The Granulocyte-Macrophage Colony-Stimulating Factors

Donald Metcalf

Summary. The granulocyte-macrophage colony-stimulating factors are well-characterized specific glycoproteins that interact to control the production, differentiation, and function of two related white cell populations of the blood, the granulocytes and monocyte-macrophages. Widely produced in the body, these regulators probably play an important role in resistance to infections. The proliferation of myeloid leukemia cells remains dependent on stimulation by colony-stimulating factors, although one of them also has the ability to suppress leukemic populations by inducing terminal differentiation.

Most of the red and white cells in the circulating blood are short-lived and need to be replaced constantly throughout life. This process of blood cell formation, termed hematopoiesis, is not only enormous in scale (there are 100 times more cells in the bone marrow of an adult than there are people in the whole world) but is also complex, since cells of nine distinct hematopoietic cell lineages, each with multiple maturation stages, are admixed apparently at random in the tightly packed bone marrow. Furthermore, hematopoiesis must be capable of rapid but controlled fluctuations to meet a wide variety of emergency situations ranging from blood loss to infections. A novel element in the system is that all blood cells originate from a small common population of multipotential stem cells that is formed during one short interval in early embryonic life and thereafter maintains hematopoiesis by an extensive capacity for self-generation. Derangements of this complex process of blood cell formation do occur and result in a range of medically important diseases from anemia to leukemia. However, the hematopoietic system usually functions with remarkable fidelity as a consequence of regulation by an overlapping system of control mechanisms.

Control of Hematopoiesis

Some of the mechanisms controlling hematopoiesis, particularly the behavior of stem cell populations, appear to involve cell contact regulation by microenvironmental cells located in the sites of blood cell formation. However, it is also apparent that much of the control of blood cell formation is mediated by a group of interacting specific glycoproteins, multiple subsets of which control each of the major hematopoietic families.

Recognition and characterization of these hematopoietic regulators were made possible by the development, beginning in the mid-1960's, of a series of techniques for the clonal culture of hematopoietic cells in semisolid culture medium. In such cultures, individual progenitor cells of a particular hematopoietic lineage are able to proliferate and generate a clone of maturing progeny cells that remain in physical proximity to each other and are identifiable as a colony (Fig. 1). In the original technique, colonies of two types of white cells—granulocytes or macrophages, or both—were grown from ancestral progenitor cells (*1, 2*) but modifications of this technique now permit stem and progenitor cells of all hematopoietic lineages from humans or other animals to proliferate clonally in vitro and generate mature progeny in a manner essentially identical to the comparable process in vivo (*3*).

Analysis of the events occurring in these cultures led to the recognition that hematopoietic cells are intrinsically incapable of unstimulated cell division. All cell division is dependent on continuous stimulation by appropriate specific regulatory molecules and, since colony formation was the method used to detect and characterize these molecules, they have been named the colony-stimulating factors (CSF's). The general subject of the CSF's has recently been reviewed (*3*) and the present article will be restricted to the granulocyte-macrophage colony-stimulating factors (GM-CSF's).

Granulocyte-Macrophage Colony-Stimulating Factors

In the mouse, four major GM-CSF's have been characterized as interacting to control the formation and function of granulocytes and macrophages (*4–7*). The granulocyte-macrophage population is an interesting model system since these two quite different cell types, each with an abundance of differentiation markers, originate from common bipotential progenitor cells that, in turn, arise by differentiation commitment from multipotential stem cells. The four GM-CSF's are listed in Table 1, but the list may be incomplete because additional variant CSF's have been noted in the mouse but not yet fully characterized. Information is less complete for the corresponding regulators in humans, but at least two distinct forms, CSF-α and CSF-β, have been identified (*8*).

Each of the four murine CSF's has been purified to homogeneity in small amounts from medium conditioned by various mouse cells with the use of multistep separative procedures coupled with bioassays of all fractions in agar cultures of bone marrow cells. Success in this work depended heavily on the use of high-performance liquid chromatography since, for example, with multi-CSF purified from lymphocyte-conditioned medium it was necessary to achieve a millionfold purification before a homogeneous product was obtained yielding a single amino acid sequence. All four CSF's are glycoproteins: GM-CSF, G-CSF, and multi-CSF are monomers of molecular weight 23,000 to 28,000, approximately 40 percent of which is carbohydrate; M-CSF (molecular weight 70,000) is a dimer of two polypeptide subunits, each with a molecular weight of approximately 14,000, the remainder of the molecule being carbohydrate. Deglycosylation experiments and analysis of CSF's synthesized by cells grown in the presence of tunicamycin have suggested that the carbohydrate portion of these CSF's is not necessary for action in vitro. Digestion by peptidases destroys the biological activity of the

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CSF's and no digestion fragments of GM-CSF have been observed that can stimulate GM proliferation. Treatment with mercaptoethanol reduces M-CSF to subunits that have no biological activity, and treatment of the three monomeric CSF's also destroys biological activity, suggesting the need in these monomers for disulfide-based tertiary structure for biological activity.

All normal tissues contain and synthesize one or more CSF's, but studies on the CSF's have been restricted by the minute amounts produced by even the richest tissue sources. For example, medium conditioned by lung tissue from mice injected with endotoxin is the richest source of both GM-CSF and G-CSF, but only 5 to 12 micrograms (*4*) and 2 to 4 micrograms (*5*), respectively, of these CSF's can be obtained from 1000 mouse lungs.

Cloning of CSF Genes

Complementary DNA (cDNA) and genomic DNA clones have been isolated for both GM-CSF (*9, 10*) and multi-CSF, the latter under the alternative names for multi-CSF of mast cell growth factor (*11*) and interleukin-3 (*12*). Transfection of these cDNA or genomic clones to monkey COS cells leads to the synthesis of GM-CSF or multi-CSF with similar biological properties to the purified molecules (*10, 13, 14*). The deduced amino acid sequences for the polypeptides of GM-CSF (124 amino acids, molecular weight 13,138) and multi-CSF (134 amino acids, molecular weight 15,142) share no significant homology and the molecules differ markedly in hydrophobicity profiles and predicted secondary structure.

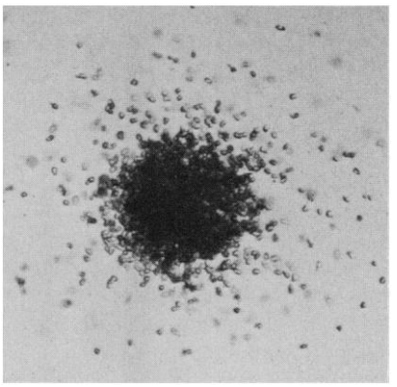

Fig. 1. A granulocyte-macrophage colony generated from a single progenitor cell stimulated to proliferate by purified GM-CSF.

This is surprising since, in the restricted context of granulocyte-macrophage populations, GM-CSF and multi-CSF function in an apparently identical manner and can stimulate the proliferation of the same granulocyte-macrophage clones. From the NH_2-terminal sequence data on G-CSF and M-CSF, it is likely that these two CSF's also differ both from each other and from GM-CSF and multi-CSF. Thus four molecules superficially resembling one another in being highly active glycoproteins with considerable overlap in their functional control of granulocyte-macrophage populations appear to have had quite diverse evolutionary origins. No sequence homology is evident between the CSF's and interleukin-2 (IL-2, or T-cell growth factor, TCGF), the analogous glycoprotein regulator for T-lymphocytes, or between the CSF's and other growth regulators, for example, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), or growth hormone.

The genes for both GM-CSF (*9*) and multi-CSF (*11, 12*) exist in single-copy form in the mouse genome. The GM-CSF's synthesized by the different adult mouse tissues appeared initially to be quite different molecules, although biochemical analysis suggested that the differences were likely to be based merely on tissue differences in glycosylation (*15*). The presence of only a single gene for GM-CSF supports this interpretation. Although GM-CSF and multi-CSF differ structurally, mitogen-stimulated T-lymphocyte clones can exhibit a closely coordinated synthesis of these two molecules (*16*). It is of interest in this context that both genes have been identified provisionally on chromosome 11, which raises the possibility that the two genes may be located adjacent to one another or that a common regulatory element is able to activate the transcription of both genes.

Responsiveness to Different CSF's

While most granulocyte-macrophage progenitors are bipotential and can respond to stimulation by more than one CSF, they exhibit considerable heterogeneity with respect to the number of progeny each generates (from 50 to 10,000 cells) and to the concentrations of CSF required to stimulate cell division (*3*). This latter variability is the basis for the familiar sigmoid dose-response curve between CSF concentration and the number of granulocyte-macrophage colonies developing in a culture dish, some clonogenic cells requiring 10- to 50-fold higher CSF concentrations than others to be stimulated to proliferate. This may be related to the tenfold variation in CSF

Table 1. The major murine GM-CSF's.

Name	Alternative acronyms*	Major progeny resulting from CSF stimulation	Molecular weight	Source of conditioned medium used in CSF purification	Amino acid sequence data obtained
Granulocyte-macrophage colony-stimulating factor, or GM-CSF	MGI-IGM (*57*)	Granulocytes and macrophages	23,000	Mouse lung (*4*)	Full (*9, 56*)
Granulocyte colony-stimulating factor, or G-CSF†	MGI-IG (*57*)	Granulocytes	25,000	Mouse lung (*5*)	NH_2-terminal
Multipotential colony-stimulating factor, or multi-CSF	IL-3 (*6*), BPA (*58*), HCGF (*59*), MCGF (*60*), PSF (*61*)	Granulocytes, macrophages, erythroid cells, eosinophils, megakaryocytes, mast cells, stem cells	23,000 to 28,000	WEHI-3B leukemia cell (*6*)	Full (*11, 12*)
Macrophage colony-stimulating factor, or M-CSF†	CSF-1 (*20*)	Macrophages	70,000	L cell (*7*)	NH_2-terminal

*MGI, macrophage-granulocyte inducer; IL-3, interleukin 3; BPA, burst-promoting activity; HCGF, hematopoietic cell growth factor; MCGF, mast cell growth factor; PSF, persisting cell-stimulating factor. †For NH_2-terminal sequence data on G-CSF and M-CSF (*62*).

receptor numbers observed by autoradiography on individual normal progenitor cells (Fig. 2).

There are also differences in the responsiveness of subsets of granulocyte-macrophage progenitor cells to stimulation by the different CSF's. This heterogeneity in target cell populations could provide some explanation for the existence of multiple types of CSF but, despite this, reciprocal clone transfer studies indicate that most progenitors can be stimulated by more than one CSF. This highly redundant control system might seem an unnecessarily complex manner in which to regulate cell proliferation but, as shall be discussed shortly, the CSF's have other important actions on granulocyte-macrophage cells allowing subtle competitive and potentiating interactions between the CSF's and thus a fine control of the production and activation of selected subsets of mature progeny. Furthermore, multi-CSF is not normally detectable in the circulation and may be produced and act primarily on local target cells adjacent to sites of multi-CSF production, whereas other CSF's, for example, G-CSF, circulate and may act simultaneously in multiple locations.

Receptors for CSF's

The membranes of responding granulocytes and macrophages exhibit specific high-affinity receptors for the CSF's. Each CSF receptor appears to bind only a single species of CSF and since most granulocytes and macrophages are able to respond to more than one CSF, these cells simultaneously exhibit more than one type of CSF receptor. Until more detailed autoradiographic data are available, it remains uncertain how many hematopoietic cells exhibit only a single species of CSF receptor. Stimulation of granulocyte and macrophages by a combination of two different CSF's enhances the resulting proliferation (*3*), but these interactions are likely to be complex since exposure of cells to one CSF can lead to down-regulation of other CSF receptors (*17*). Receptors for the different CSF's differ in molecular weight: M-CSF, 165,000 (*18*); G-CSF, 150,000; multi-CSF, 50,000 to 70,000; and GM-CSF, 50,000 (*17*). There are also differences in the mean number of receptors on normal responsive cells, receptors for GM-CSF, G-CSF, and multi-CSF being low in number (100 to 500 per cell) (*17*) and those for M-CSF being somewhat higher (3,000 to 16,000 per cell) (*19*). Despite these low receptor numbers, half-maximal proliferative effects are achieved by G-CSF and GM-CSF with a receptor occupancy of only 5 to 10 percent. Degradation of M-CSF-receptor complexes appears to be very rapid (*20*), but G-CSF–receptor complexes are degraded much more slowly with a half-life of at least 6 hours (*21*). A slow turnover of CSF-receptor complexes of this latter type may permit sustained intracytoplasmic activation of mediator molecules to compensate for the low absolute number of bound receptors.

Considerable species-specificity is exhibited by the CSF's, but there are exceptions. In general, murine CSF's do not stimulate the proliferation of human granulocytes and macrophages. Sequence data from a cloned human GM-CSF cDNA (*22*) indicate 54 percent homology with murine GM-CSF in the protein coding region and conservation of the four cysteines present in the mouse polypeptide that appear to allow the disulfide bridging necessary for biological activity (Fig. 3). Despite these structural homologies, murine GM-CSF neither binds to nor stimulates the proliferation of human granulocytes and macrophages (*23*).

However, murine G-CSF and human CSF-β are exceptional in showing considerable cross-species reactivity. G-CSF is able to bind to human cells and stimulate the proliferation of one subset of human granulocyte-macrophage cells. Conversely, human CSF-β stimulates murine granulocytes and macrophages to form colonies resembling those stimulated by G-CSF and competes for G-CSF binding to both murine and human cells (*23*).

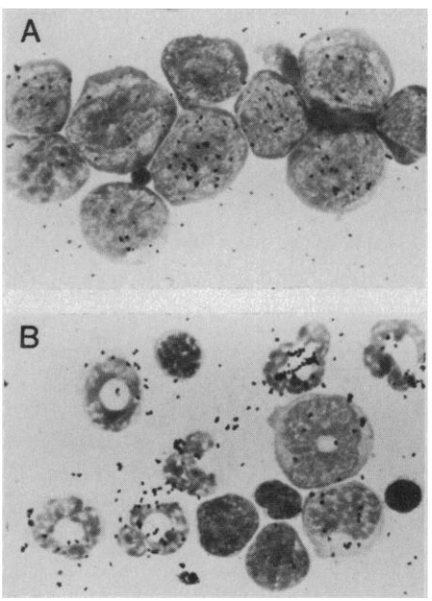

Fig. 2. Autoradiographs of ^{125}I-labeled G-CSF bound by purified granulocyte-macrophage progenitor cells (A) and normal marrow cells (B). Binding to granulocyte-macrophage progenitor cells permits stimulation of cell division while binding to post-mitotic polymorphs permits stimulation of functional activity.

Multiple Functions of the CSF's

The CSF's were first detected because of their mandatory and unique role in stimulating hematopoietic cell proliferation. However, each CSF also exhibits three other important actions on responding cells: (i) promotion of cell survival, (ii) differentiation commitment, and (iii) stimulation of end-cell functional activity.

Most hematopoietic cells that are in active cell cycle when placed in cultures lacking an appropriate CSF usually fail to complete the cycle in progress. A minority of some cell types, for example, immature granulocytes, can slowly progress through one and occasionally two divisions in the absence of added CSF (*24, 25*), but this may reflect the slow turnover of CSF-receptor complexes existing prior to the removal of the cells for culture. Withdrawal of CSF from hematopoietic cells in vitro is usually followed by rapid cessation of DNA and protein synthesis (*20*), and cell death occurs exponentially with half-life periods varying from 6 to 24 hours according to cell type (*24, 25*). This may be due to failure to maintain cytoplasmic concentrations of adenosine triphosphate and a general breakdown in the membrane glucose transport system (*26*).

Colony-stimulating factor is required continuously to stimulate sustained hematopoietic cell proliferation in vitro. The proliferative effects of the CSF's are not the passive consequence of CSF-promoted cell survival. Cell survival maintained by other means does not result in hematopoietic cell proliferation (*26*), and there is a precise dose-response relation between CSF concentration and the magnitude of proliferation shown by responding granulocytes and macrophages, half-maximal proliferative effects for all four murine CSF's being observed at molar concentrations of approximately 10^{-12} (*3*).

There is only fragmentary information on the biochemical events occurring when CSF's stimulate cells to pass through a cell cycle and divide. Autophosphorylation of M-CSF receptors has been noted following binding of M-CSF (*27*), and the CSF's have been shown to induce changes in the rate of synthesis of a number of cytoplasmic and nuclear proteins (*28*). GM-CSF has also been

observed to increase the phosphorylation of a number of proteins, prominent among which are the p21 *ras* gene product and a protein of 53,000 molecular weight (*28*). However, the complexity of these changes has thus far prevented identification of the events crucial for cell division.

Studies with micromanipulated paired daughter cells of granulocyte-macrophage progenitor cells have shown that the concentration of CSF determines the mean cell cycle time and the total number of progeny produced by a particular cell (*5, 29*). Culture of granulocyte-macrophage progenitor cells purified by fluorescence-activated cell sorting or of manipulated single progenitor cells has shown that the proliferative action of the CSF's is a direct one on responding cells (*3*), a conclusion supported by the presence of CSF receptors on these cells (Fig. 2).

Fig. 3. Comparison of amino acid sequence of mouse GM-CSF (*56*) with human GM-CSF (*22*) deduced from sequencing of cDNA clones. Asterisks indicate homologous amino acids. Fifty-four percent homology is evident as is conservation of the four cysteine residues.

Although CSF-stimulated proliferation of granulocytes and macrophages is accompanied by maturation of the cells in the resulting clones to mature granulocytes and macrophages, it remains uncertain whether the CSF's have a direct capacity to influence these maturation events. This is a difficult problem to resolve since hematopoietic cell survival in vitro depends on CSF, and the behavior of healthy cells cannot be analyzed in the absence of CSF. The question is of relevance because of the ability of certain of the CSF's to stimulate the proliferation of cells in other hematopoietic lineages. For example, GM-CSF can stimulate the formation of eosinophil colonies from fetal progenitor cells (*30*). GM-CSF can also stimulate the initial cell divisions of erythroid progenitor cells without altering their ultimate ability to form mature red cells (*31*). It may be that the genetic and biochemical programs controlling cell divisions are separate from those directing highly specialized functions in maturing cells such as the synthesis of eosinophil granules or hemoglobin.

The four granulocyte-macrophage CSF's differ widely in their ability to stimulate the proliferation of cells in other hematopoietic lineages (*3*). At one extreme, multi-CSF can also stimulate the proliferation of multipotential, erythroid, eosinophil, megakaryocyte, and mast cells (*13, 14*), while at the other extreme the proliferative effects of M-CSF are largely restricted to macrophage precursors (*7*). GM-CSF and G-CSF occupy an intermediate position in being able to initiate, but not sustain, proliferation in some multipotential, erythroid, eosinophilic, and megakaryocytic precursors (*30, 31*). Autoradiographic studies with ^{125}I-labeled CSF's indicate that these differences are based on differing distributions of membrane receptors for the various CSF's on different hematopoietic subpopulations.

However, the CSF's are not simply regulators of cell proliferation. Where granulocyte-macrophage progenitor cells are bipotential and able to form both granulocytic and macrophage progeny, paired daughter cell and reciprocal clone transfer studies have shown that the CSF's are able to induce irreversible commitment to one or other restricted pathway of differentiation. Thus, high GM-CSF concentrations force many cells to enter the granulocytic pathway whereas low concentrations permit the formation only of macrophage progeny (*29*). Similarly, stimulation by M-CSF of a bipotential cell to pass through two to three cell divisions irreversibly commits the cells to form macrophage progeny regardless of the CSF used subsequently to maintain proliferative stimulation (*32*). Exposure of a bipotential granulocyte-macrophage progenitor cell simultaneously to two CSF's, for example, M-CSF and G-CSF, allows competitive commitment to occur (*33*). The commitment process in these situations seems to require cell division, and commonly occurs asymmetrically with one daughter cell becoming committed while initially the other daughter remains bipotential (*33*). A similar phenomenon has been documented in an analysis of the manner in which G-CSF suppresses self-generation by myeloid leukemic stem cells and forces the production of differentiating progeny. Again, the process requires the presence of G-CSF for one or two cell cycles, is asymmetrical, and, having occurred, commitment to the production of differentiating progeny is irreversible (*34*). These observations on the irreversible effects of the CSF's suggest that, to mediate such effects, CSF's or, more likely, amplified cellular mediators of CSF action, may move to the nucleus and bind to regions of S-phase extended chromosomes adjacent to genes involved in these processes.

The CSF's can also stimulate a variety of functional activities of mature granulocytes and macrophages, for example, phagocytosis of bacteria or yeast by granulocytes and macrophages, antibody-dependent cytotoxic killing of tumor cells by granulocytes, or synthesis of prostaglandin E, plasminogen-activator, and other regulators by macrophages (*3, 35*). These effects occur rapidly, are associated with obvious membrane changes, for example, in adherence and self-agglutination, and could in many cases be mediated by changes occurring in regions close to the membrane.

Two important events in the life history of white cells are release from the marrow and, where necessary, entry of the cells into the tissues. There is no evidence that the CSF's influence release of cells from the marrow, but a purified human CSF can inhibit neutrophil migration (*36*). Since CSF is produced locally at inflammatory sites, this local CSF could functionally activate mature cells that have migrated to inflammatory foci and, by inhibiting further migration, ensure their retention in the region of inflammation.

Location and Control of CSF Production

All mouse tissues contain extractable CSF in concentrations higher than are present in the serum and, where various organs have been tested, all have been found to synthesize one or other form of CSF in vitro (*37*). Until in situ hybridization studies are performed with the use of CSF cDNA's as probes to identify messenger RNA, information is restricted on the specific cell types able to synthesize CSF. Only a few cell types can be purified and tested in the cell concentrations (10^5 to 10^6 per milliliter) required to detect low levels of CSF synthesis. Of cell types so tested, macrophages, T lymphoyctes, endothelial cells, fibroblasts, and skin epithelial cells have all been shown to synthesize one or more of the four major CSF's. A variety of tumors can sometimes synthesize CSF, and in animals or patients bearing such tumors, granulocyte levels can be grossly elevated (*3*).

Because macrophages produce CSF and both polymorphs and macrophages produce inhibitors of granulocyte-macrophage production, such as lactoferrin and prostaglandin E, it has been proposed that the granulocyte-macrophage system could be internally self-regulating with levels of mature cells controlling new cell production (*38*). There may be some situations in which self-regulation is prominent, but most of the evidence suggests that many, and possibly all, cell types have the ability to synthesize CSF, and that the level of CSF production is dictated by signals extrinsic to the granulocyte-macrophage system. For example, CSF production by macrophages and endothelial cells is strongly stimulated by exposure to endotoxin and other bacterial products, CSF production by T lymphocytes is stimulated by lectins or alloantigens, and CSF production by skin epithelial cells is increased after contact with promoting agents [for a review, see (*3*)].

The serum half-life of injected CSF is short, with the level falling in a biphasic mode with an initial half-life of 5 to 15 minutes followed by a slower phase with a half-life of 1 to 7 hours (*3, 17*). From studies with radioactively labeled GM-CSF and G-CSF, initial tissue localization is mainly in the liver, but this is followed by progressive relocation in and degradation by the kidney.

Two types of tissue control of granulocyte-macrophage populations by the CSF's have become evident. In the first, basal levels of cells are likely to be maintained by CSF production by stromal cells within the marrow cavity (*39*), a system apparently able to respond in a compensatory manner to depopulation of hematopoietic cells (*40*).

The second type of control system involves tissues throughout the body and is activated most commonly by exposure to microorganisms and their products, such as endotoxin. After the injection of endotoxin, a 1000-fold increase occurs in circulating CSF (*41*) and all tissues contain and synthesize elevated levels of CSF (*42*). These changes occur within minutes, and peak levels of serum and tissue CSF are attained by 3 to 6 hours. Where infections are minor and localized, it seems probable that locally involved tissues are the major source of the additional CSF. When infections are resolved, CSF levels promptly return to normal (*3*). This response pattern to clinical or subclinical infections appears to be a major factor in determining the "normal" circulating levels of CSF in humans and other animals. The increased CSF can be regarded as a defense response that is self-limiting when the stimulated granulocytes and macrophages have eliminated the microorganisms initiating the response.

Radioactively labeled CSF, when injected intravenously, can be shown to be bound by marrow cell populations (*21*) so the high circulating CSF levels elicited by microbial products can influence granulocyte and macrophage proliferation in the marrow and spleen. However, since the CSF's also have important stimulating effects on mature granulocytes and macrophages, it may be that, in the initial phases of an acute infection, the primary purpose of the elevated CSF levels in the circulation and tissues is to deliver an ultrarapid stimulus for increased functional activity by preexisting mature granulocytes and macrophages.

The CSF's and Myeloid Leukemia

Myeloid leukemias are clonal neoplasms of granulocyte-macrophage precursor cells. A currently popular view of the cancerous state is that the uncontrolled proliferation exhibited by a cancer cell population is ascribable to the action of viral or cellular oncogenes whose products are either specific growth factors or receptors for such factors. Cancer cell proliferation is viewed, not as an autonomous process, but as the response of cells to oncogene-induced autosynthesis of specific growth factors or receptors. It is postulated that the polypeptides involved could in some cases be structurally normal with a cellular oncogene becoming dysregulated by translocation or activation by viral enhancers, while in other situations the oncogene may be structurally abnormal because of mutational events.

Since the CSF's are the only known proliferative stimuli for granulocytes and macrophages, it is of interest to determine whether the myeloid leukemias are ascribable to oncogene-deranged autosynthesis of CSF or CSF receptors. There is no sequence homology between the CSF's and the known oncogenes, but no sequence data are yet available for CSF receptors.

Is there any evidence that myeloid leukemia cells are autostimulating because of an acquired capacity to synthesize their own CSF's? Data on this question are extensive and quite unambiguous for leukemia cells from patients with either acute or chronic myeloid leukemia. In no instance has it been documented that myeloid leukemic cells are capable of sustained autonomous proliferation in vitro. The proliferation of leukemic cells in vitro is, like normal cells, absolutely dependent on the addition of exogenous CSF (*43*), and the concentrations of CSF required are similar to those required to stimulate the proliferation of normal cells (*44*). These observations should not be misinterpreted as indicating that leukemia cells are unable to produce CSF. Studies on monocytes of leukemic origin have clearly documented that they have the capacity to produce CSF (*45*) and, if sufficient numbers of such cells are crowded together in a culture, the CSF's they produce can lead to apparently spontaneous proliferation by clonogenic leukemic or normal cells in the culture. However, the levels of CSF produced under these circumstances do not differ from those produced by corresponding normal monocytes. Furthermore, from the dependency of autostimulation on cell crowding it can be concluded that, to be active, CSF must be secreted and must then bind to appropriate membrane receptors. The absolute levels of CSF that can be produced by an emerging leukemic clone of a few cells would be insignificant compared with the CSF normally produced by adjacent stromal cells or with the amount reaching the cells via the circulation, and it is improbable that autoproduction of CSF by leukemia cells would represent a significant event in the emergence of a leukemic clone.

It must be emphasized, however, that

since all primary myeloid leukemias are CSF-dependent, the emergence of a myeloid leukemic clone is absolutely dependent on CSF stimulation—whatever the source. This makes the CSF's essential cofactors in leukemia induction or the reemergence of leukemia in a relapse following unsuccessful therapy.

Little information is available on CSF receptor numbers on human myeloid leukemia cells, but studies with the cross-reactive murine G-CSF have indicated no significant difference in receptor numbers from those on comparable normal cells (*46*), a situation quite unlike that documented for IL-2 receptors on human T-leukemic cells transformed by human T-cell leukemia virus (*47*). With the murine myelomonocytic leukemia WEHI-3B, a cell line known to synthesize M-CSF and multi-CSF, receptor numbers for ^{125}I-labeled G-CSF are within the normal range (*48*); the numbers for M-CSF and multi-CSF are low, however (*49*), although it is possible that they are down-regulated. With monocytic leukemia cell lines not capable of constitutive CSF synthesis (*50*), CSF receptors are either undetectable or within the normal range (*49*).

Taken together, the data on the lack of autonomous growth ability and the unremarkable receptor numbers on leukemic cells argue strongly against a simple autocrine growth model for myeloid leukemia cells. A more likely possibility, regardless of whether or not leukemic cells themselves can synthesize CSF, is that an intrinsic abnormality exists in leukemic stem cells as a consequence of which CSF-stimulated proliferation results in an abnormally high ratio of self-generative divisions versus divisions leading to the production of differentiating progeny. In this model, CSF would be a necessary cofactor in the emergence of a leukemic clone since it is mandatory for cell proliferation, but an intrinsic defect, possibly caused by an oncogene product, would be responsible for the aberrant pattern of resulting cell divisions.

Evidence for the importance of abnormally high self-generation by clonogenic leukemic cells has come (i) from recloning studies that have amply documented the high capacity of leukemic stem (clonogenic) cells to self-generate and (ii) from the observations by Ichikawa (*51*) that certain cell-conditioned media are able to induce cells of the myeloid leukemia cell line M1 to differentiate to mature granulocytes and macrophages and simultaneously to lose their leukemogenicity when injected into mice. In subsequent studies, the ability of a wide variety of chemical and biological agents to induce terminal differentiation in murine myeloid leukemia cells has been extensively analyzed (*52*) as has the comparable process in human myeloid leukemia cell lines (*53*).

While the CSF's are not the only biological agents able to influence the differentiation of myeloid leukemic cells, the ability of various conditioned media containing CSF to induce differentiation and the dependency of leukemic cells on CSF raised the likelihood that one or other of the CSF's might be able to induce differentiation in myeloid leukemia cells. Analysis of the four purified murine CSF's using the WEHI-3B myelomonocytic leukemia showed that M-CSF and multi-CSF had no differentiation-inducing activity, GM-CSF had weak activity, and G-CSF had a remarkable capacity to induce differentiation.

In line with the proliferative effects of CSF's, G-CSF initially enhances the proliferation of WEHI-3B cells, but rapidly superimposed is a suppressive effect from the differentiation-inducing action of G-CSF. G-CSF has the capacity to irreversibly shift the ratio of self-generative versus differentiative divisions in WEHI-3B leukemic stem cells with progressive suppression of self-generative divisions and the production of nondividing differentiated progeny (*5, 34*) (Fig. 4). As a consequence of this action, the leukemic population can be completely suppressed in vitro by quite normal concentrations of G-CSF (*54*). At present, the available amounts of purified G-CSF are too small to permit corresponding experiments in vivo, but the injection of crude preparations containing G-CSF has been shown to suppress other established transplanted myeloid leukemias in mice (*55*).

Conclusions

With the purification and cloning of several of the GM-CSF's, these glycoproteins are now firmly established as specific growth factors. The CSF's are the only known agents able to stimulate the proliferation of granulocyte-macrophage precursors, and they also have the capacity to induce differentiation commitment in these precursors and to stimulate the functional activity of mature granulocytes and macrophages. The hematopoietic culture systems that permitted the detection and characterization of the CSF's are also now well established as robust but elegant techniques permitting studies in defined medium on single cells producing differentiating progeny in response to stimulation by low concentrations of a single purified regulator. For the general cell biologist, clonal cultures of granulocytes or macrophages represent a model system with a potential possibly greater than any other for providing answers to some of the most fundamental questions in cell biology relating to the control of cell division and differentiation.

Given the advantages of the granulo-

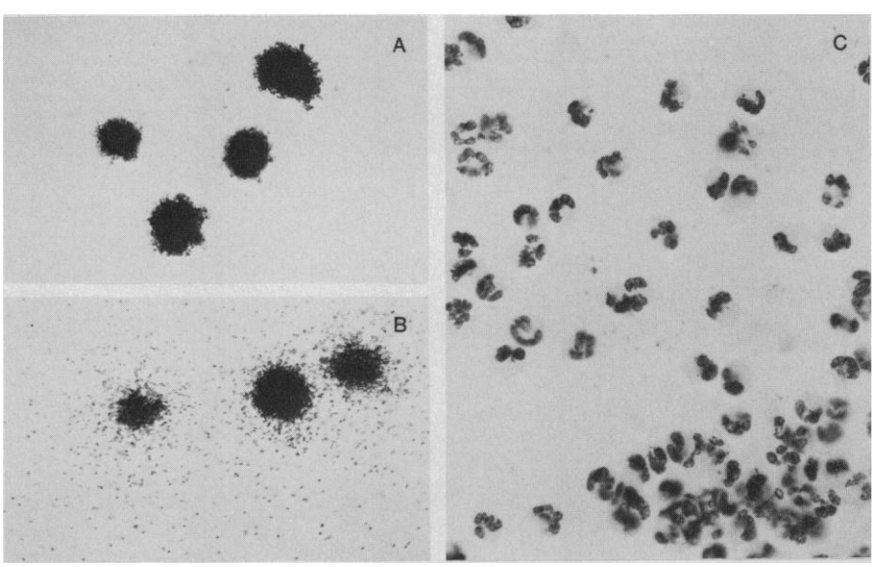

Fig. 4. Induction by purified G-CSF of differentiation in colonies of WEHI-3B murine myelomonocytic leukemic cells. Leukemic cells usually form compact colonies of undifferentiated cells (A), but in the presence of G-CSF colonies are surrounded by a corona of dispersing cells (B) because of differentiation of leukemic cells to maturing granulocytes and macrophages (C).

cyte-macrophage system in having many well-defined differentiation markers, it should be possible to make useful progress in a molecular analysis of the complex events occurring in responding cells following binding of the CSF's to their receptors and for this the availability of four similar, but competing, CSF's may be of particular value. It should also prove feasible to analyze the cellular events regulating CSF synthesis since, unlike some growth factors, a spectacular increase in CSF synthesis can be induced by agents such as endotoxin, lectins, and antigens (*9, 16, 41, 42*).

The availability of mass-produced recombinant CSF will now permit detailed studies in vivo on the actions of the CSF's. Two goals of high priority are to determine whether the CSF's will prove useful in enhancing hematopoietic regeneration following marrow damage or transplantation and in increasing host resistance to established infections. Of equal importance, in view of the demonstrated capacity of G-CSF to suppress myeloid leukemia populations, will be studies to establish whether the clinical use of CSF's will provide an effective adjuvant therapy in the management of myeloid leukemia. Here it will be necessary to keep in mind that the proliferation of myeloid leukemia cells is CSF-dependent and only CSF's with pronounced differentiation-inducing activity, such as murine G-CSF and human CSF-β, should be candidates for trial.

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