Human Myeloid Leukemia Cell Lines: A Review

By H. P. Koeffler and D. W. Golde

Several human acute myeloid leukemia cell lines were recently established. These lines provide useful model systems to study the control of differentiation in human myelogenous leukemia and, in a broader framework, the controls of normal myeloid development. The K562 line is composed of undifferentiated blast cells that are rich in glycophorin and may be induced to produce fetal and embryonic hemoglobin in the presence of hemin. The KG-1 cell line is composed predominantly of myeloblasts and promyelocytes. A unique characteristic of the KG-1 cells is their almost complete dependence on colony-stimulating factor for proliferation in soft-gel culture. The HL-60 is a promyelocytic leukemia cell line. In the presence of DMSO, the cells mature into granulocytes. Both the KG-1 and HL-60 cells differentiate into nondividing mononuclear phagocytes when exposed to phorbol esters. Investigations with these cell lines, and selected variants should provide important insights into the cell biology and perhaps therapy of human leukemia.

RESEARCH in human acute myelogenous leukemia (AML) has been impeded by the lack of adequate model systems to study. Myeloid leukemia in lower animals does not closely parallel the human disease, and studies on fresh human leukemic cells have been limited in part by the restricted survival of AML cells in vitro. Murine erythroleukemia2,3 and myelogenous leukemia4,5 cell lines have been established. These lines have been useful in studying gene expression and the modulation of hematopoietic cell proliferation and differentiation, but they have had more limited applicability in the investigation of the pathophysiology of human AML.

A number of cell lines have been established from patients with AML.4,5 These lines are usually composed of poorly differentiated lymphoblastoid appearing cells with Epstein-Barr (EB) virus-associated antigens and lymphocyte cell markers. The cell lines probably arose from EB-virus transformation of non-neoplastic lymphocytes present in the initial culture inoculum. Recently, several human myeloid cell lines have been established that will likely provide the necessary tools permitting a major increase in our knowledge of the regulation of cell growth and differentiation in AML.6–10

In 1975, Lozzio and Lozzio8 reported the development of the K562 line from the pleural fluid of a patient with chronic myeloid leukemia in blast crisis. Collins and coworkers9 established a human myelogenous cell line designated HL-60 from the peripheral blood of a woman with acute promyelocytic leukemia. Active cell growth began in liquid culture supplemented with conditioned medium from human embryonic lung cells. Later passages no longer required conditioned medium for cell growth. A third myeloid cell line, known as KG-1, was derived in our laboratory from the bone marrow of a man with erythroleukemia.10 After 24 days in liquid culture, the cells began active proliferation. Characteristics of the three myeloid cell lines are summarized in Table 1.

MORPHOLOGY AND HISTOCHEMISTRY

Representative Wright-Giemsa-stained preparations of the three cell lines are shown in Fig. 1(A, B, and C). The K562 cell is an undifferentiated blast cell with a diameter of about 20 μm (Fig. 1 A). The cell has a basophilic cytoplasm containing no granules and there are two or more prominent nucleoli. The K562 cells do not stain with cytochemical reagents normally positive in granulocytes and monocytes. Thus, there is no reaction with peroxidase, Sudan black B, or ASD-chloroacetate esterase stains. Many cells are strongly reactive for acid phosphatase.

Most of the HL-60 cells are at the promyelocytic stage of maturation and they contain prominent azurophilic granules (Fig. 1B). Myeloblasts, myelocytes, and more mature granulocytic forms are occasionally present. The HL-60 cells react strongly with cytochemical stains specific for granulocytic cells, including peroxidase, ASD-chloroacetate esterase, and Sudan black B.11 The cells do not stain for alkaline phosphatase and they appear not to contain lactoferrin.

The KG-1 line shows considerable pleomorphism with most of the cells at the myeloblast and promyelocyte stages, but 10%–20% of the cells are myelocytes or mature granulocytes (Fig. 1C). Occasional macrophages and eosinophils are also present. Clones derived from the parent line show a similar degree of pleomorphism. In later passages (greater than 1.5 yr
HUMAN MYELOID LEUKEMIA AND CELL LINES

Table 1. Properties of Human Myeloid Leukemia Cell Lines

<table>
<thead>
<tr>
<th></th>
<th>K562</th>
<th>HL-60</th>
<th>KG-1</th>
</tr>
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<tbody>
<tr>
<td>Source</td>
<td>CML in blast crisis</td>
<td>APL*</td>
<td>Erythroleukemia</td>
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<tr>
<td>Morphology and</td>
<td>Undifferentiated blast cell</td>
<td>Promyelocyte</td>
<td>Myeloblast-promyelocyte</td>
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<td>histochemistry</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean doubling time</td>
<td>12 hr</td>
<td>30-40 hr</td>
<td>40-50 hr</td>
</tr>
<tr>
<td>Chromosome number</td>
<td>Modal 70 (Ph1 chromosome)</td>
<td>Modal 45</td>
<td>47</td>
</tr>
<tr>
<td>Lymphocyte markers</td>
<td>No</td>
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<td>No</td>
</tr>
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<td>No</td>
<td>Yes</td>
</tr>
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<td>Induction of</td>
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<td>Granulocyte or macrophage</td>
<td>Macrophage</td>
</tr>
<tr>
<td>differentiation</td>
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*APL, acute promyelocytic leukemia.

in culture), almost all of the cells are morphologically at the myeloblast and promyelocyte stages. More than 90% of the cells stain strongly for ASD-chloroacetate esterase, but only 1%-2% are peroxidase-positive. The few macrophages present stain heavily for alpha naphthyl butyrate esterase.

**GROWTH KINETICS**

All three cell lines grow in suspension culture as single cells. The KG-1 and HL-60 cells have a doubling line consistent with AML cells in vivo (48-72 hr); K562 has a mean doubling time of 12 hr.

**KARYOTYPE**

A prominent feature of the three cell lines is the marked karyotypic abnormalities. Using banding techniques, no common karyotypic abnormality is seen in the three cell types. The K562 cells have nearly 1.5 times the normal number of chromosomes: 70 XXX, -13, -17, +7, +9 (p11), plus small metacentric chromosome, Xp-, 3p-, 9p-, t(15;18) (q21;q23), r(22), or 22q-. The cells have a small ring chromosome r(22) or retain the Philadelphia chromosome (22q-). Several cytogenetically different clones of HL-60 are present. The predominant karyotype is: 45x, -5, -8, -16, -x, plus an A group marker, plus an acrocentric D group marker, plus a submetacentric E group marker. The KG-1 cells have shown a consistent karyotype identical to the patient's leukemic cells: 47, xy, 7q-, 9p+, 12p+, -20, +MAR-1 (large metacentric), +MAR-2 (small fragment). The K562 cells form EA rosettes, indicating the presence of Fc receptors. Terminal deoxynucleotidyl transferase, an enzyme present in immature lymphocytes and rarely in AML cells, is not detectable in KG-1 or HL-60 cells.

**CELL SURFACE ANTIGENS**

The Ia-like or DR antigen is usually expressed on human myeloid leukemia cells. The KG-1 cells express the antigen. The antigen is not present on HL-60, which is consistent with prior studies showing that at the promyelocyte stage of development, the antigen is usually lost. The K562 cells also lack the Ia antigen. There is evidence that the K562 cells are very early erythroid precursors and that, perhaps, these cells have not matured to the stage of Ia antigen expression.

As is the case with most hematopoietic cells, the KG-1 and HL-60 cells express HLA-A and HLA-B antigens. The HL-60 cells are HLA-A1,8, B17; and the KG-1 cells express the rare antigens HLA-A30,31, B35. Neither HL-60 nor KG-1 cells express acute lymphocytic leukemia (ALL), granulocyte, or thymocyte antigens. The K562 cell population lacks HLA, ALL, and thymocyte antigens.

If a specific human acute myelogenous leukemia cell-associated antibody could be isolated, it would have great diagnostic and therapeutic potential. Rabbit antisera have been raised to K562 and KG-1 cells and a primate antiserum was produced by immunization of monkeys with K562. The KG-1 antiserum was produced by pretreating rabbits with antilymphocyte serum (ALS) and coating the immunizing KG-1 cells with antilymphocyte serum (ALS). After absorption with ALL cells, 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, CML, or CLL cells, or normal granulocyte-monocyte precursor cells (CFU-C). The K562 antiserum react with peripheral blood leukocytes from
Fig. 1. Morphological characteristics of cells from human AML lines. (A) K562—undifferentiated blasts. (B) HL-60—cells at promyelocyte stage. (C) KG-1—majority of cells are myeloblasts and promyelocytes.
patients with CML. These antisera react with occasional neoplastic lymphoid and normal myeloid cells and, therefore, a specificity for neoplastic myeloid antigens has not been conclusively demonstrated.

Recently, it has become possible to produce large amounts of monoclonal, monospecific antibody by hybridization of a myeloma cell line with hyperimmunized spleen cells. This technique has been applied to the development of antibodies to certain normal and neoplastic cellular antigens. Hybridization with spleen cells from mice immunized with the human myeloid lines may produce useful monoclonal antibodies.

HORMONAL MODULATION

The cloned AML cell lines provide a good model system to elucidate the interaction of hormones with human myeloid leukemia cells. Colony-stimulating factor (CSF) is probably an in vivo granulopoietin, and in vitro it stimulates the proliferation and maturation of early granulocyte-monocyte precursor cells (CFU-C). In semisolid culture, the CFU-C are stimulated by CSF to undergo a series of replicative and maturation steps to produce a colony of mature granulocytes or macrophages. Colony-stimulating factor stimulates colony formation by the HL-60 and KG-1 cells in vitro. A striking characteristic of the KG-1 cells is their nearly complete dependence on CSF for colony formation in soft-gel culture. In the absence of added CSF, only a rare KG-1 colony forms in soft-gel culture. There is a clear dose–response relationship between CSF concentrations and the number of colonies formed. At optimal CSF concentrations, the KG-1 cells have a cloning efficiency of 3%. The KG-1 cells also respond to CSF exposure in liquid culture with an increased thymidine labeling index LI and an increased rate of precursor incorporation into RNA and DNA. We have utilized the CSF-dependent stimulation of thymidine incorporation by KG-1 to develop a sensitive microassay for human CSFs; the assay is quantitative and requires only 1 day as compared with 10–14 days for conventional colony formation assays. CSF has no effect on KG-1 cell maturation. A stable subline of the KG-1 line spontaneously developed from the parent line and shows little response to CSF. These cells are morphologically and functionally undifferentiated blast cells. The cells retain a number of constitutive markers of the parent cells.

The HL-60 cells are capable of forming colonies of promyelocytes in the absence of any added factor with a plating efficiency of approximately 3.5%. The addition of CSF increased colony number approximately 2–3-fold. The K562 cells form colonies in soft-gel culture, but do not respond to CSF. The KG-1 and HL-60 lines and their variants provide a homogeneous cell population to study the mechanism of CSF action at the cellular level and investigations of a cellular CSF receptor will be possible.

The effect of other hormones on myeloid leukemia cell proliferation has not been extensively studied. KG-1 and HL-60 clonal proliferation in vitro is inhibited by prostaglandin of the E series, but not of the F group. KG-1 cell growth is inhibited by dibutyryl cyclic AMP and the extracellular agonists of this nucleotide, but not by dibutyl cyclic GMP and its extracellular stimulators. These findings parallel the response of normal human granulocyte-monocyte progenitor cells (CFU-C). Likewise, KG-1 clonal growth is slightly inhibited by pharmacologic concentrations of dexamethasone (10^{-7} M), while HL-60 cells are resistant to the glucocorticoid's effect. Both KG-1 and HL-60 have high affinity (Kd = 3–4 x 10^{-9} M) glucocorticoid receptors with a mean of 10,300 and 15,600 3H-dexamethasone binding sites per cell, respectively. These binding data are comparable to the glucocorticoid receptor activity seen in acute lymphocytic leukemia cells. In contrast to ALL cells, however, no correlation was observed between the number of glucocorticoid receptors and in vitro inhibition of myelogenous leukemic cell proliferation. Recently, it was shown that dexamethasone inhibits the expression of Fc receptors on HL-60 cells.

MATURATION AND CELL FUNCTION

Myeloid leukemia cells have a growth advantage over normal cells. The growth advantage is not due to
the rapid rate of proliferation of the leukemia blast cells, as these cells usually divide more slowly than normal cells. The leukemic cells accumulate because of their inability to mature to functional nondividing end cells. Since maturation and proliferation are linked events, leukemic cell growth could be greatly diminished if cellular maturation could be induced.

Many of the agents that are active in causing differentiation of the mouse erythroleukemia (Friend) and myeloid leukemia (MI) cells \(^{3,11}\) can induce granu-
locyte maturation of the HL-60 cells, including dimethyl sulfoxide, butyric acid, triethylene glycol, N,N-dimethylformamide, and N,N,N′-dimethylacetamide (Fig. 3A). The mature HL-60 cells respond to chemotaxins, phagocytose, develop complement receptors, produce superoxide, and reduce NBT dye. The HL-60 cells do not have alkaline phosphatase or lactoferrin, and do not appear to develop secondary cytoplasmic granules.

Both HL-60 and KG-1 differentiate into nondisruptive macrophage-appearing cells when exposed to phorbol esters (Fig. 3B). The cells from both lines become adherent and develop long pseudopodia, assume the morphological characteristics of macrophages, produce NADase, non-specific acid esterase and lysozyme, and develop Fc receptors and the ability to phagocytize Candida albicans and kill Straphylococcus aureus. Thus, these cell lines should be useful in studying the mechanism of early myeloid differentiation along the granulocytic and mononuclear patho-cytic pathway.

Recent evidence suggests that some K562 sublines may be a human erythroleukemia cell line with characteristics similar to the mouse Friend erythroleukemia cell line. The K562 cell membrane glycoproteins show many similarities to erythrocytes and, in particular, the cells synthesize glycophorin A, which is found exclusively in human erythrocytes. In the presence of sodium butyrate, some of the cells become benzidine positive, and hemoglobin can be detected in these cells using a radioimmunoassay. The K562 cells produce fetal and embryonic hemoglobin when grown with 0.1 mM hemin. The K562 cell appears to be an excellent tool for the study of human erythroid differentiation and globin gene expression. It seems clear, however, that there are a number of strains of K562 and these various strains may have different properties.

It is curious that human myeloid cell lines are so difficult to establish when contrasted to the ease with which lymphoid cell lines are developed. The development of B-lymphoblast lines is clearly related to EB-virus infection, but there also are various T- and null-lymphoid lines available that are not obviously virus infected. The difficulty in establishing myeloid lines as compared to permanent lymphoid cultures may relate to intrinsic cellular factors or requirements for as yet unidentified growth factors. Possibly, the use of somatic hybridization or DNA transformation using existing myeloid cell lines will permit establishment of new myeloid lines with interesting and useful features.

The human myelogenous leukemia cell lines may provide the framework for studies leading to a deeper understanding of the control of differentiation in human myeloid leukemia and, in a broader perspective, the control of normal cellular differentiation. Future studies with these cell lines and selected variants should shed new light on the cell biology of human myeloid leukemia and perhaps lead to new therapeutic modalities. The cells will be useful in the development of monoclonal antibodies reactive with myeloid leukemia cells and normal myeloid cells of different stages of maturation. The lines and their variants will help elucidate the interaction of CSF with myeloid cells and provide a convenient tool for pharmacologic and chemotherapeutic investigation. The cells should also be of use in studies of the control of granulocytic and macrophage differentiation. Finally, it is possible that modern molecular biologic techniques may permit the isolation from these cell lines of genes controlling myeloid differentiation.

REFERENCES

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