An Undifferentiated Variant Derived From the Human Acute Myelogenous Leukemia Cell Line (KG-1)

By H. P. Koeffler, R. Billing, A. J. Lusis, R. Sparkes, and D. W. Golde

A variant subline (KG-1a) of the human acute myelogenous leukemia (AML) cell line (KG-1) has been isolated. The cells retain the same constitutive markers as the parent line, including HLA antigens, isoenzymes, and karyotype. The cells from the subline are morphologically and histochemically undifferentiated blast cells, while the parent cells and several of its clones are at the myeloblast and promyelocyte stages of development. The variant cells do not respond to colony-stimulating factor (CSF), and they do not express the human Ia antigen, nor a recently characterized AML antigen. The parent KG-1 cells are stimulated to proliferate in the presence of CSF and the cells express the Ia and AML antigen. Variant AML cell lines, such as KG-1a, will be useful in vitro models for investigating cellular response to CSF and for studying antigen expression in leukemic cells.

PERMANENT LINES of murine erythroid and myeloid cells have been useful for studying the regulation of neoplastic hematopoietic cell proliferation and differentiation. Investigation of the growth and maturation of human myelogenous leukemia has been slowed by the lack of adequate model systems, but recently, several human myelogenous leukemia cell lines have been developed. The first human myeloid leukemia cell line, known as K562, was established by Lozzio and Lozzio from the pleural fluid of a patient with chronic myelogenous leukemia in blast crisis. The K562 cells are morphologically and cytochemically undifferentiated blast cells that have the Philadelphia chromosome, form tumors when injected into nude mice, and do not respond to colony-stimulating factor (CSF) in vitro. Recent findings also indicate that the K562 cells can be induced to express erythroid characteristics. Collins and coworkers established a human promyelocytic leukemia cell line known as HL-60, which responds to CSF in soft-gel culture and differentiates into functional granulocytes in the presence of dimethyl sulfoxide (DMSO) and several other experiments. We developed a cell line, KG-1, from a patient with erythroleukemia evolving into acute myelogenous leukemia. The cells have been grown in liquid culture for over 2 yr, and they retain the myeloid characteristics and karyotype of the patient’s neoplastic cells. The KG-1 cells do not show granulocytic maturation in response to DMSO, but they mature to macrophages in the presence of phorbol derivatives. The unique characteristic of the KG-1 cells is their nearly complete dependence on CSF for colony formation in soft-gel culture. We have isolated a strain of this line (KG-1a) that is composed of undifferentiated blast cells that have lost responsiveness to CSF. We now report in detail the characteristics of this subline (KG-1a).

MATERIALS AND METHODS

Growth in Suspension Culture

The KG-1 and KG-1a cells (undifferentiated strain) were grown in T flasks (Lux, Newbury Park, Calif.) with alpha medium (Flow) containing 20% fetal calf serum, penicillin, and streptomycin. The cultures were incubated at 37°C in a humidified atmosphere of 5% CO2 in air and reseeded every 6–7 days in fresh medium at a concentration of 2 × 105 cells/ml. Several other cell lines used in this investigation were cultured similarly.

Three clones (KG-1 clones 2, 3, 4) were established from the KG-1 parent line by plating the KG-1 cells in 0.3% agar, isolating individual colonies, and reculturing single colonies in liquid culture, as described above.

Histochemical Staining and Electron Microscopy

Cyto centrifuge preparations of the parent KG-1 and variant KG-1a cell lines were made bimonthly, and the following cytochemical stains were done: Wright-Giemsa, naphthol ASD-chloroacetate esterase (Sigma, St. Louis, Mo.), Sudan black B, and peroxidase. Cells were tested for hemoglobin production by benzidine stain. For electron microscopy, the KG-1 cells were fixed for 30 min in 2% glutaraldehyde with 0.1 M sodium cacodylate buffer and 1% sucrose at pH 7.3, washed in buffer, postfixed in osmium tetroxide, and embedded in Epon. They were then sectioned, stained with uranyl acetate and lead citrate, and examined with a Hitachi HS9 electron microscope at 75 kV.

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Isoenzyme Analysis

The following isoenzymes were analyzed by starch gel electrophoresis: adenosine deaminase, phosphoglucomutase 1, phosphoglucomutase 3, acid phosphatase, esterase D, GOT, and glyoxalase 1 (Table 1). Briefly, the KG-1 and KG-1a cells were washed in buffered saline and lysates were prepared by adding 0.2 ml Triton X-100 solution to approximately 8 x 10^6 cells, followed by freeze-thawing. The lysate supernatant was absorbed onto Whatman #17 filter paper and placed in 12% Electrostarch gel made with a 1:10 solution to approximately 8 x 10^6 cells, followed by freeze-thawing. Horizontal electrophoresis at 6 V/cm was run for 18 hr, and the isoenzyme migrated toward the anode. The staining reactions were performed as previously described.25-27

Cell Surface Markers

The E and EA rosette assay utilized sheep erythrocytes.19,20 The presence of Fc receptors was also determined by fluorescent antibody studies. A fluorescein-conjugated rabbit anti-human IgG (Cappel Laboratories, Cochranville, Pa.) was heat-aggregated at 62°C for 20 min and then gently sonicated with a Biosonic 1V sonicator (Browning-WVR Scientific) for 2.5 min. The antibody (diluted with phosphate-buffered saline containing 0.02% sodium azide) was incubated with the cells for 15 min at 4°C, washed 3 times with phosphate-buffered saline (PBS), and the cells observed for membrane fluorescence. Three-hundred cells were counted. Approximately 90% of K562 cells fluoresced using this technique, as previously reported,20 while no T lymphocytes (Mo, human T-lymphocyte line)20 were positive. Surface membrane immunoglobulin was identified by immunofluorescence.21

The HLA antigens were analyzed using human monospecific antisera.23 The common acute lymphocytic leukemia (ALL) antigen was analyzed using a previously tested antiserum that reacts with non-B-ALL, non-T-ALL cells, but not with normal peripheral blood B and T lymphocytes, or normal bone marrow cells.24 The presence of a T-lymphocyte antigen was tested using a heteroantiserum that reacts with T-ALL cells, but not with B-ALL or null acute lymphoblastic leukemia cells.26 Expression of an AML antigen was tested using an antiserum produced by injecting KG-1 cells into a rabbit.26 The KG-1 antiserum reacted with leukemic cells from 10 of 46 patients with AML, and 2 of 23 ALL patients. The antiserum had no activity against normal B or T lymphocytes, granulocytes, or normal granulocyte-monocyte precursor cells (CFU-C). The Ia (la-like) antigen was analyzed using rabbit anti-human B serum.27 The antigen recognized by the B antiserum has many analogies to the murine I region antigen system (Ia). In this report, the antigen will simply be referred to as Ia antigen.

Quantitative Ia antiserum absorption studies were performed using 20 µl anti-B-cell serum at a dilution of 1:16, which was absorbed at 4°C for 2 hr with various numbers of KG-1 and KG-1a cells.22 The absorbing cells were removed by centrifugation, and 1 µl of the supernatant was tested against two different normal B-lymphocyte target cells.

Table 1. Biochemical Markers

<table>
<thead>
<tr>
<th>Isoenzyme</th>
<th>KG-1</th>
<th>KG-1a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine deaminase</td>
<td>1-1</td>
<td>1-1</td>
</tr>
<tr>
<td>Phosphoglucomutase 1</td>
<td>2-1</td>
<td>2-1</td>
</tr>
<tr>
<td>Phosphoglucomutase 3</td>
<td>2-2</td>
<td>2-2</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Esterase D</td>
<td>1-1</td>
<td>1-1</td>
</tr>
<tr>
<td>GOT</td>
<td>1-1</td>
<td>1-1</td>
</tr>
<tr>
<td>Glyoxalase 1</td>
<td>2-2</td>
<td>2-2</td>
</tr>
</tbody>
</table>

Cell surface antigen expression was tested using the eosin dye exclusion microcytotoxicity assay.23 Positive cytolyis was arbitrarily chosen to be ≥80% cell killing. The Ia antigen expression was also tested by indirect immunofluorescence.26 One million KG-1 or KG-1a cells were incubated for 30 min at room temperature with 50 µl of anti-B-cell serum at a dilution of 1:200 in phosphate-buffered saline with 0.02% sodium azide (PBS azide). After washing 3 times in PBS azide, the cells were incubated for 30 min with 50 µl fluorescein-conjugated goat anti-rabbit IgG (Meloy Laboratories, Springfield, Va.) at a dilution of 1:20. After washing 3 times to remove the excess fluorescein reagent, the cells were examined under u.v. light with a Leitz orthoplan microscope.

Partial Purification of CSF

The CSF used in these studies was produced by a human T-lymphoblast cell line (Mo).22 The Mo-conditioned medium (50 ml) was lyophilized, resuspended in 5 ml distilled water, and centrifuged at 15,000 g for 30 min. The supernatant was applied to an Ultragel ACA 44 column (2.5 x 55 cm) equilibrated with PBS buffer, and 7.5-ml fractions were collected at a rate of 20 ml/hr. The CSF was recovered in a fraction corresponding to a molecular weight of about 35,000, and peak fractions were pooled and used as the source of partially purified T-cell CSF. The specific activity of pooled fractions was about 1.6 x 10^5 U/mg protein, as compared with about 4.0 x 10^5 U/mg for crude conditioned medium. One unit of CSF activity is defined as the amount required to stimulate one normal human myeloid colony when 10^3 light density nonadherent bone marrow cells are plated in soft agar.

Cloning in Semisolid Medium

The agar culture methodology has been described in detail previously.4,23,24 Briefly, the leukemic cells were plated in 35-mm dishes containing alpha medium, 20% fetal calf serum, 10^-5 M alpha thiglycerol, and various concentrations of partially purified Mo CSF. Colonies (>50 cells) were enumerated after 14-day incubation using an inverted microscope.

Labeling Index

KG-1, KG-1a, and cells of other lines were suspended in alpha medium at a concentration of 2 x 10^7 cells/ml in 3 ml and incubated for varying periods of time at 37°C in a humidified atmosphere of 5% CO2 in air. At the end of this period, tritiated thymidine (3H-TdR; 55 Ci/mM; New England Nuclear, Boston, Mass.) was added to the cells to a final concentration of 100 µCi/ml. The incubation continued for 20 min, and the cells were washed 5 times with 25 vol of cold PBS. Cyto centrifugation preparations were then made and processed for radioautography. Labeled cells were defined as having 5 or more grains over their nucleus. Three-hundred cells were counted for each sample.

Protein Synthesis and Labeling

Cells were cultured at a density of 3 x 10^3 cells/ml in alpha medium containing 20% fetal calf serum in the presence of partially purified Mo CSF (150 U activity/ml). After 6 days, the cells were placed in methionine-free medium and incubated with 10 µCi/ml 35S-methionine (790 Ci/mM; New England Nuclear) for 16 hr. Cells were harvested, washed twice with PBS buffer, resuspended in 0.5% Triton X-100, 1.0 mM phenylmethylsulfonylfluoride (PMSF), and frozen. Radioactivity incorporated into protein was determined by precipitating aliquots of the cell lysate with 10% trichloroacetic acid, collecting the precipitate on glass fiber filters (Whatman, GF/A), and washing with 5.0% trichloroacetic acid and then absolute ethanol. The filters were extracted with 1 ml "NCS" tissue

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solubilizer (Amersham), and radioactivity was measured following addition of 10 ml Aquasol scintillation cocktail (New England Nuclear).

Sodium dodecylsulfate polyacrylamide gel electrophoresis was performed as described by Laemmli,10 except that the separation gel contained 15% acrylamide. Normally, about 500,000 cpm of radio-labeled protein was applied per well. Gels were stained with Coomassie brilliant blue, destained, dried, and subjected to autoradiography using Kodak XR-2 x-ray film. Molecular weight standards used were *Escherichia coli* β-galactosidase (subunit $M_r = 135,000$), bovine serum albumin ($M_r = 68,000$), ovalbumin ($M_r = 45,000$), carbonic anhydrase ($M_r = 32,000$), and cytochrome-c ($M_r = 12,000$). The tracking dye was bromphenol blue.

Two-dimensional polyacrylamide gel electrophoresis was carried out using the procedure of O’Farrell3 with the following modifications: the ampholine mixture used in the gel and lysis buffer contained 1% pH 3.5-10 and 1% pH 5-7 ampholines (LKB), and the second-dimension gel contained 15% acrylamide.

RESULTS

**Morphological and Biochemical Characterization**

The typical KG-1 cell is at the myeloblast or promyelocyte stage of maturation (Fig. 1). Approximately 20% myelocytes and more mature granulocytes are seen in early passages (1–66) (Fig. 1A). About 1%–3% eosinophil-appearing cells are prominent in these passages. These cells have large red cytoplasmic granules after Giemsa staining, and the nucleus is either bilobed or kidney-shaped and eccentrically positioned. The KG-1 cells stain heavily with ASD chloroacetate esterase, and 1%–2% of the cells stain with peroxidase and Sudan black B, although in later passages (later than passage 66), only rare cells were myeloperoxidase-positive. Three clones (2,3,4) isolated from the parent line have the same morphological and histochemical characteristics.

Ultrastructural studies of the KG-1 cells demonstrate numerous cytoplasmic granules, scattered rough endoplasmic reticulum, free ribosomes, and occasional mitochondria (Fig. 1B). Some of the large granules contain a central core with a linear periodic substructure. Cytoplasmic vacuoles are present in many of the cells. Peroxidase is found in 5% of the cells in the granules, but is present only in the endoplasmic reticulum and Golgi apparatus in many other cells. The nucleus frequently has peripheral margination of chromatin, and some of the cells have prominent nuclear clefts and blebs.

After the tenth passage, the KG-1 cells were grown in two laboratories in our department under the same...
culture conditions as the parent line. After passage 35, the cells in one laboratory showed important morphological differences from the parent line (Fig. 2A). The variant strain (called KG-1a) is composed of undifferentiated blasts. The cells have deep basophilic cytoplasm containing no granules, and the nucleus is round with loose perinuclear chromatin and 3 or more prominent nucleoli. The cells do not stain for ASD chloroacetate esterase, alpha-naphthyl butyrate esterase, or peroxidase. Ultrastructurally, the cytoplasm contains a large number of free ribosomes, some rough endoplasmic reticulum, and mitochondria (Fig. 2B). The Golgi apparatus is well developed, and nuclear clefts and blebs are present.

To rule out the possibility that the undifferentiated blast cells were a contaminant, a number of cell markers were compared between the parent line (KG-1) and the variant strain (KG-1a). The karyotype of both KG-1 and KG-1a are identical. There are 47 chromosomes, and Glemsa banding of 7 consecutive cells from the parent line and KG-1a show 47, XY, 7q−, 8p+, 12p+, −20, +MAR-1 (large submetacentric), +MAR-2 (small fragment). Also, the isoenzyme patterns are identical in KG-1 and KG-1a (Table 1). The cells from the variant or parent line could not be induced to produce hemoglobin as judged by benzidine staining after the cells were cultured 4–6 days in alpha medium containing DMSO (0.9%–
1.3%) or sodium butyrate (1.0–0.001 mM). Both of these agents are potent inducers of hemoglobin synthesis in the mouse Friend cells, and sodium butyrate stimulates fetal and embryonic synthesis in the human erythroleukemia cells, K562. Other common characteristics of both KG-1 and KG-1a cells are listed in Table 2.

Several differences between KG-1 and KG-1a were observed (Table 3). Three percent of the KG-1 cells were Fc receptor positive as determined by two methods, while less than 0.5% KG-1a expressed the Fc receptor. Three percent of the cells from the parent line were able to phagocytize opsonized Candida albicans, while less than 0.5% KG-1a cells were able to engulf yeast.

The KG-1 cell population and clones 2, 3, and 4 have the Ia-like antigen as demonstrated by the eosin-dye exclusion microcytotoxicity studies (Fig. 3). Ninety-five percent of the cells were killed at an antiserum titer of 1:32. The cytotoxicity was complement-dependent, and antiserum activity was removed by absorption with B, but not T, lymphocytes. Less than 2% of the KG-1a cell population was killed by the Ia antiserum at any titer tested. Absorption of the Ia antiserum (titer 1:16) with 2.0 and 4.0 × 10⁸ KG-1 cells decreased cytotoxicity against B lymphocytes by 90%. Absorption with KG-1a cells had no effect on Ia antiserum reactivity. These results were confirmed using indirect immunofluorescence. Seventy percent of the KG-1 cells were Ia-positive by immunofluorescence as compared with less than 3% of the KG-1a cells.

An acute myelogenous leukemia antiserum was developed that kills greater than 95% of the KG-1 cells at a dilution of 1:16 using the standard cytototoxicity assay. Cytotoxicity is complement dependent. The KG-1a cells did not react with the antiserum at any titer tested. The parent and variant KG cells did not react with either the common ALL, granulocyte, or T-lymphocyte antisera.

The proteins synthesized by the parent and variant lines were compared using one-dimensional (data not shown) and two-dimensional polyacrylamide gel electrophoresis (Fig. 4). Approximately 300 protein spots were detected by biosynthetic labeling of both KG-1 and KG-1a with 35S-methionine. A major protein band with an apparent molecular weight of 42,000 and an isoelectric point of about pH 5.7 was seen from both cells and probably corresponds to actin. Several clear differences in the proteins synthesized by KG-1 and KG-1a were reproducibly observed after two-dimensional electrophoretic separations (Fig. 4). There were 9 distinct qualitative differences between KG-1 and KG-1a detectable by two-dimensional electrophoresis, as well as numerous quantitative differences. These protein changes were seen in 4 separate experiments.

**CSF Responsiveness**

As previously reported, KG-1 cells are dependent on CSF for colony formation in soft-gel culture. Only rare KG-1 colonies were observed in the absence of added CSF. In the presence of optimal CSF concentrations, 140 ± 18 (mean ± SE) colonies were formed.

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**Table 3. Contrast KG-1 and KG-1a**

<table>
<thead>
<tr>
<th>Marker</th>
<th>KG-1</th>
<th>KG-1a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expression of Fc receptor*</td>
<td>3% ± 0.5%†</td>
<td>&lt;0.5% ± 0.025%</td>
</tr>
<tr>
<td>Phagocytosis of Opsonized Candida albicans</td>
<td>3% ± 0.5%</td>
<td>0%</td>
</tr>
<tr>
<td>Ia antigen</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>AML antigen</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CSF response</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*EA rosette formation and binding of aggregated IgG.†Mean ± SE of three separate experiments.

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**Table 2. Common Characteristics of KG-1 and KG-1a**

1. Growth in a single-cell suspension with a doubling time of 55–65 hr and a saturation density of 2.5–3.0 × 10⁶ cells/ml
2. No Epstein-Barr virus nuclear or capsid antigen
3. No surface membrane immunoglobulin, sheep erythrocyte rosette formation, or increased [3H]-thymidine incorporation with PHA exposure
4. No chemotaxis in Boyden chamber to zymosan-activated serum or AB serum
5. No measurable terminal deoxynucleotidyl transferase
6. HLA antigens A30, 33, B35 expressed
7. No ALL or T-lymphocyte antigens expressed
8. Identical karyotype
9. Identical cytoplasmic isoenzymes

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**Fig. 3.** Ia antiserum cytotoxicity data.
when $5 \times 10^3$ cells were plated, corresponding to a plating efficiency of 2.8% (Fig. 5). Similar colony-forming capacity was observed with KG-1 clones 2, 3, and 4.

The KG-1a population is unresponsive to CSF in soft-gel culture (Fig. 5). In the presence of optimal CSF concentrations (25 μl), $5 \pm 2$ (mean ± SE) colonies were formed when $5 \times 10^3$ cells were plated. Spontaneous cloning efficiency without added CSF was 0.05% when $10^4$ or fewer KG-1a cells were plated.

We investigated the effect of partially purified CSF on KG-1 and KG-1a cells in liquid culture using $^3$H-thymidine autoradiography (Fig. 6). The KG-1 population had a labeling index (LI) of 17% ± 2% (± SE) in liquid culture without CSF, and the LI increased to 35% ± 2.5% (± SE) after 24-hr exposure to CSF. The KG-1a had a LI slightly higher than the
KG-1 in the absence of CSF (20% ± 3%), but there was no change in the LI when the KG-1a cells were grown in the presence of CSF. Exposure of KG-1 or KG-1a with partially purified CSF does not discernibly change the morphology, cytochemical staining, nor the qualitative pattern of proteins synthesized by the cell lines as resolved by one- or two-dimensional electrophoresis (data not shown).

The effect of CSF on the 3H-thymidine LI of KG-1 and the other available human myeloid cell lines was compared (Table 4). Only the KG-1 cells showed a marked increase in LI in the presence of CSF. The HL-60 cells showed a slight, but not significant (p > 0.05), increase in labeling after CSF exposure. The cells from the K562 and ML-3 (composed of myeloperoxidase-positive myeloblasts; J. Minowada, personal communication) lines did not respond to CSF.

### Table 4. Labeling Indices (3H-Tdr) of Human Myeloid Leukemia Cell Lines

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>No CSF</th>
<th>CSF</th>
<th>Percent Augmentation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>KG-1</td>
<td>16 ± 2t</td>
<td>37 ± 3</td>
<td>131</td>
</tr>
<tr>
<td>KG-1a</td>
<td>20 ± 3</td>
<td>21 ± 2</td>
<td>5</td>
</tr>
<tr>
<td>HL-60</td>
<td>29 ± 3</td>
<td>35 ± 4</td>
<td>21</td>
</tr>
<tr>
<td>ML-3†</td>
<td>34 ± 2</td>
<td>36 ± 2</td>
<td>6</td>
</tr>
<tr>
<td>K562</td>
<td>46 ± 3</td>
<td>42 ± 3</td>
<td>—</td>
</tr>
<tr>
<td>B cell§</td>
<td>24 ± 2</td>
<td>26 ± 3</td>
<td>8</td>
</tr>
</tbody>
</table>

*CSF exposed (48 hr) — control/control x 100.
†Mean ± SE of three experiments.
§ML-3, AML cell line, gift of J. Minowada.

### DISCUSSION

In this report we have documented the establishment of a variant cell line (KG-1a) from the parent human AML cell line known as KG-1. The KG-1a cells are morphologically and functionally undifferentiated. The variant line was established between passages 15 and 35 of the original KG-1 cell line. Because the variant differed greatly from the parent line, we examined the possibility that it might be a contaminant. This possibility was ruled out on the basis of identity of karyotype, HLA antigens, and isoenzyme patterns. Also, it is unlikely that KG-1a is a lymphoblastoid cell that emerged from the original cell line because the line is not infected with EB virus; it has no SMIG; it does not form EA rosettes; and it does not express the null ALL antigen. It does not have T-cell characteristics.

The KG-1a cells do not express the Ia antigen, whereas the KG-1 cells retain this antigen. The Ia antigen has been considered a "differentiation marker," since it is expressed in certain stages of hematopoietic cell maturation. The Ia antigen represents the expression of the common region of the HLA-D gene complex located on the sixth chromosomes. To understand the differential expression of the Ia antigen, several studies were performed. Giemsa banding demonstrated that the sixth chromosomes of both KG-1 and KG-1a were present. Genes syntenic to the HLA-D gene locus were intact. The HLA-D gene locus is approximately 0.8 centimorgans (cM) from the HLA-B gene, and 1.6 cM from the HLA-A gene locus. Both KG-1 and KG-1a retain the patient's HLA-A and B antigens on the sixth chromosome. Likewise, the enzymes phosphoglucomutase 3 (PGM3) and glyoxalase each have two common alleles near the HLA-D locus. Starch gel electrophoresis of cell extracts of KG-1 and KG-1a demonstrated the same isoenzymes PGM3, 2-1 and glyoxalase 2-2.

The explanation for the failure of the KG-1a cells to express the Ia antigen is uncertain. A mutation at both HLA-D gene loci is unlikely because the closely linked...
HL-A and B and PGM1, and glyoxalase enzyme genes are intact, and because of the low statistical chance of two similar mutations occurring on both number six chromosomes (approximately $6 \times 10^{-13}$). The KG-1a cells are morphologically, cytologically, and functionally less mature than the KG-1 cells. Myeloblasts and early myeloid colony-forming cells have the Ia antigen. The expression of the Ia antigen on human pluripotent hematopoietic stem cells is not known, although the murine pluripotent antigen on human pluripotent hematopoietic stem cell is reported not to express the Ia antigen. It is possible that very immature human hematopoietic cells, like KG-1a and K562, do not express the Ia antigen.

Analysis of cellular proteins using one- (data not shown) and two-dimensional gel electrophoresis allowed the detection of over 300 proteins produced both by KG-1 and KG-1a. A protein with the molecular weight and isoelectric point of actin was quite prominent in both cell types. This is a major protein in macrophages, granulocytes, and murine myeloid leukemic cells and has importance in cell contraction and movement. There were extensive differences in the KG-1 and KG-1a cytoplasmic proteins. Most of these changes were quantitative increases and decreases in proteins, but, using two-dimensional electrophoresis, nine qualitative differences in proteins were resolved between the parent and the undifferentiated variant. Some of these proteins may represent gene products that are important markers of myeloid differentiation. The KG-1a cells showed little response to CSF. The cells formed few spontaneous colonies in agar, and in the presence of optimal concentrations of CSF, the cloning efficiency did not increase appreciably. The LI of KG-1a did not increase in liquid culture containing CSF. This contrasts sharply with KG-1 cells, which are dependent on CSF for colony formation in soft-gel culture and have a marked rise in LI upon exposure to CSF. While CSF stimulates proliferation of KG-1 cells, it does not induce cell maturation.

The mechanism by which KG-1a emerged from the parent line is not clear. It is possible that very immature cells were present in the original line and that these cells overgrew the more differentiated KG-1 cells. This is difficult, however, to reconcile with the fact that while KG-1 does become morphologically less mature in later passages, the cells continue to express the Ia antigen and to respond to CSF. It is also possible that clonal evolution occurred; a mutation or mutations that were undetectable could have occurred that gave the cell and its progeny a growth advantage over the general population of KG-1 cells.

The availability of immortalized erythroid and myeloid murine cells and their various clones have enhanced the knowledge of regulatory mechanisms involved in hematopoietic cell proliferation and differentiation. Human myelogenous leukemia cell lines and their variants should provide important in vitro models for investigating the membrane and cellular responses to CSF and antigen expression, and thus, provide a framework for understanding the control of differentiation in AML, and in a broader perspective, the control of normal cellular differentiation.

ACKNOWLEDGMENT

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