Biosynthetic (Recombinant) Human Granulocyte-Macrophage Colony-Stimulating Factor: Effect on Normal Bone Marrow and Leukemia Cell Lines

By Masao Tomonaga, David W. Golde, and Judith C. Gasson

To examine the biologic properties of the molecule encoded by the human gene for granulocyte-macrophage colony-stimulating factor (GM-CSF), we expressed the cloned complementary DNA (cDNA) in transfected monkey COS cells and purified the resultant protein. Purified biosynthetic human GM-CSF was added to cultures of normal hematopoietic progenitor cells in semisolid media, and the resulting colonies were characterized cytochemically. Nonadherent light-density bone marrow cells from healthy adult volunteers were maximally stimulated with GM-CSF (approximately 250 pmoL/L), and four types of colonies were consistently identified by aspirating the individual colonies and staining with a triple stain for specific and nonspecific esterases and eosinophilic granules. Pure neutrophilic granulocyte (G), mixed granulocyte-macrophage (GM), pure macrophage (M), and pure eosinophil (EO) colonies were observed, the mean incidences on day 8 being 70%, 20%, 5%, and 5%, and on day 14, 7.5%, 16.6%, 50.9%, and 25.0%, respectively. In all types of colonies, complete maturation to segmented forms or typical macrophages was detected. GM-CSF did not enhance the growth of BFU-E from normal peripheral blood buffy coat cells in the simultaneous presence of erythropoietin alone or erythropoietin with purified erythroid-potentiating activity. GM-CSF stimulated HL-60 and KG-1 colony formation twofold and fivefold, respectively; consistent differentiation induction towards monocytic and eosinophilic lineages was observed in HL-60 but not in KG-1. These in vitro findings indicate that GM-CSF is a multilineage stimulator for progenitor cells of G, GM, M, and EO colonies.

MATERIALS AND METHODS

Cell Preparations

Bone marrow was obtained with informed consent from healthy volunteers by aspiration from iliac crest. Light-density cells were separated by Ficoll-Hypaque density gradient centrifugation. Five million light-density cells suspended in 5 mL of Iscove's modified Eagle's medium (MEM) (Irvine Scientific, Santa Ana, Calif) containing 20% fetal bovine serum (FBS; Hyclone, Logan, Utah) were incubated in a Falcon T-flask (Becton Dickinson, Oxnard, Calif) for two hours at 37 °C in 5% CO2 in air to deplete adherent cells. Nonadherent light-density cells were gently harvested, washed twice in complete medium, and used for the CFU-GM assay.

For the BFU-E assay, peripheral blood buffy coat cells were treated with Tris-HCl (pH 7.56)-buffered 0.89% ammonium chloride for 9.5 minutes at 37 °C to lyse the contaminating red blood cells. The nucleated cells were washed twice with Iscove's MEM containing 10% FBS.

Two human myeloid cell lines, HL-60 and KG-1, were used for differentiation-inducing experiments by GM-CSF. Both cell lines have been maintained in Iscove's MEM containing 10% FBS. Cells harvested during exponential growth were used for the experiments.

Hematopoietic Stimulators

Purified biosynthetic GM-CSF was obtained from serum-free medium conditioned by COS monkey kidney cells transfected with the GM-CSF cDNA clone in the expression vector p91023 (B). The GM-CSF was purified from the conditioned medium by a

OCT cell line.

We recently purified a 22,000-molecular weight (mol wt) GM-CSF from medium conditioned by the COS cells and purified the resultant protein. Purified biosynthetic human GM-CSF has been added to cultures of normal hematopoietic progenitor cells in semisolid media, and the resulting colonies were characterized cytochemically. Nonadherent light-density bone marrow cells from healthy adult volunteers were maximally stimulated with GM-CSF (approximately 250 pmoL/L), and four types of colonies were consistently identified by aspirating the individual colonies and staining with a triple stain for specific and nonspecific esterases and eosinophilic granules. Pure neutrophilic granulocyte (G), mixed granulocyte-macrophage (GM), pure macrophage (M), and pure eosinophil (EO) colonies were observed, the mean incidences on day 8 being 70%, 20%, 5%, and 5%, and on day 14, 7.5%, 16.6%, 50.9%, and 25.0%, respectively. In all types of colonies, complete maturation to segmented forms or typical macrophages was detected. GM-CSF did not enhance the growth of BFU-E from normal peripheral blood buffy coat cells in the simultaneous presence of erythropoietin alone or erythropoietin with purified erythroid-potentiating activity. GM-CSF stimulated HL-60 and KG-1 colony formation twofold and fivefold, respectively; consistent differentiation induction towards monocytic and eosinophilic lineages was observed in HL-60 but not in KG-1. These in vitro findings indicate that GM-CSF is a multilineage stimulator for progenitor cells of G, GM, M, and EO colonies.

Human CSFs have been well characterized. Nicola et al separated two types of CSF from human placental-conditioned medium on the basis of their hydrophobicity: the first activity (CSFα) contains GM-CSF, EO-CSF, and possibly erythroid-CSF activities; and the second one (CSFβ) is very similar to murine G-CSF. Comparable results were reported with conditioned medium from the GCT cell line. We recently purified a 22,000-molecular weight (mol wt) GM-CSF from medium conditioned by the Mo-T-lymphoblast cell line. Complementary DNA clones encoding this human T cell-derived GM-CSF have been isolated, and there is about 60% homology with the nucleotide sequence of the murine GM-CSF cDNA. Purified natural and biosynthetic human GM-CSF have activities on precursor cells, as well as mature neutrophils. In this study we define the biologic activity of the purified biosynthetic GM-CSF on human hematopoietic progenitors using in vitro clonal culture of normal blood and bone marrow cells and the human myeloid leukemia cell lines HL-60 and KG-1.

From the Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles.

Supports: US Public Health Service Grants No. CA32737, CA40163, and CA30388, and JFRA-134 from the American Cancer Society.

Address reprint requests to Dr Judith C. Gasson, Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

© 1986 by Grune & Stratton, Inc.

www.bloodjournal.orgFrom the Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles.

Submitted April 10, 1985; accepted June 24, 1985.

Supported by US Public Health Service Grants No. CA32737, CA40163, and CA30388, and JFRA-134 from the American Cancer Society.

Address reprint requests to Dr Judith C. Gasson, Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

© 1986 by Grune & Stratton, Inc.

0006-4971/86/6701-0005$03.00/0
modification of the previous method using only gel filtration and reverse-phase high-performance liquid chromatography. The lectin affinity column could be eliminated because of the greater abundance of biosynthetic GM-CSF in the conditioned medium. The purified GM-CSF appeared as a single protein band of 22,000 mol wt on sodium dodecylsulfate polyacrylamide gel electrophoresis, the same molecular weight as the purified natural GM-CSF. The concentration of GM-CSF in a reference preparation was estimated by amino acid analysis; the specific activity of this purified material on normal bone marrow cells is 2 x 10^4 U/mg. Half maximal activity on bone marrow cells is seen at 10^{-11} mol/L.

Erythroid-potentiating activity (EPA) used in the BFU-E assay was purified from Mo-T cell-conditioned medium as previously reported. Human urinary erythropoietin (EPO) was provided by the Heart, Lung and Blood Institute (Bethesda, Md). The EPO preparation (CAT-1) had a specific activity of 1,140 U/mg protein. The hematopoietic stimulators were serially diluted to various concentrations with Iscove's MEM just before addition to the cultures.

Clonal Culture of Hematopoietic Progenitors in Methylcellulose

Light-density nonadherent bone marrow cells (final concentration, 2.5 to 5 x 10^6/mL) were plated in Iscove's MEM containing 0.88% methylcellulose, 20% FBS, and GM-CSF at various dilutions in Iscove's MEM. One-half milliliter of semisolid medium was plated in 2.0-cm² Falcon well for colony analysis, and 0.1 ml was plated in a Falcon microtiter well for the GM-CSF dose-response studies.

BFU-E assays were performed according to the micromethod previously reported. Peripheral blood buffy coat cells (final concentration, 3.3 x 10^7/mL) were plated in Iscove's MEM containing 0.8% methylcellulose, 20% FBS, and GM-CSF at various dilutions in Iscove's MEM. One-half milliliter of semisolid medium was plated in 2.0-cm² Falcon well for colony analysis, and 0.1 ml was plated in a Falcon microtiter well for the GM-CSF dose-response studies.

Clonal Culture of Leukemic Cell Lines in Methylcellulose

HL-60 and KG-1 cells were plated at a final concentration of 3,000/mL and 10,000/mL, respectively, in Iscove's MEM containing 0.92% methylcellulose, 20% FBS, 10⁻⁴ mol/L alpha-thioglycerol, 0.33 U/mL EPO, and 10% volumes of diluted EPO and/or GM-CSF.

CFU-GM colonies were enumerated on day 8 in all cultures and also on day 14 in some, counting cell aggregates containing 20 cells or more as CFU-GM. Erythroid bursts were enumerated on day 14. Well-hemoglobinized colonies containing 100 or more cells were regarded as BFU-E. They were confirmed as having hemoglobin with diaminobenzidine according to Ogawa et al.

Cytochemical Staining of Individual CFU-GM Colonies

After enumerating the number of CFU-GM, individual colonies were aspirated using a finely drawn-out micropipettary tip, carefully avoiding contamination with an adjacent colony or cluster. The aspirated colony was then transferred into a 10-μL droplet of Iscove's MEM placed on a poly-L-lysine-coated glass slide. After complete attachment of colony cells onto the glass surface, medium was removed with absorbent paper and the slides dried rapidly with air. A slide with ten consecutively aspirated colonies was fixed with phosphate-buffered (pH 6.6) formalin-acetone for 30 seconds at 7 °C and subjected to cytochemical staining.

Each slide was triple stained, combining a double stain for specific and nonspecific esterases and an eosinophilic granule stain with Biebrich scarlet to analyze the lineages of the cells composing the colonies. For alpha-naphthyl butyrate esterase (a nonspecific esterase characteristic of the monocyte-macrophage lineage), the slide was covered with a substrate solution containing 1 mg/mL of Fast garnet GBC (Sigma Chemical Co, St Louis) for 15 minutes at room temperature and then washed with distilled water and air dried. For naphthol-ASD chloracetate esterase (a specific esterase characteristic of neutrophilic granulocytes), the slide was covered with a substrate solution containing 0.5 mg/mL of Fast blue RR (Sigma) for 20 minutes at room temperature and washed with distilled water and air dried. For eosinophilic granule stain with Biebrich scarlet (Sigma), the slide was dipped in a 0.1% Biebrich scarlet Tris-HCL (0.1 mol/L, pH 7.2) solution for two hours at room temperature. The slide was washed with distilled water, air dried, and mounted with glycerin gelatin. Using this triple stain, neutrophilic granulocytes from promyelocytes to segmented neutrophils appear as cells with discrete blue cytoplasmic staining (chloroacetate esterase), monocytes and macrophages have diffuse brown cytoplasmic staining (butyrate esterase), and eosinophils appear with coarse red granules (Biebrich scarlet).

In experiments for differentiation induction of leukemic cell lines by GM-CSF, poly-L-lysine-coated slides of pooled colony cells were subjected to the triple stain. In some experiments, single staining with Biebrich scarlet or Luxol fast blue and also performed to detect eosinophilic differentiation. The cyanide-resistant myeloperoxidase reaction was used to further confirm enzymatically the eosinophilic nature of Biebrich scarlet– and Luxol fast blue–positive cells.

RESULTS

Response of CFU-GM to Biosynthetic GM-CSF

GM-CSF dose response. Because of our previous results showing that preparations of natural GM-CSF partially purified from Mo-conditioned medium had no activity on mouse or rat bone marrow, all experiments were performed using human peripheral blood and bone marrow cells. We determined the relationship of the number of CFU-GM colonies observed to the amount of GM-CSF added to two samples of normal light-density nonadherent bone marrow cells. As seen in Fig 1, the dose-response curve was sigmoid. Further studies using known molar concentrations of the GM-CSF sample (as determined by amino acid analysis) revealed that a 1:1,000 dilution was approximately equal to 250 pmol/L. Stimulation of colony growth was seen at concentrations as low as 2 pmol/L. At submaximal concentrations of GM-CSF, the colony sizes tended to be smaller along with the lower colony numbers. Control cultures without GM-CSF did not show any colony formation.

Cytochemical analysis of individual CFU-GM colonies. Colonies that developed at maximal stimulation with GM-CSF were analyzed using five separate bone marrow samples. The colonies were classified into four subtypes: pure neutrophilic granulocyte (G) colonies showing only chloroacetate esterase–positive cells, mixed granulocyte-macrophage (GM) colonies with a mixture of chloroacetate esterase–positive cells and butyrate esterase–positive cells, pure macrophage (M) colonies with only butyrate esterase–positive cells, and pure eosinophil (EO) colonies with only Biebrich scarlet–positive cells. In general, the sizes of the individual colonies varied from 20 to more than 300 cells.
BIOSYNTHETIC HUMAN GM-CSF

Fig 1. Biosynthetic GM-CSF dose response by normal human CFU-GM. Each point represents the mean value of two normal bone marrow samples. For each sample, triplicate cultures were performed at each dilution of GM-CSF. A 1:1,000 dilution of GM-CSF in this experiment was approximately equivalent to 250 pmol/L.

GM and M colonies tended to be larger than G and EO colonies. No unstained colonies were found. To obtain the distribution pattern of these cytochemically defined subtypes of colonies in each sample, 76 to 117 colonies were randomly aspirated and stained. Day 8 colonies were analyzed in all samples, and day 14 colonies in two samples. To avoid the development of fusion colonies, the number of light-density bone marrow cells plated was reduced to as low as 2.5 x 10^4/mL. This concentration usually yielded about 20 colonies per well. As shown in Table 1, the mean number of CFU-GM colonies formed was 102/5 x 10^4 cells. The four subtypes were consistently observed in all samples. G colonies predominated (70%) in all samples. GM colonies comprised 20%, and M and EO colonies about 5%.

The G colonies consisted of granulocytes of various maturation stages: promyelocytes and myelocytes being predominant, but band and segmented forms were consistently observed. Chloroacetate esterase-negative cells, indicative of immature blast cells, were not observed. GM colonies consisted of two types: one with a minor population of granulocytes among predominant monocytes-macrophages, and the other with almost equal numbers of cells of the two lineages. GM colonies with monocytes-macrophages as a minor population were rarely observed. The M colonies were usually composed of monocytes with moderate butyrate esterase activity, large macrophages with marked butyrate esterase activity, and intermediate cells showing maturation transition.13 Rare M colonies, however, showed a pure population of monocytes that contained a few butyrate esterase-negative cells, probably representing immature monocytes such as monoblasts or promonocytes.31

The EO colonies showed substantial variability in frequency among the five samples. The lowest value of 0.9% observed in sample 5, however, increased to 12% in the day 14 analysis. The EO colonies usually consisted of 20 to 40 cells; mononuclear cells predominated, but a few banded forms also developed.

Two samples analyzed on day 14 exhibited a similar tendency in that the percentages of M and/or EO colonies became higher compared with those on day 8. Control culture without GM-CSF never yielded distinct colonies consisting of more than 20 cells in any sample.

Response of HL-60 and KG-1 Cell Lines to Biosynthetic GM-CSF

Although in control CFU-GM assays no detectable colonies were formed, the possibility remains that accessory cells such as a small number of phagocytic cells and lymphocytes in the light-density nonadherent bone marrow cell samples played some role in giving rise to colonies in GM-CSF-containing cultures.32 This consideration led us to use HL-60 and KG-1 as homogenous target cells. HL-60 may be induced to differentiate toward mature granulocytes and/or monocytes-macrophages by various compounds such as dimethyl sulfoxide, retinoic acid, tetradecanoyl phorbol ester, chemotherapeutic agents, and crude and purified lymphokines.33-41 The KG-1 cell line is also known to be inducible toward macrophage differentiation by phorbol diesters and vitamin D derivatives.40,41

In HL-60, the liquid culture sample and unstimulated colony culture cells showed almost the same pattern of enzymatic expression: chloroacetate esterase-positive cells predominated and butyrate esterase-positive cells were negligible in number. However, a small proportion of cells was found to stain weakly with Biebrich scarlet stain. In stimulated cultures, the number of Biebrich scarlet-positive cells was consistently increased with increasing concentrations of GM-CSF (Table 2). The number of butyrate esterase-positive cells also increased. In contrast to the marked change in

Table 1. Cytochemical Analysis of Individual CFU-GM Colonies

<table>
<thead>
<tr>
<th>Bone Marrow Sample</th>
<th>Colony Cells (%)</th>
<th>Cytochemical Analysis of Pooled Colony Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture Day</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 2. Response to GM-CSF of Human Myeloid Leukemia Cell Lines in Methylcellulose Culture

<table>
<thead>
<tr>
<th>Culture Condition</th>
<th>Colony Number</th>
<th>Negative* Es-chl</th>
<th>Es-b+ B-+</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL-60</td>
<td>Preculture</td>
<td>34.6 61.4 0</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Stimulated</td>
<td>35.6 59.8 2.3</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>Maximal†</td>
<td>39.6 32.1 16.3</td>
<td>12.0</td>
</tr>
<tr>
<td>KG-1</td>
<td>Preculture</td>
<td>32.2 64.7 3.1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Unstimulated</td>
<td>42.0 56.0 2.0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Stimulated</td>
<td>35.2 61.5 3.3</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: Es-chl, naphthol-ASD chloroacetate esterase; Es-b, alpha-naphthyl butyrate esterase; B-s, Biebrich scarlet stain for eosinophil granules.

* Lacking any reaction to the triple stain.
† GM-CSF was added to give a final dilution of 1:8,000.
‡ GM-CSF was added to give a final dilution of 1:1,000 (Fig 1).

Each culture was performed in triplicate. Colony number is the mean ± SEM. For cytochemical analysis of pooled colony cells, 300 cells were examined on a triple-stained smear, and the positive rate for each reaction was calculated.
HL-60, KG-1 did not show a change in enzymatic pattern, and Biebrich scarlet–positive cells never appeared (Table 2).

To confirm the eosinophilic nature of the Biebrich scarlet–positive cells in the HL-60 cell line, Luxol fast blue and Wright-Giemsa staining was performed. Distinct Luxol fast blue positivity and eosinophilic stain with Wright-Giemsa were recognized in almost the same percentages as with Biebrich scarlet stain. The percentage of cyanide-resistant peroxidase-positive cells in HL-60 cells stimulated with GM-CSF was also equal to that of Biebrich scarlet– and Luxol fast blue–positive cells.

Response of BFU-E to Biosynthetic GM-CSF

The effect of GM-CSF on erythroid burst formation was examined in cultures containing low concentrations (0.33 U/mL) of EPO (Fig 2). No enhancement of erythroid bursts was seen in cultures containing amounts of GM-CSF shown to be stimulatory on CFU-GM (Fig 2B). This observation is in contrast to the consistent burst formation in the standard EPA- and EPO-containing cultures (Fig 2A). The addition of GM-CSF to the standard EPA- and EPO-containing cultures did not enhance BFU-E numbers, but rather caused a measurable decrease in cloning efficiency (Fig 2C).

DISCUSSION

Purified biosynthetic (recombinant) GM-CSF produced by COS monkey kidney cells transfected with a cDNA clone encoding human GM-CSF caused consistent stimulation of normal CFU-GM proliferation and differentiation. The GM-CSF dose-response curve was typically sigmoid, and in several experiments we have seen no inhibitory effect at concentrations up to 1 nmol/L. All of the three G, GM, and M colony types were stimulated, as well as eosinophil colonies. G colonies predominated in day 8 cultures, most becoming diffuse and losing colony configuration by day 14. In contrast, M and EO and some of the GM colonies continued their growth to reach larger-colony sizes during the second week of incubation. The resultant colony number was considerably decreased on day 14. This difference in the rate of colony formation may reflect the kinetic characteristics of each CFU-GM subpopulation in response to GM-CSF. It remains to be seen whether the GM-CSF can stimulate all CFU-GM or a specific subset. Theoretically, purification of all human CSFs is a prerequisite for such a study.

The presence in the light-density nonadherent bone marrow cell sample of accessory cells capable of releasing CSFs makes it difficult to draw unambiguously the conclusion that GM-CSF has direct activities on CFU-GM and CFU-EO. An ideal experimental system would require single cells capable of differentiation to granulocytes/macrophages and eosinophils in the presence of pure CSF. To examine the direct actions of GM-CSF, we chose human myeloid leukemia cell lines as readily available homogeneous target cells for GM-CSF. Both HL-60 and KG-1 responded to GM-CSF by increasing their proliferative rates in liquid culture (data not shown) and plating efficiencies in methylcellulose culture.

Cytotoxic examination of GM-CSF–stimulated HL-60 cells and KG-1 cells showed that HL-60 can be induced to differentiate towards monocytic and eosinophilic lineages. Some of the stimulated HL-60 cells became fully mature eosinophils or macrophages. Eosinophilic granules stained with Biebrich scarlet or Luxol fast blue were also positive for cyanide-resistant peroxidase, confirming the eosinophilic nature of these HL-60 cells. As the unstimulated cultures of HL-60 contained small proportions of Biebrich scarlet–positive cells, the stem cells of this cell line appear to have a multipotential ability to differentiate toward monocytic and eosinophilic as well as neutrophilic lineages when properly stimulated. Induced differentiation of the HL-60 cell line has been extensively investigated; however, there are only a few published analyses of eosinophilic differentiation. Lu et al observed an increase of eosinophilic colonies after 14 days of methylcellulose culture stimulated by crude sources of CSF. In contrast, Metcalf did not note such an increase in agar culture stimulated by GM-CSF. In the present study, the total increase in the number of eosinophilic cells and the appearance of more mature forms with confluent large granules were apparent. In contrast to HL-60, the enhanced proliferation of the KG-1 cell line by GM-CSF was not accompanied by differentiation induction. The consistent differentiation induction of HL-60 by GM-CSF toward monocytic and eosinophilic lineages is compatible with the findings obtained in the CFU-GM assay of normal hematopoietic progenitor cells.

In the present study, we were unable to detect basophilic colonies from normal bone marrow samples or basophilic differentiation in GM-CSF–stimulated HL-60 cells. We stained 100 colonies from sample 4 and pooled HL-60 colony cells with toluidine blue. No colony nor HL-60 cells showed a distinct metachromasia for this dye, suggesting that basophil stimulation is not a functional activity of the GM-CSF. However, there still remains a possibility that CFU-Baso was present at a rate lower than 1% of the CFU-GM colonies and missed from the present aspiration analysis.

Fig 2. Response to biosynthetic GM-CSF by normal human BFU-E. (A) EPA dose response of BFU-E at an EPO concentration of 0.33 U/mL. (B) GM-CSF dose response of BFU-E in a culture containing EPO (0.33 U/mL) only. (C) GM-CSF dose response of BFU-E in a culture containing EPA (×10,000 dilution:peak stimulation in Panel A) and EPO (0.33 U/mL). The mean number of BFU-E obtained in the control cultures (from two different donors) with only EPO (0.33 U/mL) were 17 and 56 per well (3.3 × 10⁴ cells in 0.1 mL). These values were defined as 100% in each experiment. Each point represents the mean value of two normal peripheral blood buffy coat samples assayed in duplicate. A 1:1,000 dilution of GM-CSF in these experiments was approximately equivalent to 250 pmol/L.
It has been reported that purified murine GM-CSF is capable of stimulating BFU-E. Metcalf et al showed by the sequential addition of purified GM-CSF, a burst-promoting activity (BPA) and EPO in a single hematopoietic progenitor cell culture assay, that the GM-CSF can stimulate the initial divisions of BFU-E, although it did not induce terminal maturation of BFU-E leading to EPO responsiveness. Our observations using simultaneous addition of human biosynthetic GM-CSF to BFU-E assays containing EPA and EPO or EPO alone did not result in stimulation of BFU-E. Since the addition of large amounts of GM-CSF to the EPO plus EPA assay caused a detectable inhibition of burst formation, some unidentified interactions between these stimulators and progenitor or accessory cells are suggested. In this context, others using the same recombinant GM-CSF sample observed a consistent stimulatory activity on BFU-E growth (David Nathan and Colin Steff, Dana-Farber Cancer Institute, Harvard Medical Society, personal communication, July 1985). Further experiments are required to elucidate the interactions of EPA, EPO, and GM-CSF on human BFU-E. Human GM-CSF also differs from murine multi-CSF (IL-3), which exerts stimulatory activity on multiple progenitors of erythroid, mega-karyocyte, eosinophil, and mast cell lineages as well as on granulocyte and monocyte lineages.

REFERENCES

23. Golde DW, Bersch N, Quan SG, Lusis AJ: Production of erythroblast-potentiating activity by a human T-lymphoblast cell line. Proc Natl Acad Sci USA 77:593, 1980
25. Rajendra BR, Sciorra LJ, Lee M-L: A new and simple technique for chromosomal preparations from peripheral blood lymphocytes, amniotic cell cultures, skin fibroblasts, bone marrow and single cell clones when the yield from harvests are low. Hum Genet 55:363, 1980

ACKNOWLEDGMENT

We thank Noelle Bersch, Sue Kaufman, and Shirley D. Quan for technical assistance; Steve Clark and Gordon Wong for their help; and Betty Koers and Wendy Aft for preparation of the manuscript.
Biosynthetic (recombinant) human granulocyte-macrophage colony-stimulating factor: effect on normal bone marrow and leukemia cell lines

M Tomonaga, DW Golde and JC Gasson