving this chromosome, particularly trisomy, have frequently been associated with human acute leukemia.^{62,63}

DNA herpesviruses have been associated with the transformation of human leukocytes and immortalization of lymphoblast cells in vitro.^{13,64} RNA retroviral information has also been associated with some fresh and cultured human leukemic cells, especially those of myeloid cell type,^{9,65-68} and recent experiments in our laboratory suggest that some oncogenic primate retroviruses may have the potential of transforming human leukocytes.⁶⁹ However, no evidence of information related to known herpesviruses or retroviruses has been found in fresh or cultured HL-60 cells.

A remarkable feature of the cultured HL-60 cells is their capacity to terminally differentiate despite their aggressive malignant growth potential in vitro and in vivo. This suggests a dissociation of genetic control elements for replication and commitment to differentiation. Such a dissociation has also been suggested for murine (Friend) erythroleukemia cells.⁴ In mouse myelogenous leukemia cultured cells, the capacity for myeloid differentiation has been related to alterations in numbers of specific chromosomes.⁷ So far, the differentiated phenotype of the HL-60 cells has remained quite stable (see Table 1) despite progressive adaptation of the cells to continued culture and despite clonal selection either spontaneously in suspension culture or by deliberate cloning in methylcellulose. This suggests that the observed cytogenetic abnormalities are not directly involved with the differentiative capacity of the cells. Certainly, however, phenotypic variations could be present in different clones that have not been detected by the limited methods so far utilized to characterize the state of differentiation of the HL-60 cells. In this regard, the differentiative capacity of HL-60 cells appears to differ from normal neutrophils, since completely differentiated segmented neutrophils are rarely seen even after chemical induction⁷⁰ and since at least one enzyme characteristic of normal granulocytes, alkaline phosphatase, was not histochemically detectable in HL-60 cells. Thus, this human cell line seems of unusual interest for studying intrinsic and extrinsic elements that may affect or perturb the processes of myeloid growth and differentiation.

ACKNOWLEDGMENT

We thank the following persons for their contributions to the studies presented in this manuscript: Dr. L. Glick, Associated Biomedics Systems, Buffalo, N. Y. for rosette tests and EBNA assays; Drs. M. Reitz, R. Smith, and F. Wong-Staal, NCI, Bethesda, Md., for molecular hybridization tests for retrovirus nucleic acids; Dr. C. Saxinger, NCI, for chromatographic tests for reverse transcriptase; Dr. K. Harewood, Pfizer Corp., Maywood, N. J., for radioimmunoassays for viral p30 protein; and Miss Ann Cork, research associate, M. D. Anderson Hospital for assistance with the cytogenetic analysis.

REFERENCES

1. Friend C, Sher W, Holland JG, Soto T: Hemoglobin synthesis in murine virus-induced leukemic cells in vitro: Stimulation of erythroid differentiation by dimethyl sulfoxide. *Proc Natl Acad Sci USA* 68:378-381, 1971
2. Ichikawa Y: Differentiation of a cell line of myeloid leukemia. *J Cell Physiol* 74:223-234, 1969
3. Haran-Ghera N, Kotler M, Meshorer A: Studies on leukemia development in the SJL/J strain of mice. *J Natl Cancer Inst* 39:653-662, 1967
4. Marks PA, Rifkind RA, Bank A, Terada M, Reuben R, Fibach E, Nudel U, Salmon W, Gazitt Y: Induction of differentiation of murine erythroleukemia cells. In Saunders GF (ed): *Cell Differ-*

entiation and Neoplasia. New York. Raven, 1978, p 453

5. Sachs L: Control of normal cell differentiation and the phenotypic reversion of malignancy in myeloid leukaemia. *Nature* 274:535-539, 1978

6. Moore MAS: Regulation of leukocyte differentiation and leukemia as a disorder of differentiation, in Gallo RC (ed): *Recent Advances in Cancer Research: Cell Biology and Tumor Virology*. Cleveland. CRC, 1977, p 79

7. Gallagher RE, Salahuddin SZ, Hall WT, McCredie KB, Gallo RC: Growth and differentiation in culture of leukemic leukocytes from a patient with acute myelogenous leukemia and reidentification of type-C virus. *Proc Natl Acad Sci USA* 72:4137-4141, 1975

8. Gallagher R, Ruscetti F, Collins S, Gallo R: Growth and differentiation of human myelogenous leukemia cells in conditioned medium from human embryo cultured cells, in Bentvelzen P, Hilgers J, Yohn D (eds): *Advances in Comparative Leukemia Research*. Amsterdam. Elsevier/North Holland Biomedical, 1978, p 203

9. Gallo RC, Ruscetti FW, Gallagher RE: Human hematopoietic cells: A search for retrovirus-related information in primary tissues and development of liquid suspension culture systems for studies of cell growth and differentiation and of virus-cell interactions, in Clarkson B, Marks PA, Till J (eds): *Cold Spring Harbor Conferences on Cell Proliferation: Hematopoietic Mechanisms*. New York. Cold Spring Harbor, 1978

10. Collins SJ, Gallo RC, Gallagher RE: Continuous growth and differentiation of human myeloid leukemic cells in suspension culture. *Nature* 270:347-349, 1977

11. Collins SJ, Ruscetti FW, Gallagher RE, Gallo RC: Terminal differentiation of human promyelocytic leukemic cells induced by dimethyl sulfoxide and other polar compounds. *Proc Natl Acad Sci USA* 75:2458-2462, 1978

12. Koefler HP, Golde DW: Acute myelogenous leukemia: A human cell line responsive to colony-stimulating activity. *Science* 200:1153-1154, 1978

13. Nilsson K, Ponten J: Classification and biological nature of established human hematopoietic cell lines. *Int J Cancer* 15:321-341, 1975

14. Barak Y, Shore NA, Higgins GR, Vada-kan VV: Functional cellular maturation in cultures of human hematopoietic cells. *Br J Haematol* 27:543-549, 1974

15. Luzzio CB, Luzzio BB: Human chronic myelogenous leukemic cell-line with positive Philadelphia chromosome. *Blood* 45:321-334, 1975

16. Karpas A, Hayhoe FAJ, Greenberger JS, Barker CR, Cawley JC, Lowenthal RM, Moloney

WC: The establishment and cytological, cytochemical and immunological characterization of human haemic cell lines: Evidence for heterogeneity. *Leuk Res* 1:35-39, 1977

17. Trujillo JM, Ahearn MJ, Cork A: General implications of chromosomal alterations in human leukemia. *Human Pathol* 5:675-686, 1974

18. Yam LT, Li CY, Crosby WW: Cytochemical identification of monocytes and granulocytes. *Am J Pathol* 55:283, 1971

19. Williams WJ: in Williams WJ (ed): *Hematology*. New York, McGraw-Hill, 1972, p 1395

20. Kaplow LA: Simplified myeloperoxidase stain using benzidine dihydrochloride. *Blood* 26:215-219, 1965

21. Goldberg AF: Acid phosphatase activity in Auer rods. *Blood* 24:305-308, 1964

22. McManus JFA: Histological demonstration of mucin after periodic acid. *Nature* 158:202-203, 1946

23. Glick JL: *Human Lymphoid Cell Culture: The Fundamentals*. New York. Marcel Dekker. (in press)

24. Amos DB, Bashir H, Boyle W, MacQueen M, Tillikainen A: A simple microcytotoxicity test. *Transplantation* 7:220, 1969

25. Mittal KK, Mickey MR, Singal DP, Terasaki PI: Serotyping for homotransplantation. XVIII. Refinement of microdroplet lymphocyte cytotoxicity test. *Transplantation* 6:913, 1968

26. Anderson JK, Metzgar RS: Detection and partial characterization of human T and B lymphocyte membrane antigens with antisera to HSB and SB cell lines. *J Immunol* 120:262, 1978

27. Schiffman E, Corcoran B, Wehl S: N-formylmethionyl peptides as chemoattractants for leukocytes. *Proc Natl Acad Sci USA* 72:1059-1062, 1975

28. Perry RM Jr, Chondau RC, Shaham KM: A rapid sensitive assay of muramidase. *Proc Soc Exp Biol Med* 119:384-386, 1965

29. Reedman BM, Klein G: Cellular localization of an Epstein-Barr virus (EBV)-associated complement-fixing antigen in producer and non-producer lymphoblastoid cell lines. *Int J Cancer* 11:499-520, 1973

30. Maniatus T, Jeffery A, Kleid D: Nucleotide sequence of the righthand operator of phage λ . *Proc Natl Acad Sci USA* 72:1184-1188, 1975

31. Aulakh GS, Gallo RC: Presence of Rauscher leukemia virus related sequences in DNA from some tissues from some human hematopoietic neoplasias and their absence in DNA from other tissues. *Proc Natl Acad Sci USA* 74:353-357, 1977

32. Gallagher RE, Todaro GJ, Smith RG, Livingston DM, Gallo RC: Relationship between

fromo-
acute

human
roviral
leukemic
in our
potential
related
HL-60

minally
and in
on and
ed for
cultured
ions in
of the
station
usly in
ts that
fferen-
uld be
; so far
In this
normal
y seen
stic of
ible in
adying
myeloid

ript: Dr.
Drs. M.
ests for
ase; Dr.
id Miss
ysis.

orer A:
SJL/J
53-662,

ada M,
, Gazitt
rythro-
Differ-

reverse transcriptase from human acute leukemic blood cells and primate type-C viruses. *Proc Natl Acad Sci USA* **71**:1309-1313, 1974

33. Gallo RC, Gallagher R, Wong-Staal F, Aoki T, Markham P, Schettters H, Ruscetti F, Valerio M, Walling M, O'Keefe R, Saxinger W, Smith R, Gillespie D, Reitz M: Isolation and tissue distribution of type-C virus and viral components from a gibbon ape (*Hylobates lar*) with lymphatic leukemia. *Virology* **84**:359-373, 1978

34. Teich NM, Lowy DR, Hartley JW, Rowe WP: Studies of the mechanism of induction of infectious murine leukemia virus from AKR mouse embryo lines by 5-iododeoxyuridine and 5-bromodeoxyuridine. *Virology* **51**:1963, 1973

35. Kotler M, Weinberg E, Haspel O, Olshesky N, Becker Y: Particles released from arginine-deprived human leukemic cells. *Nature (New Biol)* **244**:197-199, 1973

36. Meyskens FL Jr, Gillespie DH, Gallo RC, Saxinger WC: Human leukemic leukocytes: Direct in vitro labelling in short term tissue culture of an intracytoplasmic RNA with properties of known RNA tumor viruses. *Proc Am Assoc Cancer Res* **18**:82, 1977

37. Beckman H, Neth R, Gaedicke G, Lundbeck G, Schoch G, Wilgers U, Winkler K: Cytology and cytochemistry of the leukemic cells, in Neth R, Gallo R, Spiegelman S, Stohlman F Jr (eds): *Modern Trends in Human Leukemia*. Munich, J. F. Lehmanns. 1974. p 26

38. Trujillo JM, Cork A, Hart JS, George SL, Freireich EJ: Clinical implications of aneuploid cytogenetic profiles in adult acute leukemia. *Cancer* **33**:824-834, 1974

39. Jondal M, Holm G, Wigzell H: Surface markers on human T and B lymphocytes. I. Large populations of lymphocytes forming nonimmune rosettes with sheep red blood cells. *J Exp Med* **136**:207-215, 1972

40. Basten A, Miller JF, Sprent J, Pye J: A receptor for antibody on B lymphocytes. I. Method of detection and functional significance. *J Exp Med* **135**:610-626, 1972

41. Kerbel RS, Davies AJS: The possible biological significance of Fc receptors on mammalian lymphocytes and tumor cells. *Cell* **3**:105-112, 1974

42. Bianco C, Patrick R, Nussenzweig VJ: A population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. I. Separation and characterization. *J Exp Med* **132**:702, 1970

43. Kagan WA, O'Neill GJ, Incefy GS, Goldstein G, Good RA: Induction of human granulocyte differentiation in vitro by ubiquitin and thrombopoietin. *Blood*, **50**:275-288, 1977

44. Coombs RR, Parker BW, Janeway CA, Wilson AB, Gell PGH, Kelus AS: Immunoglobulin determinants on the lymphocytes of normal rabbits. *Immunology* **18**:417-429, 1970

45. Winchester RE, Fu SM, Warner P: Recognition by pregnancy serums of non HLA-A alloantigens selectively expressed on B lymphocytes. *J Exp Med* **141**:924-929, 1975

46. Winchester RS, Ross GD, Jarowski CI, Halper J, Broxmeyer HE: Expression of Ia-like antigen molecules on human granulocytes during early phases of differentiation. *Proc Natl Acad Sci USA* **74**:4012-4016, 1977

47. Kaplan J, Inoue S, Ottenbreitz MJ: Myeloid forming cells express human B lymphocyte antigens. *Nature* **271**:458-459, 1978

48. Perillie PE, Finch SC: Lysozyme in leukemia. *Med Clin North Am* **57**:395-407, 1973

49. Krystosek A, Sachs L: Control of lysozyme induction in the differentiation of myeloid leukemic cells. *Cell* **9**:675-684, 1976

50. Sarin PS, Anderson PN, Gallo RC: Terminal deoxynucleotidyl transferase activities in human blood leukocytes and lymphoblast cell lines and in blast cells of some patients with chronic myelogenous leukemia in acute phase. *Blood* **47**:11-20, 1976

51. Sarin PS, Gallo RC: Terminal deoxynucleotidyltransferase in chronic myelogenous leukemia. *J Biol Chem* **249**:8051-8053, 1974

52. Marks SM, Baltimore D, McCaffrey R: Terminal transferase as a predictor of initial responsiveness to vincristine and prednisone in blastic chronic myelogenous leukemia. *N Engl J Med* **298**:812-814, 1978

53. Gallo RC, Gallagher RE, Miller NR, Mondal H, Saxinger WC, Mayer RJ, Smith RG, Gillespie DH: Relationships between components in primate RNA tumor viruses and in the cytoplasm of human leukemic cells: Implications to leukemogenesis. *Cold Spring Harbor Symp Quant Biol* **39**:399, 1975

54. Chandra P, Steel L, Laube H, Kornhuber B: Oncornaviral-like DNA polymerase activity in a case of childhood myelofibrotic syndrome. *FEBS Lett* **58**:71, 1975

55. Robert-Guroff M, Schrecker AW, Brinkman BJ, Gallo RC: DNA polymerase γ of human lymphoblasts. *Biochemistry* **16**:2866-2873, 1977

56. Sarangadharan MG, Guroff M, Gallo RC: DNA polymerases of normal and neoplastic mammalian cells. *Biochem Biophys Acta* **516**:419-487, 1978

57. Ernberg I, Andersson-Anreth M, Klein G,

Lundin I
amount of
antigen per
per cell. N

58. Ta
structural
ia. Blood

59. Va
Bodey GF
EJ: Clini
acute pro
1:37-50,

60. Gr
procoagul
elocytes
Haematol

61. Ro
Fukuhara
evidence
mality in
Cancer **2**

62. Ro
abnormal
Proc Natl

63. Mi
L: Non-r
myeloid l
nation of
18:31-38

64. KI
neoplasia

65. Ga
ponents c

- biquitin and
1977
aneway CA,
immunoglobu-
ins of normal
70
net P; Recog-
IL-A alloan-
lymphocytes.
- Jarowski CI,
on of Ia-like
ocytes during
e Natl Acad
- nbreitz MJ:
in B lympho-
1978
lysozyme in
35-407, 1973
of lysozyme
of myeloid
- o RC: Termi-
activities in
phoblast cell
patients with
acute phase.
- inal dcoxynu-
logenous leu-
1974
McCaffrey R:
tor of initial
prednisone in
nia. N Engl J
- Miller NR,
J, Smith RG,
in components
d in the cyto-
implications to
r Symp Quant
- H, Kornhuber
ase activity in
tic syndrome.
- r AW, Brink-
se > of human
6-2873, 1977
M. Gallo RC:
and neoplastic
Biophys Acta
- at M, Klein G,
Lundin L, Killander D: Relationship between
amount of Epstein-Barr virus-determined nuclear
antigen per cell and number of EBV-DNA copies
per cell. *Nature* 266:269-271, 1977
58. Tan HK, Wages B, Gralnick HR: Ultra-
structural studies in acute promyelocytic leukem-
ia. *Blood* 39:628-636, 1972
59. Valdivieso M, Rodriguez V, Drewinko B,
Bodey GP, Ahearn MJ, McCredie KB, Freireich
EJ: Clinical and morphological correlations in
acute promyelocytic leukemia. *Med Ped Oncol*
1:37-50, 1975
60. Gralnick HR, Abrell E: Studies of the
procoagulant and fibrinolytic activity of promy-
elocytes in acute promyelocytic leukemia. *Br J*
Haematol 24:89-99, 1973
61. Rowley JD, Golomb HM, Vardiinan J,
Fukuhara S, Dougherty C, Potter D: Further
evidence for a non-random chromosomal abnor-
mality in acute promyelocytic leukemia. *Int J*
Cancer 20:869-872, 1977
62. Rowley JD: Non-random chromosomal
abnormalities in hematological disorders of man.
Proc Natl Acad Sci USA 72:152-156, 1975
63. Mitelman F, Nilsson PG, Levan G, Brandt
L: Non-random chromosome changes in acute
myeloid leukemia. Chromosome banding exami-
nation of 30 cases at diagnosis. *Int J Cancer*
18:31-38, 1976
64. Klein G: The Epstein-Barr virus and
neoplasia. *N Engl J Med* 293:1353-1359, 1975
65. Gallagher RE: Molecular probes for com-
ponents of type-C viruses in human leukemia. in
Gallo RC (ed): *Recent Advances in Cancer
Research: Cell Biology, Molecular Biology and
Tumor Virology*. Cleveland, CRC, 1977. p 137
66. Gallo RC, Saxinger WC, Gallagher RE,
Gillespie DH, Ruscetti FW, Reitz MS, Aulakh
GS, Wong-Staal F: Some ideas on the origin of
leukemia in man and recent evidence for the
presence of acquired and endogenous type-C
viral-related sequences, in Watson JD, Winsten
JA (eds): *Cold Spring Harbor Conferences on
Cell Proliferation: Origins of Human Cancer*.
New York, Cold Spring Harbor, 1253, 1977
67. Gallo RC: Cellular and virological studies
directed to the pathogenesis of the human my-
elogenous leukemias, in Neth R, Mannweiler KM
(eds): *Modern Trends in Human Leukemia*. Part
III. (in press)
68. Gallo RC, Reitz MS, Saxinger WC,
Jacquemin P, Ruscetti FW: Studies in human
hematopoietic cells: Cellular and virological
aspects, in Conference "Antiviral Mechanisms for
the Control of Neoplasia." NATO Conference,
1978 (in press)
69. Markham PD, Ruscetti FW, Salahuddin
SZ, Gallagher RE, Gallo RC: Enhanced induc-
tion of growth of B lymphoblasts from fresh
human blood by primate type-C retroviruses
(Gibbon ape leukemia virus and simian sarcoma
virus). *Int J Cancer* 23:148-156, 1979
70. Azumi J, Sachs L: Chromosome mapping
of the genes that control differentiation and
malignancy in myeloid leukemic cells. *Proc Natl
Acad Sci USA* 74:253-257, 1977