The Exceptional Responsiveness of Certain Human Myeloid Leukemia Cells to Colony-Stimulating Activity


We have studied the marrow cells from a patient with acute myeloid leukemia (AML) for their responsiveness to colony-stimulating activity (CSA) in vitro. The AML cells were stimulated by CSA to rapid and extended growth in liquid culture. In the absence of CSA, the majority of cells died. CSA also stimulated the clonal growth of AML cells, and the minimum requirement for CSA was one-tenth to one-fiftieth that required to stimulate the growth of normal marrow CFU-C. CSA for AML cells was eluted from Sephacryl S-200 columns in fractions that represented an apparent molecular weight of 45,000 daltons. This fraction also produced optimal stimulation of normal human marrow. During remission, the patient’s marrow cells did not grow in liquid culture and produced normal numbers of granulocytic and erythroid colonies in response to CSA and erythropoietin. Extended culture of the AML cells resulted in cell differentiation evidenced by decreasing proliferative capacity and by morphological and histochemical changes. These studies indicate that certain AML cells are extraordinarily responsive to CSA, an in vitro mediator of normal granulopoiesis.

THE GROWTH and differentiation of human granulocyte-monocyte progenitor cells (CFU-C) in viscous culture requires colony-stimulating activity (CSA), a mediator elaborated by monocytes and macrophages. The role of CSA in acute myelogenous leukemia cell function is less clear. Although CSA may facilitate the clonal growth of some AML cell populations, proliferation and differentiation are usually reduced. The addition of conditioned medium (CM) containing CSA to liquid cultures of AML cells has produced, at most, brief increases in tritiated thymidine (3HTdr) incorporation and cell recoveries. In those unusual instances where long-term culture of AML cells has occurred, exogenous CSA has not been used.

In this article, we describe a case of AML in which leukemic cells were extraordinarily responsive to CSA.

MATERIALS AND METHODS

Clinical Data

Leukemia cells were cultured from the marrow of G.M., a 31-yr-old white male who presented with acute myelomonocytic leukemia in April 1978. At diagnosis, the total white cell count was 22,800/µl, of which 63% were blasts. The marrow was extensively infiltrated with leukemic cells. Marrow was...
obtained for cell culture prior to initial therapy with daunomycin/cytosine arabinoside, during a complete remission in May 1978, and after relapses in August and November 1978. The patient died in December 1978. The cells are referred to by the patient’s initials. GM-1 cells were obtained prior to initial treatment; GM-C, during the first remission; GM-2, prior to treatment of the first relapse; and GM-3, prior to treatment for the second relapse. Cytogenetic analyses of GM-R and GM-2 cells revealed diploid karyotypes.

Maintenance of GM Cells

After heparinized marrow cell suspensions were sedimented at room temperature for 2 hr, the buffy coats were removed and resuspended in McCoy’s 5A medium at a concentration of 2 x 10^7/ml. Aliquots of cell suspensions were added to 250 sq cm plastic tissue culture flasks containing either McCoy’s medium with 20% fetal bovine serum (standard medium) or to flasks containing standard medium plus 10% by volume of GCT cell line-conditioned medium (GCT-CM). Conditioned medium from GCT (giant cell tumor) cells, a human monocyte-like cell line, is a potent source of CSA for normal human marrow. The final cell concentration in cultures was 2 x 10^6/ml. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO2 in air. Medium exchanges were made at weekly intervals.

Properties of GM Cells

Growth in liquid culture. Cells at 5 x 10^5 to 5 x 10^6/ml were cultured in replicate 75 sq cm flasks in standard medium with 0%-10% GCT-CM. At 48-hr intervals, cells were enumerated with a model ZBI Coulter counter and multiplied by the proportion of cells excluding trypan blue to determine viable cell concentration. Experiments were terminated at 2 wk. The number of population doublings over a 48-hr period of exponential growth was calculated. In some studies, CSA semipurified from GCT-CM was substituted for GCT-CM.

Colonial growth. In the leukemia cell and CFU-C assay, cells at 5 x 10^3 to 5 x 10^5/ml were cultured in 35 x 12 mm tissue culture dishes. Each 1-ml culture contained 0.70-0.80 ml standard medium, 0.2 ml fetal bovine serum, 0-0.1 ml GCT-CM or semipurified CSA, and 16 mg methylcellulose or 3 mg agar. After 14 days of culture in a humidified atmosphere of 5% CO2 in air, colony number was determined with an inverted microscope. All clusters of greater than 40 cells were counted as colonies. Cloning efficiency was expressed as the ratio of colonies to cells cultured.

In the erythroid colony assay, cells were cultured in 0.8% methocellulose in alpha medium, 30% fetal bovine serum, 10% deionized bovine serum albumin, 0.1 mM monothioglycerol, and 2 U sheep erythropoietin (Step III of Connaught). CFU-E and BFU-E colonies were quantified at 7 and 14 days of culture, respectively.

Elaboration of colony-stimulating or -inhibiting activity. Conditioned medium was clarified by filtration through a 0.2-μm pore diameter membrane. Part of each sample was concentrated fivefold by ultrafiltration through an Amicon PM10 membrane. Samples were assayed for CSA in viscous cultures of normal human marrow not containing GCT-CM and for inhibitory activity in cultures maximally stimulated with GCT-CM. Marrows were collected, processed, and cultured as described previously.

Other properties. Morphology, histochemistry, karyotype, surface receptors, and phagocytosis were studied as previously described. Lysozyme quantification in culture supernates was performed utilizing Micrococcus lysodeikticus turbidometric assay.

Preparation of GCT Cell-Line-Conditioned Medium and Purification of CSA

Serum-free McCoy’s medium, containing 0.01% polyethylene glycol 6000, was conditioned by GCT cells. CSA was purified approximately 300-fold from this conditioned medium by sequential dialysis, calcium phosphate gel absorption and elution, ultrafiltration against an Amicon PM10 membrane, and S-200 Sephacryl chromatography, as previously described. Aliquots of elution fractions were tested for their ability to stimulate the proliferation of GM cells in liquid suspension and to promote the colony growth of GM cells and normal human marrow.

RESULTS

Morphology of GM Cells

Figure 1 shows the appearance of GM-1 cells by light microscopy prior to and following 6 wk in culture. Blast cells decreased, and many cells developed a heavy
Fig. 1. Morphology of GM-1 cells. (A) Prior to culture. (B) After 6 wk of exposure to GCT-conditioned medium in culture. Blast cells have decreased. Arrows depict cells with a heavy concentration of peroxidase-positive granules in the Golgi zone. Other cells had scattered granules. (x 4000)

Fig. 2. Effect of GCT-conditioned medium (CM) and semipurified GCT-CSA on the growth and viability of GM-1 cells. Two weeks after primary culture, equal numbers of GM-1 cells were transferred from flasks containing CM to: cultures without CM or CSA (closed circles); with 10% CM (open circles); or with 10% semipurified GCT-CSA (open squares). Viable cell concentrations were determined over a 12-day period of culture.
concentration of peroxidase-positive granules in the Golgi zone during this interval (Fig 1B). Blast cells contained only scattered peroxidase-positive granules prior to culture.

The Effect of GCT-Conditioned Medium and CSA on the Viability and Growth of GM Cells in Liquid Medium

GM-1 cells were maintained in standard medium with and without GCT-CM. After 2 wk, cultures with CM, the only ones in which growth occurred, were studied further. Cells were washed in medium without CM and thereafter studied for their responsiveness to CM and semipurified CSA. As shown in Fig. 2, no growth occurred in the absence of CM. Further, the number of viable cells/ml decreased from 25,000 to 2000 by the end of 12 days. In the presence of 10% CM or CSA, after a 48–96-hr lag, the cells proliferated rapidly until a cell concentration of 1.2 million/ml was attained. Thereafter, growth slowed.

GM-1 cells did not grow in the absence of CM in subcultures initiated up to 6 wk following primary culture, whereas CM continued to stimulate growth (Table 1). During this period, the response to CM declined, however, as judged by either maximum growth rate or cell concentration at plateau phase. Growth could not be stimulated when cells had been in culture for 10 wk.

Cells studied in relapses (GM-2, GM-3) also responded to CM initially, but the intervals that GM-2 and GM-3 subcultures could be stimulated to grow were only one-half and one-quarter those of GM-1 cells (Table 1). Early subcultures of GM-2 cells, in contrast to GM-1 and GM-3, grew for up to 3 days in the absence of CM, although, thereafter, growth ceased and most cells died. Marrow cells studied during a remission (GM-R) did not grow either in the presence or absence of CM.

Table 1. Effect of GCT-Conditioned Medium (CM) on the Growth of GM Cells in Extended Culture

<table>
<thead>
<tr>
<th>Cell Population</th>
<th>Culture Time (wk)</th>
<th>Doubling Time (hr)</th>
<th>Colony Number/2.5 x 10⁶ Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No CM</td>
<td>CM*</td>
<td>No CM Cellulose</td>
</tr>
<tr>
<td>Initial (GM-1)</td>
<td>0–2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>2–4</td>
<td>–</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>4–6</td>
<td>–</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>6–8</td>
<td>–</td>
<td>100</td>
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<tr>
<td></td>
<td>10–12</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>&gt;12</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Remission (GM-R)</td>
<td>0–2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>First relapse (GM-2)</td>
<td>0–2</td>
<td>39</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>2–4</td>
<td>80</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>4–6</td>
<td>160</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>&gt;6</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Second relapse (GM-3)</td>
<td>0–2</td>
<td>–</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>&gt;2</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

No growth; + growth—not quantified; ++ definite increase in cell number without colony formation.

*Maximum stimulatory concentration—usually 5%–10% v/v.

†Normal colonies.
The Effect of GCT-Conditioned Medium on the Clonal Growth of GM Cells

The ability of GCT-CM to stimulate GM cell growth in medium made viscous by the addition of agar or methylcellulose was compared to that in liquid suspension (Table 1). Quantification of growth was sometimes difficult because of the propensity of GM leukemia cells to disperse rather than form distinct colonies. Further, most colonies were smaller than those seen in normal marrow, comprising 40–60 cells.

Growth never occurred in agar-gel without the addition of conditioned medium. In the presence of CM, colony growth was usually observed. The highest colony number per 25,000 cells cultured was 7500, attained by GM-1 at 2–4 wk of culture. As in liquid cultures, the ability of GM-1, GM-2, and GM-3 cells to proliferate in response to CM diminished with extended passage, and the point where cells no longer formed colonies occurred earlier with GM-3 than it did for GM-2 and GM-1 cells. Qualitatively similar results were obtained in methylcellulose, the only exception being that GM-2 cells showed spontaneous growth in this matrix. Even in this instance, growth was greater in cultures to which CM had been added. The morphology of colonial cells in agar and methylcellulose showed predominantly immature myelomonocytic cells. GM-R cells showed qualitatively and quantitatively normal granulocytic colony formation in response to conditioned medium—25 colonies/2.5 x 10⁴ cells. Erythroid colony growth in response to erythropoietin was also normal—15 CFU-E and 7 BFU-E colonies/2.5 x 10⁴ cells.

Dose–Response of GCT-Conditioned Medium on GM Cell Growth

The relationship of GM leukemia cell growth in liquid culture to GCT-CM concentration is shown in Fig. 3A. The growth rate in cultures without CM averaged only 6% of that observed with optimal CM. GM cells were highly
sensitive to GCT-CM. As little as 0.25% CM stimulated growth rate to 38% of maximum; 0.4% CM to 50% of maximum; and 0.5% CM to 66% of maximum. A less steep increase occurred with further increment in CM. Maximum growth rate required about 10% CM.

Figure 3B shows the results when colony growth was studied. GM cells were even more sensitive to CM in viscous culture than in liquid medium—0.2% CM stimulating 50% of maximum colony number and maximum growth usually requiring only 5% CM.

Effects of Other Sources of CSA on GM Cell Growth

Medium conditioned by mixed human leukocytes, phytohemagglutinin-stimulated mononuclear cells, and monocytes also stimulated the growth of GM cells in liquid and semisolid medium. Growth was less than that observed with GCT-CM, however.

Effects of GCT-Conditioned Medium on GM Cell Maturation

The reduction in growth rate that occurred in extended culture was accompanied by cell maturation. For example, at the start of GM-1 cultures, 52% of white cells were blasts, 20% were intermediate monocytes and granulocytes, 10% were mature neutrophils, and 18% were lymphocytes. After 4 wk, virtually all cells were blasts. By 8 wk, a new wave of maturation had occurred. Blasts had decreased to 40%, intermediate forms had increased to 18%, and mature forms accounted for 42%. By 12 wk, blasts had decreased to 20%, and the remainder of cells were mature. The mature forms comprised macrophage-like cells and smaller cells with faint specific granules and segmented but otherwise monocyte-like nuclei. Detailed histochemical and functional analysis of GM cells was not undertaken prior to culture. However, the mixed monocyte-granulocyte character of the cultured cells was supported by the observation that 70% of cells had both fluoride-inhibitable NASDA esterase and naphthol-chloracetate esterase histochemical reactions during the second wave of maturation. At this time, about 60% of cells had functional Fc receptors; 30% ingested E. coli, B. subtilis, Candida albicans, or antibody-coated red cells; and 15% of cells had leukocyte alkaline phosphatase activity. Culture supernates were strongly positive for lysozyme.

The morphology of cells maintained in medium without GCT-CM (i.e., the small proportion of surviving cells) was compared to the morphology of cells in medium with CM at 4, 8, and 12 wk of culture. The proportions of blasts, intermediate stages, and mature stages were similar. Thus, those GM-1 cells that did not require GCT-CM for survival also did not require CM for maturation.

Maturation occurred sooner in GM-2 and GM-3 cultures, accounting in part for the shorter lifespan of these populations.

Effects of GM-Conditioned Medium on the Colony Growth of Normal Marrow

The ability of conditioned media from GM cells to stimulate normal human marrow colony growth or to inhibit colony growth stimulated by GCT-CM was studied. Seven-day harvests from GM cultures without GCT-CM were used for these experiments, the results of which are shown in Fig. 4. Growth in the absence of conditioned medium (10% control medium) was nil. Eight of nine GM-
conditioned mediums tested failed to stimulate colony growth, the only exception being the first GM-3 harvest. Overall, the addition of 10% GM-CM to cultures stimulated an average of only 4 colonies. In contrast, 10% GCT-CM stimulated about 40 colonies/10⁵ marrow cells. Addition of 10% GM-CM to GCT-CM-stimulated cultures did not decrease colony growth. Similar findings were obtained when GM-CM were concentrated fivefold and studied in concentrations up to 10% of culture volume. Thus, most GM cells elaborated little or no CSA, and none elaborated detectable inhibitors for human marrow in culture.

Identity of CSA for GM Leukemic Cells With That for Normal Human Marrow Cells

Fractions of GCT-CM were eluted from Sephacryl S-200 and studied for their ability to stimulate the colony growth of GM cells and normal marrow in agar-gel.
CSA AND MYELOID LEUKEMIA CELL GROWTH

As shown in Fig. 5, CSA for GM cells cochromatographed with that for normal marrow. CSA eluting after bovine albumin (mol wt, 68,000 daltons) in a position corresponding to an apparent molecular weight of 45,000 daltons. GCT-CM also contains two CSAs for mouse marrow, the lower molecular weight CSA eluting in the same position as the CSA for human marrow. Inactivation of mouse activity with anti-human urinary CSF immunoglobulin did not reduce activity for GM cells or human marrow (data not shown).

DISCUSSION

GM cells resembled normal granulocyte-monocyte progenitors in that they required colony-stimulating activity for sustained growth and differentiation in viscous culture. They differed from normal cells in their extraordinary ability to proliferate in liquid culture, production of abortive colonies in semisolid medium and in their delayed and incomplete differentiation. These abnormalities and the normal growth during remission support the presumption that the cells were leukemic, since the absence of marker chromosomes precluded cytogenetic confirmation.

Leukocyte-conditioned medium (LCM), a source of CSA, may stimulate a transient increase of normal marrow CFU-C in liquid culture. LCM also stimulates tritiated thymidine incorporation in some AML cultures. The responsiveness of GM leukemia cells to CSA in liquid culture is unprecedented. Indeed, the cells from four other AML patients we have studied have not shown similar responsivity to CSA. In other instances, where extended culture of AML cells has occurred, AML cells have not required exogenous CSA for growth in liquid culture or have responded to factors other than CSA, such as embryo-conditioned medium for initial propagation. Presumably, the variation in CSA-responsiveness shown by AML cells reflects, in part, the degree of block in cell differentiation induced by the leukemic lesion. The defect in AML is believed to reside in a primitive hematopoietic precursor cell pool, a level of cell differentiation in which proliferation is stimulated by factors other than CSA. The lack of these factors in vitro may account for the eventual demise of virtually all marrow cell cultures, including those of GM. CSA responsivity develops following commitment of marrow cells to granulocyte-monocyte differentiation when receptors for CSA become functional. The marked responsivity of GM cells to CSA indicates that most of the cells had attained this level of differentiation.

In contrast to the atypical growth of GM leukemia cells in liquid culture, their growth in viscous culture was similar to most AML populations in that colony size and cell differentiation were subnormal. GM, like most AML patients, showed normal colony growth in remission, reflecting the reemergence of normal, or at least less leukemic, hemopoiesis.

GM leukemia cells formed colonies with less GCT medium than that required to stimulate normal human marrow. A similar finding has been reported in AML culture by others.

GM-1 and GM-2 cells did not elaborate detectable CSA for normal human marrow cells. AML cells with monocytic features usually elaborate CSA, whereas those with granulocytic features do not. GM cells were myelomonocytic, and the failure to find CSA in most conditioned media was surprising. GM-1 and GM-2 may have utilized all the CSA for human marrow they produced. This would also
help to explain the survival and maturation of some GM-1 and GM-2 cells in the absence of exogenous CSA. The finding of weak CSA in medium conditioned by GM-3 cells is consistent with this interpretation. The masking of CSA by an inhibitor in CM appears less likely since GM-CM did not inhibit normal CFU-C growth maximally stimulated with exogenous CSA.

GM leukemia cells underwent maturation in liquid culture. Maturation occurred earlier when cells from later stages of the patient’s disease were studied, although the morphology, histochemistry, and percentage of blasts in cultured samples were similar. Thus, culture was detecting changes in leukemia cell populations not apparent on routine studies. Other studies have shown that some AML cell populations retain the capacity for differentiation in viscous culture, since the same cytogenetic markers present in leukemic blasts were found in colonies containing maturing cells.30 Certain permanent AML cultures of murine and human origin can also be induced to undergo various degrees of differentiation in culture by CSA31 33 or by certain chemicals, such as dimethylosulfoxide (DMSO).31 32

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The exceptional responsiveness of certain human myeloid leukemia cells to colony-stimulating activity

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