Rapid progress has occurred recently in characterizing the molecular nature of the specific glycoprotein colony-stimulating factors (CSFs) controlling the proliferation and some functional activities of granulocytes and monocyte-macrophages. All four known murine CSFs have been purified, and cDNAs for two have been cloned and expressed by mammalian and bacterial cells. Similarly, three human CSFs have been purified, and cDNAs for two cloned and expressed. This work has opened up the exciting prospects of testing the effects of these recombinant CSFs on hematopoiesis in vivo. Each CSF has a broader range of hematopoietic target cells than previously suspected, and it is now clear that the CSFs are not simply proliferative stimuli but can also regulate the functional activity of mature cells. There are increasing reasons to believe that these CSFs will be useful therapeutic agents in stimulating hematopoietic regeneration in leukopenic states and the functional activity of granulocytes and monocytes in infections.

A major consequence of the development of semisolid culture systems supporting the clonal growth of hematopoietic colonies was the recognition that hematopoietic precursor cells are unable to survive or proliferate in vitro unless specifically stimulated. This led to the discovery of a group of specific regulatory glycoproteins that stimulate cell proliferation and at least some aspects of the functional activity of these various hematopoietic subpopulations.

Since these regulatory glycoproteins were first identified because of their ability to stimulate precursor cells to form colonies of progeny cells, they are usually referred to as the colony-stimulating factors (CSFs). The best characterized of these CSFs are those controlling granulocyte-macrophage (GM) populations, the granulocyte-macrophage CSFs. It is useful to comment in passing that analogous regulatory glycoproteins exist for hematopoietic cells in other lineages, bearing a variety of names. For example, erythropoietin has the typical general properties of a CSF, as does the T cell regulator, interleukin 2 (IL 2 or TCGF), and less well-characterized regulators with the general properties of CSFs have been identified for cells of the B lymphocyte lineage (eg, BCGF) and the eosinophil series (EDF).

The granulocyte-macrophage colony-stimulating factors

Four CSFs have so far been clearly identified as interacting to control the proliferation of mouse granulocytes and macrophages (Table 1). As indicated in the table, there is no uniformly accepted nomenclature for these CSFs and, because of the multiple properties of the CSFs, there are difficulties in deciding on a terminology that will be both descriptive and unambiguous. For the present, we prefer at least for the murine CSFs to use a prefix to indicate the major cell populations produced as a consequence of their action at low to moderate concentrations. Thus GM-CSF stimulates the production of both granulocytic and macrophage cells, G-CSF preferentially stimulates the formation of granulocytes and M-CSF the formation of macrophages, and Multi-CSF was chosen as an appropriate abbreviation for the molecule that has not only the capacity to stimulate granulocyte and macrophage formation but also an exceptionally broad range of proliferative effects, including actions on erythroid, megakaryocytic, eosinophil, mast, stem, and multipotential cells.

IL 3 is a term commonly used for Multi-CSF but carries with it an unsubstantiated assumption that it is an “interleukocyte” (IL) factor exclusively of leukocyte (T lymphocyte) origin and does not indicate the action of this molecule on erythroid and stem cell populations. An alternative numbering system has been advanced—so far only CSF-1 has been used extensively (for M-CSF)—but this system has the disadvantage of giving no indication of a factor’s major action. It is probably premature to attempt a definitive nomenclature at this time since amino acid sequence and
membrane receptor cross-binding studies in progress may ultimately allow a rational grouping and acceptable nomenclature for these CSFs.

For human granulocytes and macrophages, four CSFs have been identified so far as being active on these populations, and these are also listed in Table 1. The general biology of these CSFs, the sites and control of CSF production, and other details have recently been reviewed extensively elsewhere.3 Here attention will be concentrated on recent work on the molecular biology of these CSFs and certain aspects of their function.

MURINE GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR (GM-CSF)

Mouse GM-CSF able to stimulate granulocyte and/or macrophage colony formation was purified to homogeneity from mouse lung conditioned medium as a glycoprotein of mol wt 23,000.12 Many mouse tissues contain and can synthesize GM-CSF with similar biologic properties, but these tissue-derived GM-CSFs differed widely in mol wt, from 23,000 to 200,000.3 However, studies on the GM-CSFs semipurified from these various organ sources suggested that the differences were ascribable to a combination of technical artifacts and varying patterns of glycosylation.33 Sequence homology exists between human and murine GM-CSFs,32 and sequence homology and partial cross-reactivity between human urine CSF and murine M-CSF.3

Mouse genomic GM-CSF exists in single-copy form, confirming the biochemical conclusions on the essential identity of the various tissue-derived GM-CSFs, and the gene has been localized on chromosome 11.14 The GM-CSF gene contains four exons,38,39 but the long mRNA form appears to be initiated from a further exon and promoter located at least 10 kilobases (kb) S’ to the main body of the gene.48 Comparison of the nucleotide sequence of the GM-CSF gene with that of other hematopoietic growth factor genes (mouse Multi-CSF, mouse IL 2, human IL 2, and human interferon γ) has revealed a common decanucleotide (5’-GPuGPuTTPyCAPy-3’) within their respective S’ flanking regions18 that could be involved in some types of coordinate regulation of the production of these factors, for example, that following lectin stimulation of T lymphocytes.40-42

Expression of GM-CSF cDNA has been obtained using monkey COS cells43,44 or a bacterial expression system35 (Metcalf D, Burgess AW, Johnson GR, DeLamarter J, unpublished data). In both cases, the recombinant GM-CSF has the typical in vitro properties of native GM-CSF. These are the ability (1) to stimulate granulocyte and macrophage colony formation and, at high to very high concentrations, eosinophil, megakaryocyte, and erythroid colony formation, (2) to stimulate the proliferation of GM-CSF-dependent continuous cell lines, such as FDC-P1, and (3) to compete for receptor binding on hematopoietic cells by 125I-labeled native GM-CSF. Antiserum against recombinant GM-CSF also neutralizes the biologic activity of native GM-CSF.35

Based on NH₂-terminus amino acid sequence data obtained from purified lung GM-CSF,11 oligonucleotide probes were used to isolate cDNAs for GM-CSF, initially from a library constructed from lung mRNA and subsequently from a library constructed from mRNA from a concanavalin A–primed T lymphocyte clone (LB3).34 Sequencing of these cDNA clones indicated that the mature GM-CSF polypeptide contains 124 amino acids and is of mol wt 14,138.34 The polypeptide contains four cysteine residues, likely to be linked by mandatory disulphide bridges since mercaptoethanol destroys the biologic activity of GM-CSF. In subsequent studies, an essentially identical cDNA clone has been isolated from a mouse EL-4-derived library.35

Deletion and survey studies on GM-CSF cDNA clones indicated that the mRNA of GM-CSF can contain two AUG initiation codons.34 From the amino acid sequences involved, the longer translation product could be displayed on the cell membrane, a possibility of interest in view of evidence suggesting that some cells, particularly in the marrow stroma, may control granulocyte proliferation by cell contact processes.36,37

Table 1. The Known Granulocyte-Macrophage Colony-Stimulating Factors

<table>
<thead>
<tr>
<th>Species</th>
<th>Name</th>
<th>Alternative Nomenclature</th>
<th>Mol Wt of Glycoprotein</th>
<th>Purified</th>
<th>cDNA Cloned</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>GM-CSF</td>
<td>MGI-1G 10</td>
<td>23,000</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>G-CSF</td>
<td>MGI-1G 11</td>
<td>25,000</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>M-CSF</td>
<td>CSF-1,14 MGI-1M 11</td>
<td>40,000–70,000</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Multi-CSF</td>
<td>IL 3,18 BPA,19 SAF,20 HCF,21 PSF,22 mast cell growth factor 23</td>
<td>23,000–30,000</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Human</td>
<td>GM-CSF 28</td>
<td>NIF-T,17 CSFa</td>
<td>22,000</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>G-CSF</td>
<td>CSFβ</td>
<td>≤ 30,000</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Human urine CSF</td>
<td></td>
<td>45,000</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Pluripoietin</td>
<td></td>
<td>18,000</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

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<tr>
<td></td>
<td>Pluripoietin</td>
<td></td>
<td>18,000</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Sequence homology exists between human CSFa and murine GM-CSF,14,28 complete functional cross-reactivity between human CSFa and murine G-CSF,32 and sequence homology and partial cross-reactivity between human urine CSF and murine M-CSF.30

Based on granulocyte-macrophage colony formation in bone marrow cultures, native GM-CSF has an activity of 12 × 10⁹ U/mg44 (50 units is the concentration stimulating half-maximal numbers of colonies to develop in cultures of mouse bone marrow cells), while bacterially synthesized recombinant GM-CSF has an activity of 5 × 10⁹ U/mg. This indicates that for both forms of GM-CSF, 50% colony formation is stimulated by a concentration of approximately 10⁻¹² mol/L. The data demonstrating the in vitro activity of nonglycosylated bacterially synthesized GM-CSF35 confirm earlier evidence that the quite large carbohydrate moiety of
the molecule is not necessary for biologic activity in vitro, a conclusion true also for the other murine CSFs.

MURINE MULTIPOTENTIAL COLONY-STIMULATING FACTOR (MULTI-CSF)

The only normal cell type so far documented as being able to synthesize Multi-CSF (also referred to as IL 3, BPA, PSF, HCGF, SAF, and mast cell growth factor) is the antigen- or mitogen-primed T lymphocyte. However, Multi-CSF is also synthesized constitutively by the myelomonocytic leukemia cell line, WEHI-3B,22'45 and by a T hybridoma.46 Multi-CSF has been purified to homogeneity under this24 and other conditions,25'26 with or without NH2-terminus amino acid sequence analysis. Estimates of mol wt ranged from 23,000 to 30,000, possibly due to a varying carbohydrate content, and Multi-CSF stimulates half-maximal proliferative responses at $10^{-12}$ to $10^{-13}$ mol/L, according to the type of target population used.

Clones of cDNA for murine Multi-CSF (IL 3) have been isolated from a WEHI-3B-derived cDNA library25 or a T lymphocyte-derived cDNA library.26 Clones were identified by hybrid selection of mRNA using highly responsive continuous cell lines to detect Multi-CSF produced by oocytes injected with the selected mRNA. The mature Multi-CSF polypeptide appears to contain 140 amino acids and to have a mol wt of approximately 15,600. Like GM-CSF, the Multi-CSF polypeptide also contains four cysteine residues, probably linked by mandatory disulfide bridges.

The Multi-CSF gene exists in single-copy form and has five exons,48'49 and linkage studies with c-erb B, known to be on chromosome 1, have located the Multi-CSF gene on chromosome 11.50 The same chromosome on which the GM-CSF gene has been located. Many T lymphocyte clones have the ability to synthesize both GM-CSF and Multi-CSF, and exposure of such clones to antigens or inducers such as concanavalin A can cause a spectacular increase in transcription of both genes,42 suggesting some type of common induction process.

A survey of different Multi-CSF cDNA clones has revealed essentially the same situation as discussed for GM-CSF. A variant of longer length, encoded by an additional exon at least 14 kb upstream of the Multi-CSF gene, has been observed, again raising the possibility of production by some cells of a longer, membrane-displayed Multi-CSF (Gough NM, King J, Metcalf D, Dunn AR, unpublished data).

Expression of Multi-CSF cDNA has been obtained using both monkey COS cells43,45 and an E. coli expression system51 (Metcalf D, Johnson GR, Nicola NA, unpublished data). In both cases, the recombinant Multi-CSF has the full range of in vitro properties previously noted for purified native Multi-CSF—not only the ability to stimulate granulocyte and/or macrophage colony formation but also an ability to stimulate (1) CFU-S self-generation, (2) colony formation by multipotential cells, BFU-E, CFU-E, eosinophil, and megakaryocyte progenitors, and (3) the proliferation of mast cell lines and Multi-CSF-dependent hematopoietic cell lines, such as 32D.31'33 Recombinant Multi-CSF binds to the same hematopoietic cells as the native form and competes on an equimolar basis for receptor binding (Nicola NA, Metcalf D, unpublished data) and is neutralized by antisera prepared against purified interleukin 3 (Multi-CSF).31

MURINE GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF)

Murine G-CSF can be synthesized in vitro by the same wide range of tissues known to synthesize GM-CSF.34 G-CSF has been purified to homogeneity from mouse lung conditioned medium as a glycoprotein of mol wt 25,000.15 The molecule appears to be monomeric in form and to require internal disulfide bridges for biologic activity.

G-CSF stimulates the formation of half-maximal colony numbers at a concentration of $5 \times 10^{-13}$ mol/L.15 At low or medium concentrations, G-CSF stimulates exclusively the formation of granulocytic colonies that are characterized by their small average size, their maturity, and their relatively small total numbers.55 Sequential observations indicated that G-CSF can initiate proliferation in many additional GM progenitor cells but fails to sustain this beyond two to four days.

G-CSF has a powerful capacity to induce terminal differentiation in WEHI-3B myelomonocytic leukemic cells,15,56 and because of this, it was initially termed differentiation factor (DF).34 This molecule needs to be distinguished from a larger molecule of mol wt 62,000, called D-factor, able to induce differentiation in M1 myeloid leukemia cells. D-factor has been purified from medium conditioned by L cells and Ehrlich ascites tumor cells,57,58 and this factor has also been termed MGI-2.11 This differentiation-inducing factor has no colony-stimulating activity for normal hematopoietic cells.

MURINE MACROPHAGE COLONY-STIMULATING FACTOR (M-CSF)

Murine M-CSF (CSF-1) has been shown to be a product of fibroblasts,17 embryonic yolk sac,59 whole embryonic, and pregnant mouse uterus.60 It was purified to homogeneity from L cell conditioned medium as a glycoprotein of mol wt 70,000,17,61,62 and partial NH2-terminus amino acid sequence data have been obtained. A varying carbohydrate content appears to be the basis for the lower mol wt forms of this CSF detected in yolk sac and pregnant mouse uterus. M-CSF stimulates half-maximal colony formation at a concentration of $5 \times 10^{-13}$ to $5 \times 10^{-12}$ mol/L,63 and differs from the other three murine CSFs in being a dimer of two equal subunits, the subunit polypeptides having a mol wt of approximately 14,000.64 The subunits have no colony-stimulating activity and do not bind anti-M-CSF serum.

M-CSF stimulates the formation of predominantly macrophage colonies, but at all concentrations in serum-containing cultures, it also stimulates the formation of a small percentage of granulocyte-containing colonies.34 When combined with hemopoietin I (not in itself a colony-stimulating factor), M-CSF has a capacity to stimulate the formation of very large macrophage-containing colonies. Analysis has suggested that hemopoietin I induces the
expression of M-CSF receptors on immature progenitor cells, possibly allowing responsiveness of such cells to M-CSF stimulation that is not seen when untreated cells are stimulated by M-CSF.63

RELATIONSHIPS BETWEEN THE MURINE CSFs

For each granulocyte-macrophage CSF there are characteristic differences in the proportions of granulocytic and macrophage colonies stimulated. Thus high concentrations of GM-CSF stimulate the formation of a high proportion of granulocyte-containing colonies, while low concentrations stimulate mainly the formation of macrophage-containing colonies.64,65 In serum-free cultures, GM-CSF exhibits little capacity to stimulate granulocyte colony formation, but this is restored by addition of hydrocortisone.66 G-CSF is a selective granulocyte colony stimulus at low concentrations and only at high concentrations stimulates the formation of macrophage-containing colonies.55 For Multi-CSF, the proportions of granulocytic-containing and macrophage-containing colonies are not altered by concentration changes,65 and with M-CSF, most colonies are composed of macrophages, although at all concentrations, a few granulocyte-containing colonies also develop.65

All four CSFs exhibit a capacity to stimulate the proliferation of cells in other hematopoietic lineages, but this varies widely in magnitude. At one extreme, Multi-CSF is also an effective proliferative stimulus for stem, multipotential, erythroid, eosinophil, megakaryocytic, and mast cells,72,24,67,51–53 while at the other extreme, M-CSF exhibits no additional actions other than an ability to act in collaboration with hemopoietin I to stimulate as yet undefined progenitor cells with a high proliferative potential.63 GM-CSF is intermediate in position. At high concentrations (>200 U/mL), it is an effective eosinophil proliferative stimulus,55,65 and, while not supporting stem cell self-generation, at relatively low concentrations it can initiate but not sustain cell division in multipotential, erythroid, and megakaryocytic progenitors.3 Use of very high concentrations of GM-CSF (>1,600 U/mL) has shown that this CSF is an effective proliferative stimulus for megakaryocyte and mixed erythroid colony formation (Metcalf D, Johnson GR, unpublished data). At the concentrations tested so far, G-CSF has no proliferative action on eosinophil populations or stem cells but can initiate proliferation in multipotential and erythroid progenitors, although this is a weaker action than that exhibited by GM-CSF.55

Care is required by using purified or single progenitor cells or clone transfer studies to establish that a particular action of a CSF is a genuine direct effect on the precursor cells involved. Certain indirect, or “cascade,” effects of CSFs have been noted that can give rise to apparent ambiguities. For example, low concentrations of purified GM-CSF can stimulate CFU-E to form erythroid colonies by such an indirect action.65 Similarly, M-CSF can stimulate macrophages to produce G-CSF, again causing misleading effects when M-CSF is used in some types of cultures.65

The proliferation of clones initiated by GM progenitor cells in response to stimulation by one CSF can be sustained by transfer of the clone to cultures containing one of the other three.68 The most striking example of this is the almost complete cross-responsiveness of most GM precursors to both GM-CSF and Multi-CSF.70 This overlapping control of GM progenitor cells is also demonstrable in cultures containing pairs of purified CSFs, e.g., M-CSF plus G-CSF, where potentiation of both granulocytic and macrophage colony formation is observed.3,71

The functional overlap on bone marrow cells of the four CSFs is the more remarkable since in the case of the two whose actions are most similar—GM-CSF and Multi-CSF—there is no sequence homology between the two molecules and quite different predicted secondary structure.14 Similarly, the sequence data for M-CSF indicate no homology with either GM-CSF or Multi-CSF, and the NH2-terminus sequence data for G-CSF again indicate no homology with the other CSFs (Nicola NA, Simpson R, unpublished data).

MEMBRANE RECEPTORS FOR MURINE CSFs

The lack of structural homology between CSFs is substantiated by the documentation of distinct but coexpressed membrane receptors for the four CSFs.

Studies using 125I-labeled M-CSF showed that a single class of specific membrane receptor existed and identified the receptor as a protein of mol wt 165,00072,73 with receptor numbers highest on cells of the monocyte-macrophage lineage.74–75 This receptor has been purified by affinity chromatography, and analysis has shown the receptor to be structurally related, and possibly identical, to the c-fms proto-oncogene product that is expressed at high levels in mature macrophages.76 This is of considerable interest in view of the possibility of links between the CSFs and oncogenes and the previous demonstration that v-erb B encodes for a protein that is a truncated form of the EGF receptor.77

Although these studies suggested limitation of M-CSF receptors to cells of the monocyte-macrophage series and some undifferentiated cells, one report has described labeling of cells of the granulocyte series following incubation with 125I M-CSF,78 a finding in better agreement with the ability of M-CSF to support the proliferation of granulocytic clones.69 Receptor numbers for M-CSF are relatively high (up to 50,000/cell) on some macrophage tumor cell lines, while lower numbers (3,000 to 15,000/cell) were demonstrated on mature monocyte-macrophages from various normal tissue sources.16,74

Use of 125I G-CSF has documented the existence of a single class of specific receptor for G-CSF on the membranes of all murine granulocytic cells, receptor density increasing as the cells mature to postmitotic polymorphs.79 Receptors were also demonstrable on some cells in the monocytic lineage but not on eosinophil, erythroid, or lymphoid cells. Average receptor numbers were 50 to 500/cell, and, with an apparent dissociation constant for binding of 60 to 80 pmol/L, G-CSF appears to be able to exert half-maximal proliferative effects on responding cells at low receptor occupancy. The G-CSF receptor is a monomer of mol wt 150,000 (Nicola NA, Peterson L, unpublished data).

Murine G-CSF binds with equal efficiency to human
granulocytic cells, although with human cells the immature promyelocytes and myelocytes exhibit the highest numbers of receptors.\textsuperscript{32} Competition studies suggested that the human receptors binding G-CSF were likely to be receptors for human CSFβ. Studies using \textsuperscript{125}I-labeled GM-CSF have demonstrated the presence on normal marrow cells of both high- and low-affinity receptors (average number 70/cell and 350/cell, respectively).\textsuperscript{30} All granulocytes, monocytes, and eosinophils bear receptors for GM-CSF, the numbers decreasing with increasing cellular maturation and with mature eosinophils exhibiting half the receptor numbers of neutrophils. No receptors were noted on lymphoid or erythroid cells (Metcalf D, Walker F, Burgess AW, unpublished data). A single molecular species of receptor has been observed of mol wt 51,000.\textsuperscript{30}

High numbers of receptors for Multi-CSF (IL 3) of a single class have been observed on continuous hematopoietic cell lines dependent on Multi-CSF (1,000–5,000/cell)\textsuperscript{31} (Nicola NA, Peterson L, unpublished data). Lower receptor numbers have been observed on normal marrow cells (50–1,000/cell), and in autoradiographs all granulocytic, monocytic, and eosinophil cells were labeled, with receptor numbers decreasing with increasing maturation and with eosinophils exhibiting twice the labeling of neutrophils. No labeling of lymphoid or nucleated erythroid cells was observed (Metcalf D, Nicola NA, unpublished data). Multi-CSF receptors of both mol wt 55,000 and mol wt 75,000 have been observed.

It appears that most murine granulocyte-macrophage cells simultaneously exhibit receptors for three or four CSFs, an apparently redundant situation whose purpose is not clear. Recent studies have indicated intriguing cross-influences between the different CSF receptors. There is no direct competition for receptor binding between the CSFs, but binding of Multi-CSF to its receptors leads to down-modulation of receptors for all other CSFs; binding of GM-CSF to its receptors does not influence Multi-CSF receptor binding but down-modulates receptors for G-CSF and M-CSF; high concentrations of M-CSF down-modulate GM-CSF receptors; and high concentrations of G-CSF down-modulate receptors for M-CSF.\textsuperscript{65}

**HUMAN GRANULOCYTE-MACROPHAGE CSFs**

Studies have identified at least two biochemically separable forms of CSF produced by various human cells that are able to stimulate granulocyte-macrophage colony formation by human cells.\textsuperscript{12} From their sequence of elution from hydrophobic columns, these were labeled α (nonbinding) and β (binding).\textsuperscript{12} CSFa preferentially stimulates day 14 colonies composed mainly of monocytes and macrophages, while CSFβ stimulates the formation mainly of day 7 colonies, a high proportion of which are granulocytic in composition. CSFa, but not CSFβ, also stimulates eosinophil colony formation.

**Human granulocyte-macrophage CSF (CSFa).** Gasson et al\textsuperscript{27} reported the purification to homogeneity of a granulocyte-macrophage CSF from medium conditioned by the Mo hairy T leukemia cell line. This was a glycoprotein of mol wt 22,000.

Clones of cDNA for human GM-CSF have been isolated by direct expression screening of cDNA libraries from mRNA from Mo leukemic cells\textsuperscript{28} or a human T lymphocyte cell line.\textsuperscript{45} Transfection of these clones to monkey COS cells led to the production of a CSF able to stimulate granulocyte-macrophage colony formation by human cells. Sequencing of these cDNA clones indicated that the mature GM-CSF polypeptide contains 127 amino acids (mol wt ± 14,000) and includes four cysteine residues. In the protein-coding region there is 70% nucleotide homology with the equivalent region of murine GM-CSF, including conservation of the four cysteine residues, but there is no species cross-reactivity between murine and human GM-CSF. From the identity of deduced and observed NH\textsubscript{2}-terminus sequence data, both cDNA clones code for the same GM-CSF as purified from Mo cell conditioned medium.\textsuperscript{27}

The recombinant CSF produced by transfected COS cells was purified to homogeneity and had mol wt 19,000 and a specific activity of 4 × 10\textsuperscript{6} U/mg protein.\textsuperscript{28} Analysis of the biologic activities of recombinant CSF showed that it stimulates granulocyte and/or macrophage colony formation by human cells, colony numbers being highest at day 14 of incubation, at which time most colonies were monocytomacrophage in composition.\textsuperscript{12,56} Recombinant CSF is also an effective proliferative stimulus for eosinophil colony formation.\textsuperscript{12,56} These actions are due to a direct action of the CSF on responding progenitor cells.\textsuperscript{12} Recombinant GM-CSF is also able, via mainly indirect action, to potentiate erythroid and multipotential (GEMM) colony formation in combination with erythropoietin and is able to stimulate the functional activity of both neutrophils and eosinophils. Based on these properties, this recombinant GM-CSF has been identified as CSFa.\textsuperscript{66}

**Human CSFβ.** Human CSFβ has not been purified to homogeneity but is a preferential stimulus for relatively mature granulocytic progenitors (promyelocytes and myelocytes)\textsuperscript{37} and stimulates the formation of comparable murine granulocytic colonies. It has the size and hydrophobic properties of murine G-CSF, exhibits a capacity to induce differentiation in murine WEHI-3B leukemic cells equivalent to that of murine G-CSF, and competes at equivalent biologic concentrations with binding of \textsuperscript{125}I-G-CSF to either murine or human cells.\textsuperscript{32} Based on these properties, it is likely to be a close analogue of murine G-CSF.

**Human pluripotin.** The purification to homogeneity has been reported of a CSF from medium conditioned by the human bladder cancer cell line 5637.\textsuperscript{31} This CSF, mol wt 18,000, was reported to have the ability to stimulate granulocyte and macrophage colony formation, to potentiate erythroid and multipotential colony formation by human cells, and to induce differentiation in HL60 and murine WEHI-3B colonies. These properties appear to be a combination of those exhibited by CSFa and β.

**Human urinary CSF.** CSF in human urine, active in stimulating granulocyte-macrophage colony formation by murine cells, was the first CSF to be purified to homogeneity.\textsuperscript{29} This CSF is a glycoprotein of mol wt 45,000, and while...
it has little direct proliferative effect on human GM progenitor cells, it was shown to be able to stimulate human granulocyte-macrophage colony formation indirectly, probably by stimulating adherent accessory cells to produce a human-active CSF.\textsuperscript{3,8,9}

Use of anti-murine M-CSF sera and competitive binding studies using \textsuperscript{125}I M-CSF indicated that the human molecule was likely to be similar to mouse M-CSF,\textsuperscript{10} and this has been confirmed by the demonstration that human urinary CSF, like M-CSF, is a dimer with two equal subunits,\textsuperscript{30} with NH\textsubscript{2}-terminus sequence data indicating homology with murine M-CSF.\textsuperscript{30}

A cDNA for a human CSF has been isolated indirectly using nucleotide probes based on NH\textsubscript{2}-terminus sequence for human urinary CSF.\textsuperscript{30} These probes were used to isolate a genomic clone, and with this probe a cDNA clone was isolated from a library constructed from mRNA from a human pancreatic cancer cell line known to be able to secrete CSF. The COS cell expression product of this clone stimulates macrophage colony formation by mouse bone marrow cells and is neutralized by anti-human urine CSF serum.

**GENERAL COMMENTS ON THE STRUCTURE OF THE CSFs**

The CSFs show no sequence homology with any other known proteins, growth factors, or oncogenes. This structural uniqueness of the CSFs is substantiated by two biologic facts: (1) The CSFs are the only agents able to stimulate directly the proliferation of granulocyte-macrophage populations.

None of the known growth factors have direct proliferative effects on GM cells, and even where agents such as the phorbol esters have been shown to stimulate colony formation,\textsuperscript{90,91} analysis has proved this to be an indirect effect mediated by stimulation of monocyte-macrophages in the culture to produce an active CSF.\textsuperscript{92} (2) A wide range of polypeptide growth factors have been shown to be unable to compete with CSFs for binding to their membrane receptors.

The sequence dissimilarity between the CSFs within one species indicates that although these molecules have highly specific biologic activity, often of a similar nature, they are not derivatives of some common evolutionary ancestral regulatory molecule.

**MULTIPLE FUNCTIONS OF THE CSFs**

Although the GM colony-stimulating factors were identified on the basis of their ability to stimulate the clonal proliferation of hematopoietic precursor cells, each exhibits three other functional activities: (1) They are necessary for, or promote, cell survival in vitro of both progenitor and mature end cells,\textsuperscript{94,95} (2) they can commit bipotential GM progenitors to a restricted differentiation pathway of granulocyte or macrophage formation, an irreversible asymmetric process requiring passage of the cells through one or two cell divisions in the presence of the committing CSF,\textsuperscript{85} and (3) they can stimulate a number of functional aspects of mature granulocytes, eosinophils, and macrophages. These effects include survival, cell mobility, shape, phagocytic activity, synthesis of biologically active molecules, antibody-dependent cytotoxicity, autofluorescence, and expression of various membrane markers.\textsuperscript{3,7,8,9,5-108}

While the CSFs are not the only agents able to stimulate end cell functional activity, their action is rapid and readily measurable and can often be demonstrated using CSF concentrations lower than those required for proliferative stimulation. The CSFs should not, therefore, be viewed solely as proliferative agents but also as being capable of stimulating significant changes in the functional activity of existing mature cells.

**THE CSFs, ONCOGENES, AUTOCRINE GROWTH, AND MYELOID LEUKEMIA**

Since the CSFs are the only known proliferative regulators for granulocytic and macrophage cells, it has been logical to raise certain questions with respect to the CSFs and myeloid leukemia: (1) Are the genes for CSF or CSF receptors oncogenes? (2) Does autocrine production of CSF lead to myeloid leukemia? (3) Can myeloid leukemias be suppressed by manipulation of CSF levels?

There is no sequence homology between the CSFs and known oncogenes, but the list of known oncogenes is probably incomplete, particularly in respect to hematopoietic neoplasms, because of the overuse of a fibroblast (NIH 3T3) detection system. One striking observation, however, has been the demonstration of homology between the M-CSF (CSF-1) receptor and the translation product of c-fms,\textsuperscript{76} v-fms being the viral oncogene of the McDonough strain of feline sarcoma virus.

The possible role of CSFs as autocrine stimulators of the proliferation in myeloid leukemia cells is a complex question on which available evidence is quite conflicting. A striking feature of primary myeloid leukemias in man and mouse (and here the situation with long-passaged myeloid leukemias needs to be discounted) is that the leukemic cells remain absolutely dependent on exogenous CSF for survival and continued proliferation in vitro (see review\textsuperscript{3}). This situation persists throughout the clinical course of the disease. The data on the dependency of myeloid leukemia cells on extrinsic CSF are quite unequivocal and would lead to the firm conclusion that myeloid leukemias are unlikely to be examples of autocrine growth, given only that the in vitro data reliably reflect the situation in vivo. It must also be emphasized that in vivo there are multiple normal-tissue sources of CSF, producing CSF levels greatly in excess of those conceivably able to be produced by the first emerging leukemic cell. Whether or not the first leukemic cell produces CSF is likely to be irrelevant if it is bathed in fluid containing CSF.

Leukemic cells, both primary and passaged, can often be shown to synthesize CSF. In primary myeloid human leukemias, this is most clearly demonstrable for the mature monocytes of the leukemic clone, but most cultured cell lines of monocytic leukemias can also produce one or another CSF, and here undifferentiated cells are likely to be the cellular source of the CSF.\textsuperscript{109,110} (see review\textsuperscript{3}). Two comments
need to be made in assessing the significance of this production of CSF by leukemic cells: (1) There has been no evidence so far that the leukemic cells produce abnormal forms of CSFs to which leukemic cells are abnormally or selectively responsive. Where such CSFs have been biochemically characterized, they are demonstrable as being similar to the normal equivalent molecule. (2) At least some normal hematopoietic cells can also synthesize CSF, and the levels of CSF produced on a per cell basis are equivalent to those produced by leukemic cells.

Despite the strong circumstantial evidence against ascribing myeloid leukemia to autocrine CSF production, certain observations have linked acquisition of constitutive CSF production with leukemic transformation. Two groups studying the behavior of murine nonleukemic but immortalized hematopoietic cells that are dependent on extrinsic Multi-CSF for survival and proliferation noted that the emergence of subclones capable of autonomous proliferation and Multi-CSF production coincided with the acquisition by these cells of a capacity to produce transplanted leukemias in syngeneic recipients. Similarly, infection of cells of one such line (dependent on Multi-CSF or GM-CSF) with a retroviral construct containing the GM-CSF cDNA led to the emergence of autonomous, GM-CSF-producing sublines that were uniformly leukemogenic on transplantation to syngeneic recipients. In a similar vein, a second infection of v-myc-immortalized chicken macrophages with the oncogene v-mil resulted in the emergence of leukemic cells whose continued proliferation was dependent on the acquired synthesis and secretion of an avian M-CSF. These observations make a strong case implicating constitutive and possibly dysregulated CSF synthesis as being capable of transforming immortalized cells to leukemic cells.

However, in keeping with the contradictory nature of present evidence on this question, it has been shown that Abelson virus infection of immortalized CSF-dependent murine hematopoietic cell lines transformed the cells to leukemic conditions under which autocrine production of either Multi-CSF or GM-CSF could be excluded and in which membrane receptors for CSF appeared to be unaltered in the transformed cells.

Thus dysregulated CSF production by preleukemic cells may be able to lead to the transformation of such cells to fully leukemic cells, but leukemic transformation can also occur by other mechanisms, and in human myeloid leukemia development, there is still no substantial evidence for autocrine stimulation by CSF as a crucial factor in leukemia development.

Because primary myeloid leukemias are uniformly dependent on CSF for proliferation, it should be possible to suppress such leukemias by suppressing CSF levels. Unfortunately, this is not a feasible approach, since normal GM cells require stimulation by similar concentrations of CSF. However, because the CSFs can induce differentiation commitment in granulocyte-macrophage precursor cells, an intriguing alternative approach to leukemia control has emerged. With the murine WEHI-3B leukemia model, the known CSFs have been tested for their inability to induce differentiation commitment and to suppress leukemic stem cell self-generation. Of the four known CSFs, M-CSF and Multi-CSF are inactive, and GM-CSF has detectable but relatively weak activity, whereas G-CSF is a powerful inducer of differentiation. At the same concentrations used to stimulate the proliferation of normal GM cells, G-CSF can reduce leukemic stem cell self-generation, induce the formation of end cell granulocytes and monocytes, and, on continued exposure, completely suppress a leukemic population.

Analysis of these events indicated a close similarity with the irreversible, asymmetric cell cycle-dependent events during commitment of normal GM progenitor cells by CSF when these cells enter a restricted differentiation pathway.

Primary human myeloid leukemia populations express membrane receptors for G-CSF (presumably CSFβ receptors), and while the CSFs are not the only biologically derived macromolecules able to induce terminal differentiation in myeloid leukemic cells, it remains possible that the CSFs will be of value in the suppression of myeloid leukemias.

IN VIVO ACTIONS OF THE CSFs

A major criticism of past work on the CSFs has been that the effects of these regulators had been analyzed only in vitro. This situation arose because of the minute amounts of CSF produced by tissues or cell lines, the short in vivo half-lives of the CSFs, and a proper reluctance to use crude CSF-containing materials to establish likely in vivo effects of injected CSF. Indirect evidence for CSF action in vivo has come from an analysis of the effects of CSF-producing tumors on host hematopoietic populations, but while these are in line with the predicted effects of CSF, it cannot be eliminated that the effects were due to some other products of the tumor cells or to indirect responses on the part of the host to the presence of the tumor tissue.

Moderate stimulating effects on granulocyte and monocyte formation were observed in studies on normal adult mice injected with partially purified CSF from human urine and purified CSF from this source injected into leukopenic patients caused a slightly accelerated recovery of leukocyte levels.

With the development of recombinant bacterially synthesized CSFs with full in vitro biologic activity, the situation has now changed. There are no publications yet on this subject, so the material cannot be formally reviewed, but data from this and other laboratories indicate that there are demonstrable effects of injected GM-CSF and Multi-CSF on white cell levels, organ cellularity, and content of progenitor cells and marked elevations of peritoneal cell levels if, in the latter instance, the intraperitoneal route of injection is used.

This comment on unpublished data is inserted here mainly to raise a question that most workers in the field have so far not properly considered. The CSFs were detected as proliferative stimuli, and most thinking on likely in vivo uses for the
of hematopoietic regeneration following irradiation, cytotoxic drugs, and other treatments. This may or may not turn out to be the most effective application of the CSFs.

Since the CSFs have rapid stimulating effects on mature-cell functional activity, this raises the alternative possibility that a more common use of such agents will be to stimulate the functional activity of preexisting cells in situations such as acute infections, either local or systemic. It is in this context that the dramatic effects of locally injected recombinant CSF on peritoneal populations are of considerable interest.

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