We have examined the biologic and physical properties of a human T-lymphocyte granulocyte-macrophage colony-stimulating factor (CSF). The source of the factor is a T-lymphoblast cell line (Mo) that was derived from a patient with a T-cell variant of hairy-cell leukemia. The Mo line constitutively produces a number of lymphokines that are normally produced by mitogen-stimulated T lymphocytes. Medium conditioned by Mo cells grown in the absence of serum is especially rich in CSF activity, and using this source we have purified the CSF to a specific activity of about 3.5 x 10^8 colonies per 10^6 Ficoll-Hypaque-separated human bone marrow cells plated per mg protein. The Mo CSF stimulates the formation of both granulocyte and macrophage colonies in vitro (in about equal numbers) and it has a relatively steep dose-response curve. Both the crude and purified preparations stimulated the formation of eosinophil as well as neutrophil colonies; it is unclear whether this is due to the presence of multiple factors with similar physical properties or a single factor with multiple activities. The CSF has little stimulating activity for mouse bone marrow progenitors. Physically, the Mo CSF is an acidic glycoprotein of molecular weight about 34,000. It binds to concanavalin A-Sepharose, is unusually resistant to denaturing agents and heat treatment, and is not inactivated in the presence of sulfhydryl reagents. The Mo CSF is distinct from factors stimulating erythroid colony formation and inhibiting neutrophil migration that are also produced by Mo cells. It differs in several physical and biologic properties from other human CSFs that have been characterized.

COLONY-STIMULATING FACTORS (CSFs) are required for the in vitro proliferation and differentiation of granulocyte-macrophage progenitors, and evidence is accumulating that the factors serve a physiologic role in the regulation of granulopoiesis. CSFs are produced by a variety of tissues, but cells of the monocyte-macrophage lineage and activated T lymphocytes are prominent human cellular sources. There appear to be several CSF subclasses in mice and in humans that stimulate the formation of macrophage colonies, both macrophage and neutrophil colonies, and eosinophil colonies. Physically, the colony-stimulating factors are quite heterogeneous, although much of the heterogeneity could be the result of aggregation or covalent modification of a common polypeptide chain. A human CSF of the subclass stimulating predominantly macrophage colonies has been purified from urine, and recently a CSF with similar properties was isolated from medium conditioned by a human pancreatic carcinoma cell line. In order to clarify the structural and functional relationships between different CSFs, and to examine the mode of action of CSFs at the molecular level, it will be necessary to obtain purified preparations of the various CSF subtypes for biochemical analysis and target cell receptor binding assays.

In this report we describe the properties and partial purification of a human T-lymphocyte CSF. The source of the factor is a T-lymphoblast cell line (Mo) that was derived from a patient with a T-cell variant of hairy-cell leukemia. The cell line constitutively produces and liberates into the medium several modulators of hematopoiesis, including a CSF and an activity that potentiates erythroid colony formation in vitro. The Mo CSF stimulates the formation of both granulocyte and macrophage colonies in vitro, and it differs functionally and physically from CSFs obtained from other sources.

MATERIALS AND METHODS

Bioassays

CSF activity was assayed using a two-layer agar technique as previously described. The target cells were normally 1 x 10^6 Ficoll-Hypaque-separated, light-density bone marrow cells obtained from normal volunteers. In some experiments the light density cells were further fractionated by petri-dish adherence to remove CSF-producing cells. The removal of adherent cells decreased the formation of small clusters but had little or no effect on the stimulation of colony formation by Mo CSF. Colonies (containing 40 or more cells) were scored after 11 days. One unit of activity is defined as the amount stimulating one colony in 10^6 separated human bone marrow cells plated. Cellular morphology in the colonies was examined either by staining individual colonies picked from culture with Wright's stain or by staining entire culture dishes for a lipase activity. The latter method was particularly useful for distinguishing granulocyte and macrophage colonies. Eosinophil colonies were identified by staining cultures with Dominici's stain containing phloxine or with luxol fast blue in urea. The latter
method was found more reliable and was used in all experiments reported here.

Small erythroid colonies (CFU-E) and large erythroid colonies (BFU-E) were grown in methylcellulose using normal human bone marrow and peripheral blood as previously described. Human urinary erythropoietin was obtained from the Division of Blood Diseases and Resources, National Heart, Lung, and Blood Institute.

Cell Lines and Media

The Mo cells have been in continuous culture for about 3 yr. and they retain the characteristics of relatively mature T lymphoblasts. More than 60% of the Mo cells rosette with sheep erythrocytes; they do not synthesize immunoglobulin; they are lysed by antithymocyte serum in the presence of complement, and they respond to phytohemagglutinin by increased 3H-thymidine incorporation. The cells do not contain measurable terminal deoxynucleotidyl transferase but they do retain the tartrate-resistant isozyme 5 of acid phosphatase, characteristic of hairy-cell leukemia. The Mo cells are not infected with Epstein-Barr virus. The Mo cells were generally cultured in alpha medium (Flow Laboratories, Inglewood, Calif.) containing 20% fetal calf serum (screened lot). Serum-free conditioned medium (CM) was obtained by culturing unwashed Mo cells in alpha medium for 7 days. 

Comparative Studies Utilizing CSFs from Placental- and Leukocyte-conditioned medium (CM)

Human placental CM was prepared as described. Using 50 µl of the resulting CM, about 70 colonies were obtained with the clonogenic human bone marrow CFU-G,M assay described; approximately the same number of colonies was stimulated by a peripheral leukocyte underlayer. Phytohemagglutinin-stimulated leukocyte conditioned medium was prepared by culturing Ficoll-Hypaque-separated peripheral blood mononuclear cells (3 x 10⁶ cells/ml) in alpha medium containing 20% fetal calf serum (screened lot). Serum-free conditioned medium (CM) was obtained by culturing unwashed cells in alpha medium for 7 days.

Purification of Mo CSF

Serum-free Mo CM (1.8 liter) was concentrated by ultrafiltration (using an Amicon apparatus equipped with a PM10 membrane) to 8 ml. The solution was then heated at 57°C for 30 min and the resulting precipitate removed by centrifugation (9,000 rpm, 15 min, using a Sorvall HB-4 rotor). The clear supernatant solution was applied to a 1.6 x 78-cm column of Ultrogel AcA 44 (LKB) equilibrated with PBS containing 0.01% polyethylene glycol (average molecular weight 6000). Fractions of 3.8 ml were collected at a rate of 7.2 ml/hr and assayed for CSF activity (Fig. 1). The major contaminating protein in serum-free Mo CM was serum albumin, which accounts for the protein peak eluting at molecular weight about 68,000. Presumably, the albumin was associated with Mo cells upon transfer from serum-containing to serum-free medium. Peak fractions (see Fig. 1) were pooled, dialyzed against 0.02 M Tris, pH 7.4, containing 0.01% polyethylene glycol, and applied to a 0.9 x 5-cm column of DEAE-Sephadex (Pharmacia) equilibrated with the same buffer. The column was washed with several volumes of equilibration buffer and then developed with a linear NaCl gradient (from 0 to 0.4 M NaCl in equilibration buffer) with a total volume of 90 ml. Fractions of 3.6 ml were collected at a rate of 5.4 ml/hr and those with peak activity, eluting between about 0.12 and 0.20 M NaCl (total volume, 18 ml) were pooled (Fig. 2). The pooled preparation was applied directly to a 0.9 x 1.0-cm column of concanavalin-A-Sepharose (Pharmacia) equilibrated with PBS, containing 0.01% polyethylene glycol. The column was washed with equilibration buffer (4 ml) and developed with equilibration buffer containing 0.2 M methyl-α-D-mannoside at the rate of about 2 ml/hr. Essentially all of the CSF activity bound to the lectin column and the bulk of the activity was eluted in the first two column volumes after the application of methyl-α-D-mannoside (Fig. 2). Unless otherwise stated, this was the source of CSF for the biological and physical studies employing purified Mo CSF.

Analysis of the purified preparation using electrophoresis suggested that the CSF was not homogeneous. Multiple protein bands were resolved using a native polyacrylamide gel electrophoresis system (pH 8.1, 7% acrylamide, 0.2% bisacrylamide), and two major protein bands, corresponding to molecular weights of about

![Fig. 1: Gel exclusion chromatography of Mo CSF. Sample preparation and chromatography using Ultrogel AcA 44 were as described under "Purification of Mo CSF." Molecular weight standards were blue dextran (void volume), bovine serum albumin (M₆, 68,000), carbonic anhydrase (M₆, 32,000), cytochrome c (M₆, 12,000) and 4-methylumbelliferone (included volume). Fractions 24 through 29 were pooled for further purification. Protein (Ψ); CSF activity (○).](https://www.bloodjournal.org/content/105/3/14.full.html)
**Isolated and Purified Mo CSF**

**Enzyme Treatments**

Treatments were as described in figure and table legends. Vibrio cholerae neuraminidase and pronase (45 U/mg) were from Calbiochem, subtilisin (type VII, 10.4 U/mg) and Clostridium perfringens neuraminidase (type VI) were from Sigma, and trypsin (TPCK treated, 256 U/mg) and chymotrypsin (61 U/mg) were from Worthington.

**Protein Assays**

Protein was determined using a dye-binding procedure. Materials were purchased from Bio Rad Laboratories, and bovine serum albumin was used as the standard.

**RESULTS**

**Isolation of Mo CSF**

Mo-conditioned medium. When cultured in alpha medium containing 15%–20% fetal calf serum the Mo cells continuously elaborated a CSF that stimulated the formation of granulocyte-macrophage colonies in vitro. The Mo CSF stimulated colony formation using light density, nonadherent human bone marrow cells, indicating that its action is not dependent on interactions with endogenous CSF-producing cells. The specific activity of the conditioned medium (CM) prepared in the presence of serum was about 700 U/mg protein (using light density human bone marrow target cells), roughly similar to the specific activities observed using several other sources of human CSF, such as placental CM, phytohemagglutinin-stimulated leukocyte CM, and lung CM. The Mo cells continued to release CSF when transferred into serum-free alpha medium, although their growth was slowed considerably. After 7 days of incubation in serum-free culture at a density of about $5 \times 10^6$ cells/ml, the medium had a specific activity in the range of 3000–20,000 U/mg protein. The absolute amount of CSF present in serum-containing cultures was much greater than in serum-free cultures. For example, 50 μl of serum-free CM stimulated the formation of 20 to 60 colonies while 10 μl of serum-containing CM stimulated up to 100 colonies.

**Purification.** Because of the higher specific activity of serum-free Mo CM, it was used as the starting material for purification experiments. A variety of fractionation methods were first surveyed using crude CM and partially purified preparations of Mo CSF.

**Isoelectric Focusing**

Flat-bed isoelectric focusing in granulated gel was performed as previously described with the following modifications: Focusing was for 12 hr at 3°C and 200 V. Gels contained 1.8% pH 4–6 ampholines (LKB) and 1.8% pH 3.5–10 ampholines. The anode reservoir contained 0.02 M sodium hydroxide and the cathode reservoir contained 0.02 M phosphoric acid.

The pH gradients for analytical and preparative isoelectric focusing were estimated by measuring the pH of gel eluates prepared using degassed water.

**Enzyme Treatments**

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due to the removal of inhibitors of colony formation during the first steps of the purification is probably stimulated by Mo CSF was greater than for CSFs.

The concentration of CSF used (between 20 and 80 colonies per plate). The fraction of macrophage colonies predominated slightly. The ratio of colony types was not greatly dependent upon the concentration of CSF used (between 20 and 80 colonies per plate). The fraction of macrophage colonies stimulated by Mo CSF was greater than for CSFs from human PHA-stimulated leukocyte CM and human placental CM (Table 2).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Activity (U x 10^5)</th>
<th>Protein (mg)</th>
<th>Specific Activity (U/mg x 10^3)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo-conditioned medium</td>
<td>900</td>
<td>272</td>
<td>3.3</td>
<td>100</td>
</tr>
<tr>
<td>Heat treatment</td>
<td>1,130</td>
<td>184</td>
<td>6.1</td>
<td>125</td>
</tr>
<tr>
<td>Uitrogl AAc44</td>
<td>1,115</td>
<td>30</td>
<td>383</td>
<td>124</td>
</tr>
<tr>
<td>DEAE Sephadex</td>
<td>381</td>
<td>0.72</td>
<td>530</td>
<td>42</td>
</tr>
<tr>
<td>Concanavalin-A-Sephrose</td>
<td>280</td>
<td>0.08</td>
<td>3,500</td>
<td>31</td>
</tr>
</tbody>
</table>

*Details presented in Materials and Methods.

All preparations of Mo CSF, including crude CM and the most highly purified fractions, stimulated eosinophil as well as neutrophil colonies (Table 2). The proportion of each colony type was highly dependent on the length of culture. Cultures examined between 6 and 10 days contained primarily neutrophil clusters and colonies, while later cultures contained primarily eosinophil colonies. Similar results were obtained using low concentrations (20 colonies per plate) and high concentrations (80 colonies per plate) of CSF.

*Species specificity.* Crude Mo CM gave little or no stimulation of macrophage-granulocyte colonies using mouse bone marrow cells, while partially purified preparations of Mo CSF showed very weak stimulation. The relative stimulation of mouse, as compared to human, target cells was considerably less for Mo CSF than for CSFs examined from other human sources (Fig. 4).

**Physical Properties**

**Stability.** The CSF activity in crude Mo CM was stable indefinitely when stored frozen at −20°C and for weeks when stored at 4°C. However, highly purified preparations of the CSF were relatively labile when stored either frozen or at 4°C, with the bulk of the activity being lost within days. The addition of protein (0.1% bovine serum albumin) or polyethylene glycol (0.01%) stabilized CSF preparations, and polyethylene glycol was routinely included in buffers during the latter stages of the purification of CSF.

The Mo CSF was stable over a wide pH range; most of the activity was recovered after incubating the CSF in buffers between pH 3 and pH 10 at 37°C for 1 hr or

**Bioclogical Activity**

**Dose-response curves.** The responsiveness of human bone marrow cells to various concentrations of Mo CSF was determined (Fig. 3). Curves relating the number and size of colonies observed in soft-agar cultures to the concentration of CSF were sigmoidal. High doses of the crude conditioned medium resulted in slight inhibition of colony formation. For example, using serum-containing CM maximal colony formation was observed at 20–50 µl per plate and about 20% fewer colonies were obtained at 200 µl per plate. This probably results from the presence of inhibitors, since the inhibition was not observed with partially purified preparations. Inhibition was less apparent using serum-free CM (Fig. 2), but the increase in total CSF activity after partial purification (Table 1) suggests that inhibitors are present. The inhibitors have not been characterized.

**Cell Morphology.** Crude and purified Mo CSF stimulated the formation of both granulocyte and macrophage colonies (Table 2). Normally, macrophage colonies predominated slightly. The ratio of colony types was not greatly dependent upon the concentration of CSF used (between 20 and 80 colonies per plate). The fraction of macrophage colonies stimulated by Mo CSF was greater than for CSFs from human PHA-stimulated leukocyte CM and human placental CM (Table 2).
T-LYMPHOCYTE COLONY-STIMULATING FACTOR

at 4°C overnight (data not shown). It was also quite stable in the presence of denaturing agents such as 8 M urea and 6 M guanidine hydrochloride (Table 3). Exposure to concentrations of mercaptoethanol as high as 0.1 M or to 1 mM dithiothreitol had little or no effect on CSF activity (Table 3).

Treatment of Mo CSF with a variety of proteases destroyed the activity (Table 3), suggesting that the CSF is protein in nature. However, the proteases were not removed prior to assaying CSF activity and their presence could have affected cell growth by mechanisms not involving CSF.

Heat inactivation. In contrast to CSFs from placental CM and stimulated leukocyte CM, the Mo CSF was stable at temperatures up to about 70°C (Fig. 5). Even at temperatures as high as 90°C, about half the CSF activity was recovered after 5 min of incubation (Fig. 5).

The possibility that these heat stability differences resulted from differences in the state of purity of the preparations was examined using mixing experiments. Mixtures of Mo CSF and placental or leukocyte CSF exhibited additive, biphasic kinetics of heat inactivation (data not shown). Additive kinetics would be expected if the heat stability was an inherent property of the CSFs but not if the stability was determined primarily by the presence of contaminants (such as proteases) in the preparations. Moreover, fractionation of the CSFs using gel exclusion chromatography, as described under Materials and Methods, affected their heat stabilities only slightly.

Molecular weight. When subjected to gel exclusion chromatography, the Mo-CSF activity eluted as a

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Table 2. Morphology of Colonies Stimulated by Mo CSF and Other Human CSFs

<table>
<thead>
<tr>
<th>Colony Types (% of Total ± SE)</th>
<th>Colony Types (% of Total ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF Source*</td>
<td>Colony Types (% of Total ± SE)</td>
</tr>
<tr>
<td>Mo CSF (crude CM)</td>
<td>Macrophage†</td>
</tr>
<tr>
<td></td>
<td>Granulocyte</td>
</tr>
<tr>
<td></td>
<td>Eosinophil and Neutrophil</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
</tr>
<tr>
<td></td>
<td>Granulocyte-Macrophage</td>
</tr>
<tr>
<td>Mo CSF (partially purified)</td>
<td>48 ± 4</td>
</tr>
<tr>
<td>Placental CM CSF</td>
<td>20 ± 9</td>
</tr>
<tr>
<td>PHA-stimulated</td>
<td>33 ± 7</td>
</tr>
<tr>
<td>leukocyte CM CSF</td>
<td>43 ± 2</td>
</tr>
<tr>
<td></td>
<td>78 ± 7</td>
</tr>
<tr>
<td></td>
<td>62 ± 7</td>
</tr>
</tbody>
</table>

*Experiments were performed using sufficient CSF to produce 40–50 colonies per 10⁶ human bone marrow cells plated after 11 days' culture. The CSFs from placental CM and PHA-stimulated leukocyte CM were partially purified as described under Materials and Methods.

†Eosinophil colonies were identified by staining with Luxol-fast blue in urea using a modification of previously described procedures. Colonies were placed on slides, fixed with methanol for 1 hr, and allowed to air dry. They were then rinsed with tap water for 15 min and stained with hematoxylin for 2 min. They were then rinsed and air dried. Eosinophils were identified by the green staining of the cytoplasm.

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Table 3. Effect of Denaturing Agents and Enzyme Treatments of Mo CSF

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CSF Activity Recovered (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (8 M)*</td>
<td>116 ± 8</td>
</tr>
<tr>
<td>Guanidine hydrochloride (6 M)*</td>
<td>126 ± 4</td>
</tr>
<tr>
<td>β-Mercaptoethanol (10 mM)*</td>
<td>100 ± 6</td>
</tr>
<tr>
<td>Pronase (0.1 mg/ml)†</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>Subtilisin (0.1 mg/ml)†</td>
<td>1.0 ± 0.5</td>
</tr>
<tr>
<td>Trypsin (0.1 mg/ml)†</td>
<td>1.7 ± 4</td>
</tr>
<tr>
<td>α-Chymotrypsin (1.0 mg/ml)†</td>
<td>1.5 ± 1.0</td>
</tr>
<tr>
<td>Neuraminidase (8 U/ml)†</td>
<td>94 ± 5</td>
</tr>
</tbody>
</table>

*Mo CSF partially purified by gel exclusion chromatography was exposed to the agent for 1 hr at 23°C in PBS. It was then exhaustively dialyzed against PBS and assayed for CSF activity.

†Mo CSF partially purified by gel exclusion chromatography was treated with the enzyme for 1 hr at 37°C in PBS and tested for residual CSF activity. In this experiment Vibrio cholerae neuraminidase was used. Sources of enzymes are given in Materials and Methods.
Heat inactivation of human CSFs. Samples of partially purified human CSFs (see Materials and Methods) were incubated at various temperatures in 0.02 M sodium phosphate, 0.15 M NaCl, pH 7.2, containing 0.1% bovine serum albumin. At intervals aliquots were removed and assayed for CSF activity.

A single major peak, in a volume corresponding to an apparent molecular weight of about 34,000 (Fig. 1). The molecular weight of the CSF did not change during the course of purification, although the crude conditioned medium contained variable amounts of a higher molecular weight form of CSF. This latter activity had an apparent molecular weight of about 50,000 and generally comprised less than 20% of the total activity (e.g., Fig. 1).

Isoelectric point and charge heterogeneity. Using isoelectric focusing in granulated gel, the Mo CSF from serum-free CM focused between pH 4.5 and pH 5.3 (Fig. 6). The CSF from serum-containing CM, on the other hand, was slightly more acidic, focusing between about pH 4.0 and pH 4.6 (e.g., Fig. 7). A similar charge difference was noted using ion-exchange chromatography; for example, the bulk of the activity from serum-containing medium eluted from DEAE-Sephadex (equilibrated with 0.02 M Tris, pH 7.4) between 0.18 M and 0.22 M NaCl, while CSF from serum-free medium eluted over a wider range of salt concentrations, between about 0.12 and 0.22 M NaCl (e.g., Fig. 2). In contrast to the variation in charge, the CSFs from serum-containing and serum-free Mo CM were not discernibly different in size (as judged by gel exclusion chromatography) or heat stability.

Experiments involving treatment with neuraminidase under similar conditions showed a qualitative shift in charge after neuraminidase treatment. The shift was reproducible and noted using both isoelectric focusing and ion-exchange chromatography.
dase suggested that the charge heterogeneity of the CSF is due in part to the presence of varying amounts of sialic acid. When CSF prepared from serum-containing Mo CM were subjected to isoelectric focusing in adjacent wells of polyacrylamide gels, neuraminidase treatment was found to shift the charge of the CSF toward a more basic pH (Fig. 7). This effect was also observed using ion exchange chromatography (data not shown). Similar experiments using CSF from serum-free CM resulted in a smaller, although reproducible, anodal shift in isoelectric point.

Carbohydrate. The Mo CSF appears to be glycosylated, since it was bound by the lectin concanavalin-A and was eluted using methyl-α-D mannoside (see Materials and Methods). Moreover, neuraminidase treatment altered the charge, but not the activity of Mo CSF, suggesting the presence of sialic acid (Fig. 7, Table 3).

Relationship to Other Hematopoietins Present in Mo-Conditioned Medium

The Mo T-lymphocyte cells liberate into the medium several lymphokines in addition to the CSF. These include an activity that stimulates human erythroid colony formation in vitro and an activity that inhibits the migration of neutrophils. To test whether these activities resulted from distinct factors, fractions obtained after chromatographic separations of Mo CM were tested for each activity. Using gel exclusion chromatography, the CSF eluted in a volume corresponding to apparent molecular weight 34,000 and was clearly separable from the activities potentiating erythroid colony formation and inhibiting neutrophil migration, both of which eluted in a volume corresponding to molecular weight about 45,000 (Fig. 8; R. Weisbart, personal communication). Moreover, the CSF was less heat stable than the latter activities (Fig. 5; see references 11 and 20 for stabilities of erythroid potentiating activity and neutrophil migration inhibitor, respectively). The purified CSF had little or no erythroid-potentiating activity as judged by the stimulation of human CFU-E or BFU-E in vitro; in fact, the addition of 50 U of purified CSF to human erythroid cultures (BFU-E) decreased colony formation about 20%.

DISCUSSION

The Mo T-lymphoblast cell line constitutively elaborates various products known to be produced by mitogen-stimulated normal T lymphocytes. These include an activity stimulating the proliferation of erythroid progenitors, an activity inhibiting the migration of neutrophils, and a granulocyte-macrophage CSF activity. Thus, the Mo cell line provides a useful homogeneous cell source of the human T-lymphocyte hematopoietic modulators for biochemical and physiologic studies. The Mo cells continue to liberate the modulators when cultured in medium lacking serum, and such serum-free CM is especially rich in modulator activities.

The CSF from Mo-conditioned medium was purified to a specific activity of about 3.5 x 10^6 U per mg protein. This is the highest specific activity reported for a human CSF (using human bone marrow target cells), although differences in CSF clonogenic assay conditions and in bone marrow cell preparations affect the absolute activities obtained in different laboratories. The purification protocol involves a combination of heat treatment, gel exclusion chromatography, ion exchange chromatography, and concanavalin-A-Sepharose affinity chromatography. The inclusion of 0.01% polyethylene glycol during the latter stages of the purification stabilized the CSF activity, improving the yield. As judged by electrophoresis, the final product was not homogeneous, although the relatively high specific activity of CSF suggests that CSF is not a minor protein species in the final preparation. Half-maximal colony formation was obtained using about 10 ng of the partially purified Mo CSF per 2 ml culture dish, indicating that the CSF is active well below nanomolar concentrations.

The Mo CSF stimulated the formation of both granulocyte and macrophage colonies in vitro in about equal numbers using human bone marrow target cells. The ratio of colony types was not greatly dependent on

![Figure 8](image-url)
the amount of CSF used. With the exception of the removal of inhibitors, the functional and physical properties of the CSF activity did not change discernibly during purification.

All fractions of the Mo CSF examined stimulated eosinophil as well as neutrophil colonies. Thus, the factor stimulating eosinophils appears to be physically quite similar to the factor(s) stimulating neutrophils and macrophages, or, alternatively, the activities may reside in a common factor. Previous studies utilizing placental conditioned medium suggested that the CSF stimulating eosinophil colonies is distinct from that stimulating macrophages and neutrophils, since partial separation was achieved using various fractionation procedures. Using similar techniques we have been unable to resolve the activities for the different cell types in Mo CM. The presence of eosinophil CSF in Mo CM is in accordance with previous work, indicating that mitogen-stimulated lymphocytes are a rich cellular source of this CSF.

Both purified and crude Mo CSF showed very little activity toward mouse bone marrow progenitors. This is in sharp contrast to most other human CSFs that have been examined, including those from stimulated leukocyte CM (Fig. 2), lung CM, urine, placental CM (Fig. 2), a monocyte cell line, and a pancreatic carcinoma cell line. On the basis of relative activity toward mouse marrow, as compared with human marrow, these CSFs exhibited at least 10-fold greater relative activity than did the Mo CSF. However, a CSF exhibiting a species specificity similar to that of Mo CSF was described in a preliminary communication. The factor, obtained from human embryo kidney CM, had a molecular weight of 30,000–40,000 and stimulated human but not mouse colony growth.

The Mo CSF also differs in certain characteristics from human CSFs that stimulate both granulocyte and macrophage production. Most notably, it has relatively little activity toward mouse marrow (Fig. 4; discussed above) and it has exceptional heat stability (Fig. 5). In addition, unlike the CSFs from placental CM, it binds to concanavalin A. One possibility is that these differences have a “trivial” explanation, such as modification of a common polypeptide chain or the presence of multiple modulator activities. Another is that the Mo CSF is functionally and structurally distinct from other human CSFs that have been characterized.

ACKNOWLEDGMENT

We thank Shirley G. Quan, Robert Wangenstein, and Carol Paoquin for invaluable assistance with experiments, Dr. John Wells and Claire Cardin for assistance with lipase staining of agar CFU-G,M cultures, and Elizabeth P. Koers for preparing the manuscript.

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Purification and characterization of a human T-lymphocyte-derived granulocyte-macrophage colony-stimulating factor

AJ Lusis, DH Quon and DW Golde