

The Colony-Stimulating Factors and Cancer

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Abstract

The colony-stimulating factors (CSF) are the master regulators of granulocyte and macrophage populations. There are four different aspects of the connection between the CSFs and cancer: (i) the CSFs can accelerate the regeneration of protective white cells damaged by chemotherapy; (ii) the CSFs can mobilize stem cells to the peripheral blood in convenient numbers for transplantation; (iii) the CSFs can enhance anticancer immune responses; and (iv) the CSFs are potentially involved in the genesis of the myeloid leukemias. *Cancer Immunol Res*; 1(6); 351–6. ©2013 AACR.

Disclosure of Potential Conflicts of Interest

D. Metcalf is a staff member of the Walter and Eliza Hall Institute, which has a patent position for GM-CSF.

CME Staff Planners' Disclosures

The members of the planning committee have no real or apparent conflicts of interest to disclose.

Learning Objectives

Upon completion of this activity, the participant should acquire a basic knowledge of the biology of the colony-stimulating factors (CSF) and their activity as the master regulators of granulocyte and macrophage populations. Some of the CSFs are already in use in the clinic for infections and cancer; a broader understanding of the connections between the CSFs and leukemogenesis, will inform future therapeutic management of cancer.

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Introduction

Granulocytes and macrophages are key members of the innate immune system protecting the body against bacterial, viral, and fungal infections. Most of these cells are short lived and must be replaced continuously by new cells formed in widely separate locations in the bone marrow. Under stable conditions of good health, the numbers of these cells are remarkably constant, indicating the existence of tight regulatory control. However, the numbers of these cells are demand driven. In the presence of infections, the production of granulocytes and macrophages can be greatly and speedily increased. This flexibility in response to sudden demands requires a highly responsive control system.

These competing demands for stability and flexibility in controlling and coordinating cell production by widely scattered deposits of marrow cells have been achieved by a consortium of four glycoproteins—the colony-stimulating factors (CSF; ref. 1). These belong to a group of regulatory factors that are commonly referred to as cytokines. When in the

circulation, the CSFs can resemble hormones with highly specific actions on appropriate target cells. In other situations, the CSFs can be produced and act in quite localized regions. Unlike hormones, the CSFs are not the products of a single cell type and can, when needed, be produced by virtually any organ or cell type in the body (1).

In some situations, the control system can include synergistic interactions between the CSFs or with certain other cytokines. In addition, the CSFs can interact with microenvironmental cells in the bone marrow in the control of the formation of stem cells and early precursors of granulocytes and macrophages.

The Ancestors of Granulocytes and Macrophages

Hematopoietic populations are organized in a hierarchical manner (Fig. 1). A limited number of self-renewing multipotential hematopoietic stem cells serves as the ultimate origin of all blood cells. These stem cells, numbering 1 per 10^5 marrow cells, generate a 100-fold larger population of blast colony-forming cells (BL-CFC; 1 per 10^3 marrow cells) that are very likely to be the actual stem cells sustaining the daily requirements for new blood cell production. BL-CFCs can self-renew and each can generate several thousand committed progenitor cells of granulocytes, macrophages, eosinophils, megakaryocytes, dendritic cells, erythroid cells, and T and B lymphocytes (2, 3). In turn, each progenitor cell in the granulocyte-macrophage lineage can produce up to 10^4 maturing progeny that end as mature

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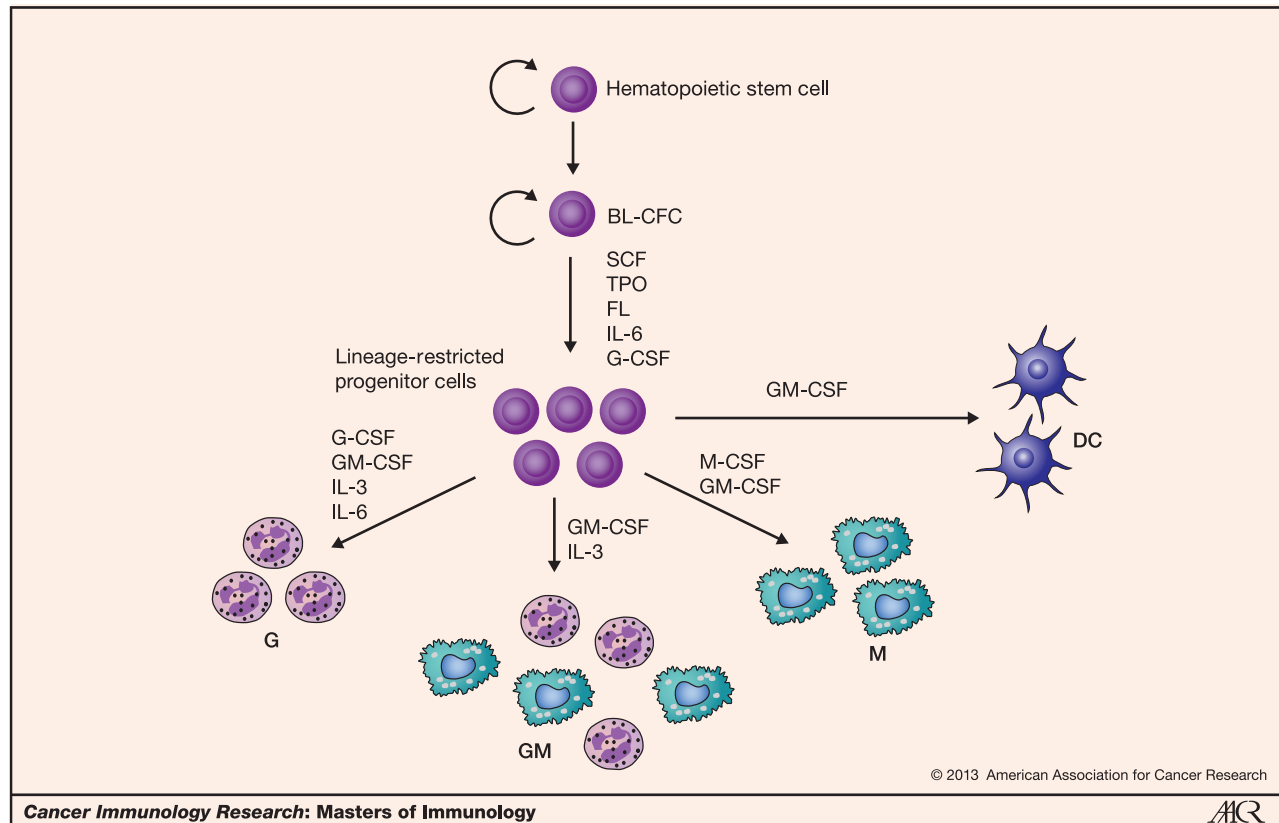


Figure 1. The family tree of granulocytes and macrophages. Hematopoietic stem cells are self-generating cells and also produce 100-fold higher numbers of BL-CFCs, each of which can generate up to a thousand committed progenitor cells in various lineages (only the granulocyte-macrophage and dendritic lineages are shown). In turn, each progenitor cell can generate up to 10^4 maturing progeny. The ability of one stem cell to produce 10^9 progeny is rarely required. Shown in boxes are the cytokines controlling each differentiation/proliferation step. DC, dendritic cell lineage; G, granulocytic lineage; GM, granulocyte-macrophage lineage; M, macrophage lineage; SCF, stem cell factor; TPO, thrombopoietin.

neutrophilic granulocytes (here termed granulocytes) and monocytes (here termed macrophages). The immediate ancestors of granulocytes and macrophages are a heterogeneous collection of progenitor cells able to form varying numbers of mature granulocytes and/or macrophages. These progenitor cells can be conveniently monitored in semisolid tissue culture by their ability to form clonal colonies of maturing granulocytes and/or macrophages (4, 5). The frequent presence in colonies of both granulocytes and macrophages indicates that these two populations are closely related and often share common immediate ancestors. Hardly any two granulocyte-macrophage progenitor cells resemble one another in the number or composition of their progeny, yet, overall, the final production of mature cells is able to be maintained at an appropriate level determined by the microbiologic demands on the body at that time. Blood cells in other lineages, for example, eosinophils, mast cells, or megakaryocytes, can also be monitored by colony formation in convenient semisolid cultures.

The Colony-Stimulating Factors

The CSFs were discovered because granulocyte and macrophage colony formation in semisolid cultures *in vitro* is

absolutely dependent on the presence of CSFs in the culture. Initially, it was thought that there would be only one type of CSF and that this would be the regulator of granulocytes and macrophages, comparable with the role of erythropoietin in controlling red cell production.

Analysis finally showed that there were in fact four distinct types of CSFs (6–9), now named after the major types of colony formation stimulated by their action—granulocyte-macrophage CSF (GM-CSF), granulocyte CSF (G-CSF), macrophage CSF (M-CSF), and multipotential CSF [most commonly termed interleukin (IL)-3]. Purification and cloning of the CSFs was achieved between 1977 and 1986 (1). The CSFs were shown to be glycoproteins of molecular weight from 18 to 70,000. Unlike erythropoietin, the carbohydrate moiety of CSFs is not necessary for biologic activity *in vitro* or *in vivo* and it merely prolongs the half-life of the molecules *in vivo*.

The CSFs have a short half-life of a few hours *in vivo*, but there is an ability to increase CSF production up to 1,000-fold within hours following stimulation, for example, by the bacterial cell wall component, endotoxin (1). CSFs can be produced locally at the site of an infection or systemically by multiple tissue types. The CSF control system is therefore

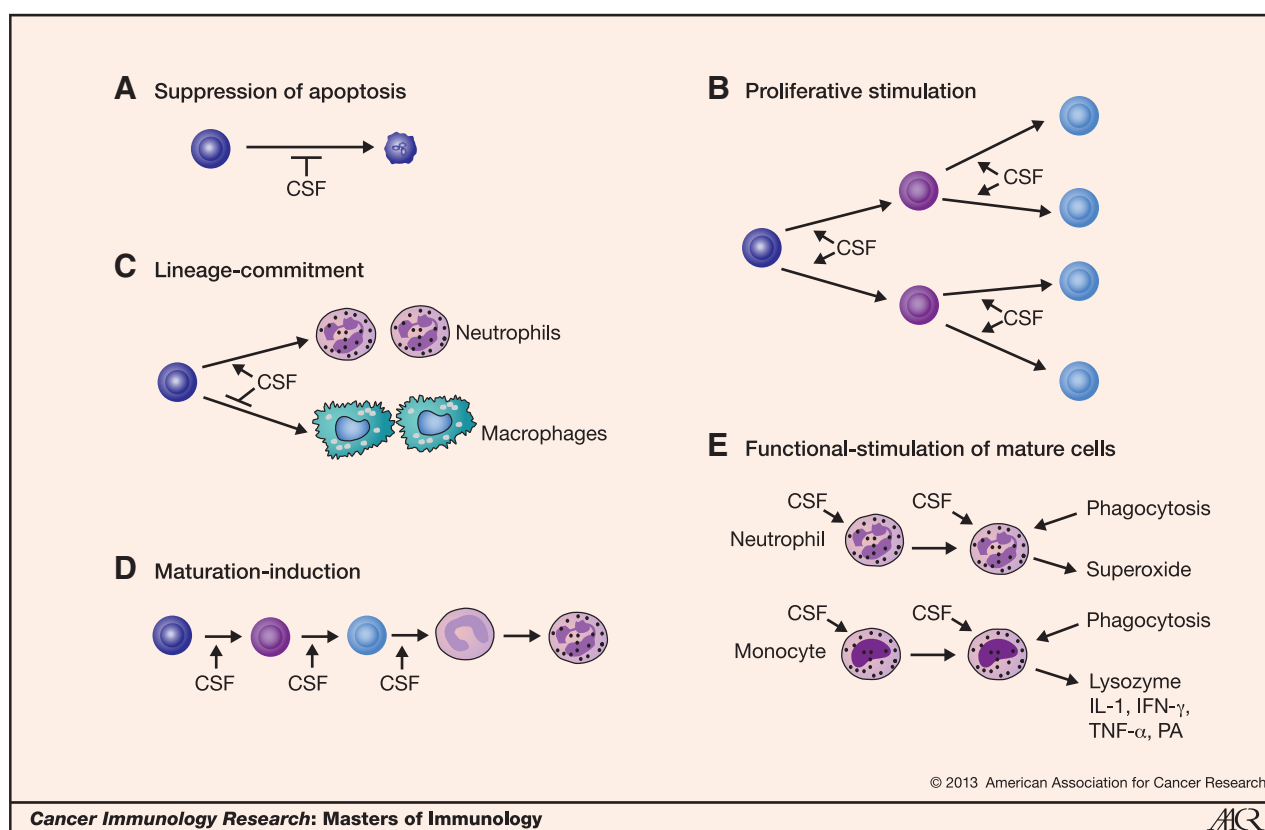


Figure 2. The five major actions of CSFs on responding granulocyte-macrophage populations. Several different regions of the cognate CSF membrane receptors are needed to initiate the various responses. A, the CSFs prevent death in the population by suppressing apoptosis. B, the CSFs are required to stimulate every cell division in a dose-responsive manner. C, the CSFs have some ability to influence lineage commitment decisions. D, the CSFs can initiate and regulate maturation of cells in these lineages. E, the CSFs can strongly stimulate the functional activity of mature granulocytes and macrophages. PA, plasminogen activator. This figure was adapted from ref. (27).

highly labile and able to respond rapidly to changing demands imposed by microorganisms.

Actions of the CSFs

The CSFs act via specific membrane receptors that define the targets that are able to respond. Despite the fact that target cells have relatively few receptors (approximately a few hundred per cell), CSFs exhibit extremely highly specific activity, these being active at picomolar concentrations (1). Two CSF receptors (for G-CSF and M-CSF) are homodimers, one of which (the M-CSF receptor) has tyrosine kinase activity. The receptors for the other two (GM-CSF and IL-3) are heterodimers that share a common signaling β chain. In each case, analysis has shown that multiple functional domains are present in the cytoplasmic regions of receptors, allowing the CSFs to exert multiple effects on responding cells (10, 11).

There are five major actions of CSFs on responding granulocyte-macrophage cells (Fig. 2): (i) they maintain the viability of progenitor cells and their more mature progeny by blocking apoptosis (12); (ii) they are mandatory for the stimulation of every cell division; the CSF concentration determines cell-cycle times and the number of mature cells produced per progenitor cell (13); (iii) it is likely, but not firmly established, that the CSFs

can influence lineage-commitment choices specifically as to whether a precursor cell produces granulocytic or macrophage progeny (14); (iv) they can influence the process by which cells mature and end (11); and (v) they have major stimulating actions on the function of mature end cells, for example, enhancing phagocytosis by neutrophils or stimulating macrophages to increased phagocytic activity or to produce various cytokines (1).

The CSFs are active when injected *in vivo* as predicted from the *in vitro* studies. The CSFs have short half-lives of 3 to 12 hours when administered subcutaneously, but in PEGylated form the half-life is extended. There is a dose-response to injected CSFs in peripheral blood levels of neutrophils and a less marked increase in monocytes. In mice, injections of G-CSF or GM-CSF increase the marrow, spleen, and blood content of granulocytes and macrophages (15, 16). In both mice and humans, the injection of G-CSF or GM-CSF has a remarkable additional action of mobilizing stem and progenitor cells from the bone marrow to the peripheral blood (17, 18). In this response, levels of these latter cells in the peripheral blood can reach as high as those in the bone marrow.

Gene knockout studies have clarified the apparent redundancy of the multiple CSFs as monitored *in vitro*. These studies clearly identified G-CSF as the most important

regulator of neutrophil production and levels (19), and similarly loss of M-CSF has the greatest effect in reducing monocyte and macrophage populations in multiple organs (20). Loss of GM-CSF has little effect on cell numbers but reduces mature cell functional activity. In particular, reduction of macrophage function leads to accumulation of surfactant in the lung and the disease alveolar proteinosis (21). Finally, loss of IL-3 has little apparent effect on the numbers of granulocytes or macrophages but reduces mast cell levels and some T-cell responses (22).

When CSFs were first tested *in vivo*, it was surprising that the quantitative effects of CSF action *in vivo* did not correspond with their actions *in vitro*. In particular, G-CSF, which, at least in murine cultures, was the weakest agent in terms of colony stimulation, turned out to have the strongest effects on neutrophil numbers and stem cell mobilization when tested *in vivo*. This effect is largely dependent on a synergistic action with stem cell factor. In the absence of stem cell factor, the actions of G-CSF *in vivo* are extremely weak (23).

Clinical Uses of the CSFs

The readily demonstrable stimulating effects of G-CSF and GM-CSF on granulocyte-macrophage populations led to the licensing by the U.S. Food and Drug Administration (FDA) of both agents for use where chemotherapy had caused marrow damage and neutropenia (1). For this purpose, G-CSF alone has been used in the management of 10 to 20 million patients with cancer and in additional patients with neutropenia from other causes or severe infections.

M-CSF has not entered the clinic because initial studies showed that thrombocytopenia occurred as a side effect, presumably because of macrophage stimulation. Similarly, IL-3 has not entered the clinic because of side effects, presumably due to IL-3 stimulation of mast cells.

An initially surprising outcome of the first clinical trials of G-CSF and GM-CSF was the observation of major increases in the blood of stem and progenitor cells (17, 18). This has led to the replacement of bone marrow cells for hematopoietic transplantation by CSF-elicited peripheral blood stem cells (PBSC). This is because the greater number of PBSC harvestable leads to more rapid recovery of hematopoietic populations, easier nursing, and lower mortality from the transplant procedure (24). The greater efficiency of PBSC has permitted transplants to be used in a broader range of patients with cancer than previously possible.

The most direct impacts of G-CSF and GM-CSF on patients with cancer are the accelerated regeneration of marrow following chemotherapy and the almost complete replacement of bone marrow cells by CSF-elicited PBSCs for transplantation both in the autologous and allogeneic settings. In these clinical uses, the CSFs have no direct action on cancer cells but merely support the use of cytotoxic therapy to eliminate cancers.

GM-CSF and Immune Responses to Cancer

Of the four CSFs, GM-CSF has a special involvement in immune responses. It is the most important cytokine in the stimulation of dendritic cell formation and the enhancement

of dendritic cell activity (25). In extensive *in vitro* studies, immune responses to many agents, including cancer cells, were clearly enhanced by the use of GM-CSF to increase dendritic cell activity. These findings have led to a variety of clinical trials in an attempt to enhance host immune responses particularly against melanomas, renal tumors, and prostate tumors in which historically there were reasons to suspect that immune responses (either adaptive or innate) could restrain tumor growth or induce remissions. GM-CSF has been combined with tumor cells or tumor-derived peptides in clinical trials to enhance possible responses. Alternatively, GM-CSF has been incorporated into agents such as vaccinia virus to enhance the induction of activated dendritic cells (26, 27). Although these trials have had some promising initial clinical responses, no definitive impact on survival times or tumor reduction has yet been obtained, but the approach is still under active study.

The CSFs and Myeloid Leukemia

It is a general tenet in cancer biology that agents able to stimulate the proliferation of cell populations, if acting for prolonged periods, can be cofactors in cancer development. This is a well-established concept for cancer development in target tissues of hormones. To the degree that CSFs are major proliferative stimuli for granulocyte and macrophage populations, the proposition that CSF action may be leukemogenic has been raised since the early 1970s. There was a considerable delay in developing animal models with sustained CSF elevations. These were achieved initially by transfecting and overexpressing CSF genes in hematopoietic cells that were then used to repopulate irradiated animals and eventually by generating transgenic animals. The outcome of studies in these animals documented the development of severe hyperplasia in the responding granulocyte-macrophage populations, in some cases with lethal consequences, but no leukemias developed (1, 28).

No studies so far seem to have determined the effects of excess CSF levels in a variety of mutant mice that exhibit hematopoietic dysplasia, such as JAK2 mutants or mice whose cells bear transduced fusion oncogenes, such as *MLL-ENL* or *MLL-AF9*. As a consequence, the possible role of CSFs as leukemogenic cofactors has not been extensively studied.

However, insertion or activation of GM-CSF or IL-3 in continuous cell lines transformed these cell lines into leukemic populations and this was observed to occur both *in vitro* and *in vivo*. Furthermore, combination of the overexpression of IL-3 and a homeobox gene produced rapidly developing leukemias. In these contexts, the CSF genes are probably classifiable as oncogenes (29).

The remarkable feature of human chronic and acute myeloid leukemia and also most murine models of acute myeloid leukemia is that the proliferation of the vast majority of the leukemic cells *in vitro* is absolutely dependent on stimulation by CSF with the same dose-response relationships as those for normal granulocyte-macrophage progenitor cells (29). The situation has become even more intriguing with the demonstration that the rare autonomous cells in murine

MLL-ENL or MLL-AF9 myeloid leukemic population give rise to large numbers of factor-dependent progeny when stimulated by GM-CSF.

The expectation that autonomous growth would be the end stage in the transformation of myeloid leukemic cells seems not to be as simple as originally envisaged. It now seems that factor dependency and autonomy are reciprocally interchangeable states, and animals and humans with myeloid leukemia have leukemic populations that are mainly CSF dependent at least *in vitro*.

To further complicate the role of cytokines in leukemic cell biology, certain long-established leukemic cell lines such as MI, Wehi-3B, or GB2 can be suppressed by cytokines active in inducing terminal differentiation and death. Foremost among these agents are leukemia-inhibitory factor (LIF) and IL-6, but in some models, G-CSF has a similar action and can synergize with both LIF and IL-6 (30–32).

The summation of these data paints a picture in which CSF stimulation seems to be necessary for the progressive emer-

gence of leukemic populations and may be a cofactor in transformation but on occasion may suppress leukemic populations. So far, no real attempt has been made to use this information clinically. However, the long-term use of G-CSF seems to be nonleukemogenic in patients with chronic neutropenia, some of whom are at risk of developing acute myeloid leukemia (33). In the latter disease, activating mutations of the G-CSF receptor seem to be associated with the transformation (34), raising again the original possibility that chronic overstimulation may be leukemogenic.

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