Autostimulation of Growth by Human Myelogenous Leukemia Cells (HL-60)

By James K. Brennan, Camille N. Abboud, John F. DiPersio, Grant H. Barlow, and Marshall A. Lichtman

We have studied the effects of medium conditioned by the human progranulocytic leukemia cell line, HL-60, on the subsequent growth of new inocula of HL-60 cells. When HL-60 cells were cultured at high cell density, optimal growth rate occurred in liquid suspension and confluent colony growth was observed in viscous medium without the addition of conditioned medium. However, when cells were cultured at lower cell density, growth rate was reduced and colony growth was nil unless conditioned medium from HL-60 culture was added. All HL-60 populations studied, including the earliest available passage, 9, both elaborated and responded to HL-60 CM. HL-60 CM did not stimulate normal human or mouse granulocyte-monocyte colony-forming cell (CFU-GM) growth. Conditioned media from other human cell lines varied in the ability to stimulate HL-60 cell and CFU-GM proliferation. Some, such as GCT CM, stimulated both HL-60 cells and normal CFU-GM, whereas others, like HL-60 CM, stimulated only HL-60 growth. The majority of cell line CMs tested did not stimulate either HL-60 or CFU-GM. Chromatography of HL-60 CM on Ultrogel AcA54 showed a single peak of HL-60 stimulating activity of apparent molecular weight 13,000. The ability of HL-60 cells to elaborate this activity provides a possible explanation for their proliferation at higher cell densities. Autostimulation may prove to be important in the high growth potential of other cell populations that undergo unrepressed proliferation.

THE CLONAL GROWTH and maturation of normal granulocyte-monocyte precursor cells in vitro requires colony-stimulating activity.1 3 Colony-stimulating activity (CSA) for human progenitors, elaborated by monocytes and other cells, appears to chiefly comprise proteins of about 30,000 daltons.4 12 Some acute myelogenous leukemia (AML) populations retain dependency on CSA,13 18 although growth and maturation in response to CSA are usually abnormal.16 18 Other AML cells do not appear to require this growth mediator or to require it only under certain conditions.19 22 An example of such a population is HL-60, a permanent cell culture derived by Collins, Gallo, and Gallagher from the blood leukocytes of a patient with acute progranulocytic leukemia.20 Although these cells have the features of primitive granulocytes23 24 and can be induced to undergo terminal maturation by certain chemical agents,25 31 they do not require exogenous CSA for growth at high population densities.21 Since HL-60 cells apparently do not elaborate detectable CSA,21 another explanation was sought for this "spontaneous" growth.

MATERIALS AND METHODS

Cultivation of HL-60 Cells

Several populations of HL-60 cells were used in these studies. The passage number of the population designated HL-60 UR was unknown. Populations designated HL-60 GP0, 23, 40, and 76 were obtained directly from Dr. Robert C. Gallo in July 1980 and were derived from frozen stocks of passages 9, 23, 40, and 76. All populations were maintained in McCoy’s 5A medium without 20% fetal bovine serum, 2 mM Hepes buffer, 100 U/ml penicillin, and 50 

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standard medium with trypsin incubated before addition of soybean trypsin inhibitor; (3) standard medium with trypsin and soybean trypsin inhibitor followed by incubation.

Periodate treatment. Oxidation of conditioned medium and standard medium was accomplished by exposing them to 50 mM sodium metaperiodate for 14 min at 4°C and then stopping the reaction by the addition of 200 µl of 50% sucrose/2 ml sample. The samples were then dialyzed against phosphate-buffered saline, pH 7.4 (PBS).

Reduction and alkylation of conditioned medium. Conditioned medium and standard medium were treated with 5 mM diithothreitol (DTT) for 2 hr at 23°C, 15 mM N-ethylmaleimide (NEM) for 2 hr at 23°C, or with DTT followed by NEM. Samples were dialyzed against distilled water.

Dialysis. Conditioned medium was dialyzed at 4°C against 50 volumes of PBS utilizing membranes with nominal exclusions limits of ~13,000 daltons and ~8,000 daltons.

Elution of conditioned medium from Ultragel AAc54. Conditioned medium was concentrated fivefold by ultrafiltration against an Amicon UM-05 membrane. The retentate was applied to a 2.5 cm by 10 cm Ultrogel AAc4S column equilibrated in phosphate-buffered saline, pH 7.4 (0.025 M PO4, 0.1 M NaCl). The column had been previously calibrated utilizing ovalbumin, molecular weight 43,000 daltons; semipurified colony stimulating activity derived from a human cell line, GCT, molecular weight 30,000 daltons; and ribonuclease, molecular weight 13,700 daltons. Following treatments or chromatographic separations of conditioned medium, samples were filtered-sterilized and stored at 4°C until used.

Preparation of other conditioned media. The derivation and type of cell lines used to generate other CMs are shown in Table 3. GCT; GCT-V1, a spontaneously arising variant of GCT that grows in suspension rather than attached to surfaces; RC-4; GM-1; PIR; and RPMI-1788 CMs were prepared in our laboratory by culturing cells in McCoy's medium with 10% fetal bovine serum as previously described.18,44 Information on the derivation of these cells can also be found in these references. KB and WI-38 CMs were prepared by the University of Rochester Microbiology Department by culturing cells in Eagle's MEM with 10% FBS. Information concerning the derivation of these cells was obtained from Flow Laboratories, McLean, Va. who provided the cells. Dr. Jun Minowada of Roswell Park Memorial Institute prepared HL-60, ML-1, NALM-16, SU-DHL-1 U-937, K-562, Reh, MOLT-4F, and RPMI-8402 CMs by culturing cells in RPMI-1640 with 10% FBS.35,37 All CMs were obtained at the time of routine weekly exchanges/subculture. Concentration, dialysis, membrane-filtration, and storage of samples were done in our laboratory as described above. Semipurified GCT CSA (specific activity ~2 x 10^8 CFU-GM colonies/mg protein) was prepared from serum-free GCT CM as described previously.31

Growth Studies

HL-60 growth: Liquid culture. Cells at 10^4-10^5/ml were cultured in replicate 25 cm flasks containing McCoy's medium with 20% fetal bovine serum and 0%-20% HL-60 conditioned medium. Growth was evaluated over a 2-wk period, and the number of population doublings over a 48-hr period of exponential growth (doubling time) was calculated.

HL-60 growth: Viscous culture. Cells at 10^4-10^5/ml were cultured in 35 by 12 mm tissue culture dishes containing 0.60-0.80 ml standard medium, 0.2 fetal bovine serum, 0-0.2 ml HL-60 conditioned medium, or other cell-line CMs, and 16 mg (0.8%) methylcellulose. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO2 in air for 1 wk. Thereafter, colonies were counted utilizing an inverted microscope, and in some experiments removed, stained, and studied for cell maturation and cell number/colony. Aggregates of less than 8 cells were rare and were not counted. The average cell number per colony was 75 with a range of 8-200.

During later studies, it was necessary to introduce a new lot of fetal bovine serum into growth experiments. Following this change, spontaneous growth of HL-60 cells occurred at fivefold lower cell density. Thus, lower starting cell densities were required to demonstrate the conditioned media effects summarized in Tables 2 and 3 than those summarized in other tables and figures in this report.

Human marrow CFU-GM assay. The nonadherent fraction of normal human marrow cells at 10^7/ml were cultured in viscous medium containing 0%-20% HL-60 or other test conditioned medium; 0%-20% GCT-conditioned medium, a potent source of CSA for normal marrow;3 or varying admixture of HL-60 CM and GCT-CM. Normal volunteers gave informed consent for iliac crest marrow aspiration. Their participation was approved by the Committee on Investigations Involving Human Subjects. Colonies were counted at days 7 and 14 of culture and comprised at least 50 cells. Colonies (clusters) of less than 50 cells did not occur.

Human marrow erythroid colony assay. Morrow cells were cultured in viscous medium containing 0%-20% HL-60 conditioned medium and 2 U/ml erythropoietin (Step 3 of Connaught).38,39 CFU-E colonies were quantified at 7 days and BFU-E colonies at 14 days of culture.

Mouse marrow colony assays. Granulocyte-monocyte and erythroid colony assays were similar to their human counterparts except that 7.5 x 10^5 nonadherent BDF1 marrow cells were cultured and colony counts were done at 3 and 7 days.3,51

Human and murine lymphoblast assays. L5178Y murine lymphoblasts and PIR human lymphoblasts were cultured at 10^2-2 x 10^5/ml in viscous medium containing 0%-20% HL-60 CM or control medium for 7 days and counted.34

RESULTS

Effect of HL-60 Conditioned Medium on HL-60 Growth

Studies were initiated to determine whether HL-60 UR cells would elaborate macromolecules that facilitated their growth. High cell density liquid cultures of HL-60 cells were used to generate conditioned medium (CM). CM was then studied for its effect on the growth of HL-60 cells in liquid and viscous culture at low, intermediate, and high starting cell densities. As shown in Table 1, when 10,000 cells/ml were cultured, 10% HL-60 CM decreased the average doubling time over days 2-4 of culture from 108 hr to 65 hr and also increased colony number from 0 to 106. HL-60 CM also augmented growth in both liquid and viscous cultures when 25,000 cells/ml were cultured. However, when 50,000 cells/ml were cultured, the doubling time in cultures without CM decreased to 41 hr and was not further decreased by CM. On the other hand, CM continued to stimulate growth in viscous culture, increasing colony number from 5 to 297 colonies. Hence, an influence of culture conditions on the requirement for CM was apparent at this cell density. When 100,000 cells/ml were cultured, no further decrease in doubling time was observed in

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The effect of HL-60 conditioned medium (CM) on HL-60 colony growth was examined at a culture density of ~25,000 cells/ml (Fig. 2). In contrast to the results in liquid culture (Fig. 1B), no colony growth of HL-60
Fig. 2. Effect of HL-60 conditioned medium (CM) concentration on HL-60 colony growth. HL-60 cells were washed free of standard liquid medium and resuspended to a concentration of $2.5 \times 10^5$ cells/ml in medium containing 0.8% methylcellulose and 0%-20% HL-60 conditioned medium (■); or control medium (○). The 1 ml cultures in 35 x 10 mm plastic tissue culture dishes were incubated at 37°C in a humidified atmosphere of 5% CO$_2$ in air. Colonies of greater than 8 cells were enumerated after 7 days. Bars: SEM of triplicate observations in three experiments.

cells occurred in cultures without CM at this cell density, and the addition of 0.5% CM stimulated only 35 colonies. Maximum growth, 233 colonies, required 10% CM. The average cell number/colony stimulated by 10% CM was $76 \pm 9.8$ after 7 days of culture. In contrast to normal marrow stimulated with CSA, HL-60 CM did not stimulate terminal cell maturation.

Studies of Other HL-60 Populations for Growth-Stimulating Activity

It was conceivable that the phenomenon of auto-stimulation was a peculiarity of our HL-60 cell population. To answer this question, we compared HL-60 GP9, 23, 40, and 76 populations to HL-60 UR for elaboration of, and responsiveness to, HL-60 growth-stimulatory activity.

As shown in Table 2, when unconcentrated conditioned media from GP9, 23, 40, and 76 cell populations were studied, none promoted GP9, 23, 40, or 76 colony growth. However, GP 76 CM did stimulate UR growth. Moreover, all CMs were found to stimulate all target cell populations when they were concentrated tenfold by ultrafiltration against an Amicon UM-05 membrane (nominal exclusion 3000 daltons) and studied. Thus, these HL-60 populations all elaborated growth-stimulating activity and all could respond to it. None of these CMs promoted cell maturation.

Specificity of HL-60 Stimulatory Activity

To evaluate the specificity of HL-60 growth-stimulating activity for HL-60 cells, we studied the effect of

HL-60 UR CM on normal human marrow growth. HL-60 CM did not stimulate granulocyte-monocyte or erythroid colony growth even when concentrated tenfold by Amicon UM-05 ultrafiltration and added in up to 20 vol % in cultures. When HL-60 CM was added to cultures stimulated by semipurified CSA or erythropoietin, colony growth was not altered. HL-60 CM also failed to stimulate or inhibit the clonal growth of mouse marrow, the mouse lymphoblastoid cell line, L5178Y, or the human lymphoblastoid cell line, P1R.

Elaboration of HL-60 Stimulatory Activity by Other Cell Populations

We next examined the question of whether HL-60 growth-promoting activity was elaborated by other cell lines or cell strains and its relationship to the elaboration of CSA. CMs were obtained from 17 different human cell populations and studied for the ability to stimulate the colony growth of HL-60 UR cells, human CFU-GM, and mouse CFU-GM. As shown in Table 3, three groups were observed. Certain cell lines such as GCT and RC-4 stimulated both HL-60 and CFU-GM growth. Other cell populations like HL-60, ML-1, NALM-16, KB, and WI-38 elaborated activity for HL-60 cells but not for normal marrow. A third group, comprising the majority of cell lines, did not stimulate either HL-60 cells or normal precursors. There was no definite association between the origin or type of cell population and the elaboration of stimulatory activities.

Biochemical Properties of the Growth-Stimulating Activities in GCT and HL-60 Conditioned Media

The finding that HL-60 CM contained autostimulatory activity that did not promote normal marrow growth suggested the possibility that the growth-promoting activity for HL-60 cells in GCT-conditioned medium might be due to a similar principle and not to the CSA contained therein. To answer this question, GCT CM and HL-60 UR CM were concentrated fivefold by ultrafiltration against an Amicon UM-05 membrane and chromatographed on polyacrylamide-agarose (Ultrogel AcA54) columns. Fractions were studied for the ability to stimulate HL-60 and CFU-GM colony growth. As shown in Fig. 3, HL-60 stimulating activity in GCT-conditioned medium eluted in two regions, one of molecular weight 30,000-50,000, which also stimulated normal CFU-GM growth, and another of molecular weight $\sim 13,000$, which did not stimulate normal marrow. In contrast, HL-60 conditioned medium contained only one region of activity that corresponded to the lower
### Table 2. Responsiveness of HL-60 Cell Populations to HL-60 Conditioned Media

<table>
<thead>
<tr>
<th>Conditioned Medium</th>
<th>Source Concentration</th>
<th>Target Cell Population</th>
<th>HL-60 Colony no.</th>
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<th>GP23</th>
<th>GP40</th>
<th>GP76</th>
<th>UR</th>
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<td>4 x 10^7 Cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incubated medium</td>
<td>(control) HL-60</td>
<td></td>
<td>1x†</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>GP9§</td>
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<td>0</td>
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<td>0</td>
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<td></td>
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<td></td>
<td></td>
<td>408</td>
<td>336</td>
<td>432</td>
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<td>600</td>
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<tr>
<td>GP23</td>
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<td>384</td>
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<td>696</td>
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<td>792</td>
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<td>GP40</td>
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<td>10x</td>
<td></td>
<td></td>
<td>551</td>
<td>816</td>
<td>1,368</td>
<td>1,056</td>
<td>1,056</td>
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<tr>
<td>GP76</td>
<td>1x</td>
<td></td>
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<td>0</td>
<td>0</td>
<td>48</td>
<td>0</td>
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<tr>
<td></td>
<td>10x</td>
<td></td>
<td></td>
<td>720</td>
<td>960</td>
<td>1,176</td>
<td>1,104</td>
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<tr>
<td>UR∥</td>
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<td>0</td>
<td>288</td>
<td>456</td>
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<td>480</td>
<td>1,152</td>
<td>888</td>
<td>816</td>
<td>936</td>
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</tbody>
</table>

*Stimulated by addition of 0.1 ml control or conditioned medium sample/1 ml culture.
†Unconcentrated.
‡Concentrated ten-fold by ultrafiltration against an Amicon UM-05 membrane.
§GP, passage number in Dr. R. C. Gallo’s laboratory.
∥UR, cells maintained in our laboratory.

### Table 3. Effects of Conditioned Media from Various Human Cell Lines on the Colony Growth of HL-60 cells and Normal Human and Mouse Granulocyte-Monocyte Progenitors (CFU-GM)

<table>
<thead>
<tr>
<th>Conditioned Medium Source</th>
<th>Activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HL-60 Colonies/4 x 10^7</td>
</tr>
<tr>
<td>Cell Line</td>
<td>Cells</td>
</tr>
<tr>
<td>GCT§</td>
<td>1,176</td>
</tr>
<tr>
<td>RC-4§</td>
<td>840</td>
</tr>
<tr>
<td>HL-60†</td>
<td>860</td>
</tr>
<tr>
<td>ML-1†</td>
<td>792</td>
</tr>
<tr>
<td>NALM-16†</td>
<td>432</td>
</tr>
<tr>
<td>KB†</td>
<td>720</td>
</tr>
<tr>
<td>WI-38†</td>
<td>648</td>
</tr>
<tr>
<td>GCT-V1§</td>
<td>0</td>
</tr>
<tr>
<td>SU-DHL-1†</td>
<td>0</td>
</tr>
<tr>
<td>U-937†</td>
<td>0</td>
</tr>
<tr>
<td>GM-1§</td>
<td>0</td>
</tr>
<tr>
<td>K-562†</td>
<td>0</td>
</tr>
<tr>
<td>Reh†</td>
<td>0</td>
</tr>
<tr>
<td>MOLT-4F†</td>
<td>0</td>
</tr>
<tr>
<td>RPMI-8402†</td>
<td>0</td>
</tr>
<tr>
<td>PIR§</td>
<td>0</td>
</tr>
<tr>
<td>RPMI-1788§</td>
<td>0</td>
</tr>
<tr>
<td>Incubated medium (control)</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: FH, fibrous histiocytoma; NB, normal blood; AML, acute myelogenous leukemia; ALL, acute lymphocytic leukemia; NPCA, nasopharangeal carcinoma; ELF, embryo lung fibroblasts; HL, histiocytic lymphoma; CML, chronic myelogenous leukemia; BL, Burkitt’s lymphoma; MA, macrophagic; PG, progranulocytic; MYB, myeloblastic; NLB, non-T, non-B lymphoblastic; CA, carcinoma; F, fibroblastic; MYMOB, myelomonoblastic; T, T lymphoblastic; BLB, B lymphoblastic.

*Stimulated by addition of 0.1 ml control or conditioned medium sample/1 ml culture.
†CM provided by Dr. J. Minowada of Roswell Park Memorial Institute.
‡CM provided by the Microbiology Department of the University of Rochester.
§CM from cells maintained in our laboratory.
FIG. 3. Elution of GCT conditioned medium from Ultrogel AcA54. GCT CM was concentrated fivefold by ultrafiltration against an Amicon UM-05 membrane (nominal exclusion 500 daltons). The retentate was applied to a 2.5 x 110 cm Ultrogel AcA54 column and eluted with phosphate-buffered saline, pH 7.4 (0.025 M PO₄, 0.1 M NaCl) at a flow rate of 1 ml/min. Fractions were added to a final concentration of 10% in viscous cultures of HL-60 UR and nonadherent human marrow cells and studied for their ability to stimulate HL-60 and CFU-GM colony growth. This figure shows the elution of activity for HL-60 cells. (O—O) Absorbance at 280 nm, (•—•) HL-60 colonies/2.5 x 10⁶ cells. Points represent mean of triplicate determinations in two experiments. Molecular weight markers: ovalbumin, 43,000 daltons; GCT colony-stimulating activity (CSA), 30,000 daltons; ribonuclease, 13,700 daltons. The higher molecular weight area of HL-60 stimulating activity also stimulated CFU-GM growth. CSA and the high molecular weight area of HL-60 activity always coelute but the apparent bimodal distribution shown in this figure is not a constant feature.

molecular area in GCT CM (Fig. 4). The active areas in GCT CM and HL-60 CM also promoted HL-60 growth in liquid culture (data not shown).

The resistance of autostimulatory activity in HL-60 CM to physical and chemical treatments is shown in Table 4. Activity was completely retained following dialysis against a membrane with a molecular exclusion of ~13,000 daltons but was partially dialyzable through a membrane with an exclusion of ~8000. It was resistant to 60°C, freeze-thawing, extraction by diisopropyl ether-butanol, and reduction by dithiothreitol but was totally inactivated by trypsin, periodate, and the alkylating agent, N-ethylmaleimide. In contrast, GCT CM continued to stimulate growth following exposure to N-ethylmaleimide, perhaps reflecting the known resistance of CSA to this agent. Otherwise, GCT CM exhibited similar properties to HL-60 CM. HL-60 stimulating activities in ML-1, NALM-16, KB, and WI-38 CMs were, like that in HL-60 CM, partially dialyzable through a 13,000 dalton membrane. These CMs were not characterized further.

DISCUSSION

These studies suggest that HL-60 cells can grow in the absence of colony-stimulating activity at high population density by virtue of their ability to elaborate autostimulatory activity, which differs in certain respects from CSA. They support previous observations that HL-60 cells exhibit growth-responsiveness to CSA but do not elaborate detectable quantities of CSA into culture medium.

Population density restriction of growth has been used for many years to identify growth-promoting nutrients in conditioned media, but the identification of autostimulatory mediators has been an infrequently reported event. Rubin and Millis and coworkers showed that fibroblasts cultured in high density elaborated factors that facilitated growth at low densities. Also, medium conditioned by mitogen-stimulated T-
lymphocyte populations has been used as a source of T-cell growth factor(s),\textsuperscript{43-45} and conditioned media from B-lymphoid cell lines has been used to facilitate the growth of B-lymphocyte colonies.\textsuperscript{46} Among leukemia cell populations, the mouse myelomonocytic leukemia cell line, WEHI-3, shows spontaneous growth that is probably due to autostimulation by CSA.\textsuperscript{47} At the low cell densities used in the primary clonal culture of AML cells, growth rarely occurs without the addition of CSA\textsuperscript{1} even though some AML populations, especially those with a monocytic component, elaborate CSA.\textsuperscript{48,49} Even with CSA added, growth of AML cells is usually poor,\textsuperscript{1,13,14,16} suggesting that other mediators may be missing in culture. Examination of high density leukemic cell CMs for autostimulators different from CSA might be a useful path to pursue in the identification of such molecules.

Several variables influenced the requirement of HL-60 cells for HL-60 conditioned media. Thus, the requirement for CM disappeared at high cell density presumably because sufficient activity was elaborated to meet growth requirements. Conversely, at very low densities, growth was reduced even when CM was added, indicating other adverse effects on growth under these conditions. The requirement for CM was more stringent in methylcellulose medium than in liquid culture, indicating an influence of culture matrix. This finding could reflect binding of the activity to methylcellulose, thus rendering it less available to cells; the fact that cells are more uniformly separated in viscous medium minimizing the effects of favorable cell–cell interactions, or to other variables yet to be defined. Serum “quality” was a third variable that influenced the population density at which spontaneous growth occurred, possibly indicating the presence of growth-promoting factors or cofactors in serum. Finally, although all passages of HL-60 cells tested elaborated and responded to HL-60 activity, it was necessary to concentrate most HL-60 CMs by ultrafiltration through a membrane with a very low exclusion limit (~500 molecular weight) to demonstrate effects.

Human cell lines other than HL-60 varied in the ability to elaborate growth-promoting activity for HL-
60 cells and normal progenitors. Two, GCT and RC-4, had both HL-60 and CFU-GM stimulating activities; five, including HL-60, had activity only for HL-60; and the majority did not stimulate either HL-60 or CFU-GM. Although there was no consistent relationship between the origin and type of cell line and the production of mediators, it is of possible relevance that those lines that had only HL-60 activity were all of either leukemic or embryonic origin. Human embryo lung fibroblast conditioned medium was used in the original isolation of HL-60 cells20,24 and is known to stimulate certain AML populations but not normal progenitors.21 HL-60 activity could represent a primitive growth-promoting activity common to embryonic and certain neoplastic cells.

Parallel chromatography of GCT CM and HL-60 CM on Ultrogel columns revealed HL-60 stimulating activity of molecular weight ~13,000 in both CMs. In addition, GCT CM had a second area of activity that corresponded to the elution of CSA and stimulated normal progenitors. Presumably, CSA stimulates HL-60 growth, although this cannot be concluded with certainty until purified CSA is available for testing.

Preliminary characterization of the HL-60 stimulating activity in HL-60 conditioned media indicated that it was inactivated by trypsin, periodation, and by exposure to N-ethylmaleimide. These data suggest that the factor(s) or an essential cofactor is protein, possibly glycoprotein, and that free sulfydryl groups are required for its activity. The resistance to dithiothreitol suggests that critical disulfide bonds are absent. Also, the molecule does not appear to be particularly hydrophobic, since it could not be extracted into an organic solvent, diisopropyl ether-butanol. In its thermal stability, susceptibility to trypsin and periodate, and resistance to organic solvents, HL-60 factor resembles GCT CSA.11 However, HL-60 factor does not stimulate normal marrow growth, is totally inactivated by N-ethylmaleimide, and has a lower molecular weight than CSA. The relationship of CSA to HL-60 activity is unclear. It is conceivable that HL-60 activity is a precursor or degradation product of CSA. Some indirect support for these possibilities is provided by the observation that HL-60 cells can be induced to become macrophage-like by certain phorbol esters, suggesting that the cell machinery for producing CSA could be present.26 28 Also, GM-CSA in the molecular weight range of HL-60 activity has been found in leukocyte conditioned medium.4 Finally, Price and coworkers have presented data that suggest that AML cell membranes may contain certain molecular weight species of CSA not detectable in AML cell-conditioned media.3 Determination of the precise relationship of HL-60 activity to CSA awaits the purification of the two activities and the preparation of specific antibodies for cross-neutralization studies.

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Autostimulation of growth by human myelogenous leukemia cells (HL-60)

JK Brennan, CN Abboud, JF DiPersio, GH Barlow and MA Lichtman

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