MINIREVIEW

The Role of Colony-Stimulating Factors in Host Defenses (43266)

STEPHEN H. GREGORY,* D. MITCHELL MAGEE,† AND EDWARD J. WING*,[1]

Department of Medicine, The University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213 and Department of Medicine,† The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284*

The colony-stimulating factors (CSF) are glycoproteins integrally involved in the production and activation of phagocytic cells. There are four well-defined CSF capable of stimulating the proliferation and differentiation of bone marrow-derived phagocytes *in vitro*. These four CSF were distinguished originally on the basis of the major cell type(s) they affected: interleukin 3 (IL-3, or multi-CSF) promotes the formation of granulocytes, eosinophils, and macrophages; granulocyte-macrophage CSF (GM-CSF) induces the formation of granulocytes and macrophages; macrophage CSF (M-CSF or CSF-1) induces macrophage formation, primarily; granulocyte CSF (G-CSF) stimulates neutrophil formation, primarily. In addition to the CSF, a number of other cytokines exhibit colony-stimulating activity. In particular, interleukin 5 (IL-5) acting alone and interleukin 1 (IL-1), interleukin 4 (IL-4), and interleukin 6 (IL-6) acting in synergy with the CSF have proliferative effects.

The phagocytes in the peripheral blood of normal individuals exhibit a relatively short half-life that ranges from several hours to a few days, necessitating the replenishment of phagocytes on a continual basis. The production of phagocytes *in vivo* appears to be at least partially dependent upon CSF activity. The CSF stimulate both the proliferation of pluripotential bone marrow stem cells and the differentiation of these stem cells to committed progenitor cells. The proliferation and further differentiation of committed progenitor cells to mature phagocytes is, in turn, mediated by CSF activity. The CSF also contribute to the accelerated production of phagocytes and the marked increase in white blood cell count observed during infection. This increase in phagocytes constitutes a major mechanism of defense against invading microorganisms. In addition to increasing phagocyte numbers, CSF modulate cell-surface receptor expression, chemotaxis, secretory activity, and the killing mechanisms of phagocytes. Thus, the CSF are important regulatory glycoproteins that enhance both the production and function of phagocytes.

[1] To whom requests for reprints should be addressed.

0037-9727/91/1974-0349/$3.00/0

Overview of Hemopoiesis

Self-renewing stem cells in the bone marrow generate the full range of mature hemopoietic cells. The CSF, acting within the bone marrow microenvironment, stimulate the differentiation of stem cells to committed progenitor cells, which further differentiate to mature phagocytes (Fig. 1). Hemopoietic stem cells are defined by an *in vivo* assay in which normal syngeneic bone marrow cells are injected into lethally irradiated mice (1–4). The colonies that develop within the spleen after 12 to 14 days contain the most primitive cells, called splenic colony-forming units (CFU-S). Although not usually proliferating, CFU-S are capable of self-renewal. Of the CSF identified, IL-3 acts on the least-differentiated cells and may be an important signal for CFU-S (5, 6). As maturation proceeds, CFU-S evolve into an additional type of progenitor cell called granulocyte-erythrocyte-monocyte-megakaryocyte colony-forming units (CFU-GEMM) and the capacity for self-renewal decreases. CFU-GEMM respond to both IL-3 and GM-CSF and evolve into committed phagocyte progenitor cells: (i) macrophage colony-forming units; (ii) granulocyte colony-forming units; and (iii) eosinophil colony-forming units. The differentiation of macrophage CFU to mature macrophages is influenced by IL-3, GM-CSF, and M-CSF. The differentiation of granulocyte CFU to mature granulocytes is influenced by IL-3, GM-CSF, and G-CSF. IL-3, GM-CSF, and IL-

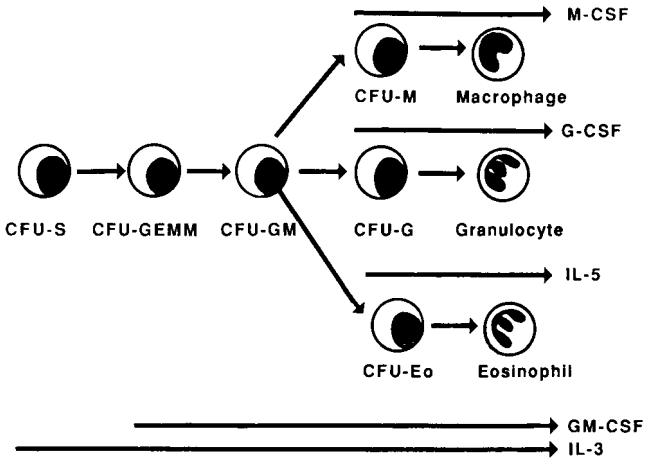

Figure 1. Hemopoiesis as a function of CSF activity.

5 promote the differentiation of eosinophil CFU to eosinophils.

The hemopoietic system is under complex control and responds to a variety of positive and negative factors (2, 7–9). The production of phagocytes may be increased up to 1000-fold by positive feedback signals. The CSF themselves act synergistically to promote the proliferation of progenitor cells (7). When used in combination, the concentrations of CSF required to stimulate cell cycling can be reduced by a factor of 10–400. In addition to the CSF, IL-1, IL-4, IL-5, and IL-6 are potent positive regulators of hemopoiesis. The interleukins may exert a direct effect on the production of phagocytes from precursor cells, or an indirect effect mediated by their actions on accessory cells (2). Bacterial endotoxin (lipopolysaccharide [LPS]) also enhances hemopoiesis (8). Experimental animals injected with LPS exhibit a transitory rise in serum CSF levels reaching 50–100 times baseline values. In addition, the numbers of progenitor cells in the bone marrow and spleen increase after LPS injection. Other microbial products, including extracts of *Listeria monocytogenes*, bacillus Calmette-Güerin, *Corynebacterium parvum*, and *Lactobacillus casei*, increase the serum CSF levels, as well as the number of progenitor cells (8, 10, 11).

As infectious organisms are effectively eliminated by host defense mechanisms, the requirement for white blood cells is diminished. A reduction in CSF activity alone may not be sufficient to decrease phagocyte production, however. A series of negative feedback mechanisms are available to facilitate the return of hemopoietic cell production to steady state levels. Prostaglandins inhibit colony formation by a variety of progenitor cells (2, 8). At physiological concentrations of prostaglandins, however, the production of macrophage CFU is suppressed primarily. Iron-binding proteins such as acid ferritins, lactoferrin, and transferrin decrease CSF production or the cycling of progenitor cells (2, 8). α, β, and γ-interferons (IFN), and α- and β-tumor necrosis factors (TNF), also interfere with colony formation (2, 8). These regulatory mechanisms enable the precise control of white blood cell production.

The Biological Properties of Colony-Stimulating Factors

The four classic CSF are glycoproteins composed of polypeptide chains of approximately equal length. IL-3, G-CSF, and GM-CSF are single chains; M-CSF is a dimer consisting of two identical chains (Table I). The carbohydrate components of the molecules are extremely variable, accounting for the differences in molecular weights reported in the literature.

A variety of cells, including T lymphocytes, endothelial cells, macrophages, and fibroblasts, produce CSF. In general, those cells that produce CSF are capable of producing more than one type and are widely distributed among tissues. The basal levels of CSF production are usually low. Increased levels in the blood and tissues occur rapidly in response to factors such as infection and antigenic stimulation. Besides stimulating the proliferation of hemopoietic cells, the CSF in general: (i) promote continued cell viability; (ii) stimulate the irreversible commitment of cells to differentiation; and (iii) promote cell function or biological activity. Analysis of the biological activity of any one CSF is greatly complicated by the capacity of that factor to provoke the synthesis of other cytokines, including other CSF. In addition to their effects on hemopoietic cells, the CSF stimulate the growth and biological activity of a number of other cell types, e.g., normal marrow fibroblasts and endothelial cells (12, 13). The importance of these effects on nonhemopoietic cells *in vivo* remains to be determined.

The response of a cell to a particular CSF is governed by the expression of a unique, noncross-reacting cell surface receptor. The receptors for all four CSF are glycoproteins composed of single polypeptide chains (14). Following CSF binding, the receptors are internalized and degraded. The molecular events that occur subsequent to CSF-receptor interaction and that culminate in cellular activation are not well understood (15). However, these events include the phosphorylation of specific cellular proteins and the enhanced expression of specific cellular genes, e.g., proto-oncogenes (16). Cells can express receptors for more than one CSF, allowing the interaction of CSF at the cell surface. Thus, the occupation of one type of receptor may modulate (usually down-regulate) CSF binding to a receptor of a different type (14).

IL-3. IL-3, or multi-CSF, is an interleukocyte factor produced exclusively by activated T4 helper lymphocytes (17). IL-3 is the least-restricted CSF in terms of the cell lineages that it affects. In addition to stimulating the formation of granulocyte and macrophage colonies, IL-3 stimulates the proliferation of erythroid, mega

Table I. Hemopoietic Colony-Stimulating Factors

Factor	Mol wt	Cell source	Cells produced
IL-3 (multi-CSF)	15,000–30,000	T lymphocyte	Erythrocyte, granulocyte, macrophage, megakaryocyte
GM-CSF	18,000–30,000	T lymphocyte, fibroblast, endothelial, epithelial, macrophage	Granulocyte, macrophage, megakaryocyte
G-CSF	20,000	Endothelial, macrophage, fibroblast	Granulocyte
M-CSF (CSF-1)	70,000–90,000 (dimer)	Macrophage, fibroblast, endothelial	Macrophage

karyocytic, mast, and pluripotential stem cells (6, 9). Although its precise role remains to be elucidated, its major function may involve regulating the proliferation and differentiation of bone marrow stem cells. The effects of IL-3 on the survival and differentiation of early progenitor cells are enhanced by other cytokines such as interleukin 1 and interleukin 6 (18). Recent studies showed that while IL-3 alone does not stimulate the proliferation of mature macrophages, IL-3 does enhance the proliferative response of macrophages to M-CSF (19).

IL-3 exerts a variety of effects on cells in addition to stimulating their proliferation. IL-3-treated monocytes, for example, exhibit elevated antimicrobial activity. Recombinant human IL-3 is as effective as GM-CSF in inducing the killing of *Candida albicans* by human monocytes *in vitro* (20). Moreover, IL-3-treated monocytes maintain their anticandidal activity for a longer period of time than do monocytes stimulated with IFN-γ. In addition, IL-3 in conjunction with bacterial endotoxin stimulates the tumoricidal activity of monocytes (21). This activity is diminished in culture by the addition of antibody specific for TNF-α, implicating TNF in the elevated tumoricidal activity observed.

GM-CSF. GM-CSF is undetectable in normal sera by current methods of analysis (22). In the bone marrow, it appears to be synthesized locally and sequestered in the extracellular matrix (23, 24). GM-CSF is synthesized and secreted by a number of cell types, i.e., T lymphocytes, macrophages, fibroblasts, and endothelial cells. GM-CSF production by T cells is induced by IL-1 (25) and antigen- or mitogen-stimulation (26, 27). Expression of the GM-CSF gene in macrophages and fibroblasts is initiated by phagocytosis, contact with microbial products, or treatment with either IL-1 or TNF (28–31). Similarly, IL-1 and TNF stimulate GM-CSF production by fibroblasts and endothelial cells (32–37).

GM-CSF affects cells predominantly, but not exclusively, of the myeloid lineage (12, 13). GM-CSF has a broad range of activities in addition to stimulating the growth of granulocyte and macrophage colonies *in vitro*. GM-CSF supports the proliferation of progenitors, including multipotential blast cells, that give rise to mixed colonies which contain all the myeloid elements (38–40). GM-CSF is chemotactic for mononuclear phagocytes (41) and is a potent activator of mononuclear phagocyte function. Monocytes treated with GM-CSF exhibit an increase in IL-1 production and the enhanced expression of cell-surface Ia antigens (42, 43). Both activities may serve to up-regulate the antigen-presenting capacity of macrophages and, thus, the capacity of cells to initiate an immune response. Indeed, prior treatment of splenic macrophages with GM-CSF increases the primary immune response to sheep red blood cells (sRBC) measured in terms of the number of anti-sRBC plaque-forming cells (42).

GM-CSF stimulates the tumoricidal activity of human peripheral blood monocytes. Human peripheral blood monocytes treated with human recombinant GM-CSF exhibit an elevated capacity to kill human malignant melanoma cells (A375) *in vitro* (44, 45). In contrast, the enhanced tumoricidal activity exhibited by monocytes in response to IFN-γ is observed only when a second signal, such as LPS, is provided. It has been suggested that activity exhibited by GM-CSF-treated monocytes may be related to the enhanced secretion of TNF-α (21, 46).

The antimicrobial activity of mononuclear phagocytes is also stimulated by GM-CSF. GM-CSF acts in synergy with IFN-γ to promote the resistance of macrophages to infection by *Leishmania major* (47). Moreover, GM-CSF-activated macrophages exhibit an elevated capacity to kill *Leishmania donovani* (48). Sub-

optimal concentrations of GM-CSF and IFN-γ are synergistic in their anti-leishmanial activity. Similarly, GM-CSF-treated macrophages inhibit the replication of *Leishmania tropica* (49) and *Trypanosoma cruzi* (50). GM-CSF stimulates the secretion of hydrogen peroxide by macrophages incubated with either phorbol esters or zymosan, providing a possible mechanism for the enhanced killing of some organisms (50, 51). GM-CSF also prevents the infection of the monocytic cell line U937 by the human immunodeficiency virus (HIV) (52). A maximal effect is observed when the cells are pretreated and GM-CSF is provided continuously throughout the culture period. HIV replication resumes when GM-CSF is removed from the culture system. Additional studies have shown a synergistic effect of GM-CSF and 3′-azido-3′-deoxythymidine (AZT) on the anti-HIV activity of macrophage cells (53, 54). Moreover, the toxic effect of AZT on human myeloid progenitor cells is ameliorated by the addition of GM-CSF (55).

In contrast to the aforementioned studies, other investigators report that GM-CSF does not induce the anti-*Toxoplasma* activity of murine tissue-derived macrophage cells, despite an increase in H_2O_2 secretion (51). In separate studies, GM-CSF did not induce H_2O_2 production by human macrophage populations or stimulate their antimicrobial activity toward either *Toxoplasma* or *Legionella pneumophila* (56, 57). Contrary to its effect on the replication of HIV in the U937 cell line noted above, GM-CSF promotes the replication of HIV in human monocytes (54).

In addition to its effects on mononuclear phagocytes, GM-CSF is a potent stimulator of eosinophil and neutrophil function. GM-CSF is chemotactic for both granulocytes and eosinophils (41, 58). Neutrophils treated with physiologic concentrations of GM-CSF express a 3-fold increase in the number of cell-surface receptors for the chemoattractant f-Met-Leu-Phe (59). In addition, 30 min of incubation with GM-CSF reversibly inhibits the migration of neutrophils (60). Thus, GM-CSF may serve to attract and to immobilize neutrophils in areas of inflammation. Both neutrophils and eosinophils exhibit enhanced antibody-dependent cellular cytotoxicity (ADCC) following GM-CSF treatment (38, 61–64). It is relevant to note that treatment with GM-CSF increases the number of Fc receptors on U937 and HL-60 cells (65, 66). Furthermore, GM-CSF stimulates the oxygen burst of neutrophils (67), a factor that could augment their killing capacity. Finally, GM-CSF-treated granulocytes are characterized by elevated production of IL-1 (68).

The antimicrobial activity of granulocytes is also increased following treatment with GM-CSF. GM-CSF stimulates oxidative metabolism (67), the phagocytosis of bacteria (69), and the uptake and killing of *T. cruzi* by human neutrophils (70). The killing of *Shistosoma mansoni* larvae by eosinophils is also stimulated by GM-CSF (71).

G-CSF. G-CSF is undetectable in the serum of most healthy human subjects, but high levels are detected in patients with a variety of blood disorders (72). The expression of G-CSF transcripts and the production of G-CSF by human monocytes, fibroblasts, and endothelial cells *in vitro* are stimulated by LPS, IL-1, and/or TNF (29, 36, 37, 73–75).

G-CSF stimulates the proliferation and differentiation of committed granulocyte precursor cells (76). Colonies of other cell types that occur when bone marrow cultures are treated with G-CSF are thought to arise as an indirect effect of accessory cells and other cytokines also present (2). G-CSF is a potent differentiating factor for some, but not all, myeloid leukemia cells (77, 78). In addition to supporting granulocyte formation, G-CSF exerts a profound influence on the biological activity of mature cells. G-CSF is a chemotactic factor for granulocytes (79). Furthermore, neutrophils treated with recombinant G-CSF exhibit: increased phagocytic activity (80); elevated superoxide anion production in response to f-Met-Leu-Phe (81); and enhanced ADCC for tumor cells (62). These biological activities are consistent with the hypothesis that the primary functions of G-CSF are to stimulate the growth and differentiation of neutrophil progenitor cells and to prime the response of mature cells to subsequent stimuli. Although granulocytes are the primary target cells of G-CSF activity, recombinant G-CSF induces both the migration and the proliferation of human endothelial cells in culture (12). These findings suggest that G-CSF may serve as a regulatory molecule outside of the hemopoietic system.

M-CSF. M-CSF is produced by macrophages, endothelial cells, and fibroblasts and is found in low concentrations in many tissues (36, 82, 83). IL-3, GM-CSF, and IFN-γ stimulate M-CSF production by macrophages (29, 75). Murine M-CSF added to murine bone marrow progenitor cells is a potent stimulator of macrophage colony formation *in vitro*. Initial studies reported a greater effect of human M-CSF on the formation of murine macrophage colonies than on human macrophage colonies (84–86). Improved culture conditions have resulted in the enhanced stimulation of human macrophage progenitor cells and the elevated production of macrophage colonies in response to human M-CSF treatment (87, 88).

M-CSF exerts a wide range of effects on macrophages in addition to promoting cell proliferation and viability. One of the most striking effects is the change in cell morphology induced by M-CSF. M-CSF-treated cells exhibit increased cell size, membrane ruffling, and cytoplasmic vacuolization (89). M-CSF is chemotactic for macrophages (90) and has been shown to enhance their tumoricidal activity in several studies. M-CSF-

treated murine macrophages were found to be tumoristatic in an early report (91). In more recent studies, M-CSF-treated murine macrophages exhibited enhanced tumor cytotoxicity and ADCC (92, 93). In the latter case, the greatest effect was observed when M-CSF and lymphocyte supernatants were used as costimulants. An increase in TNF secretion by macrophages following treatment with M-CSF may contribute to the increase in tumor cytotoxicity observed (94). Likewise, an increase in Fc receptor expression could augment ADCC by treated macrophages (95). Human recombinant M-CSF is also a potent stimulator of human macrophage cytotoxicity (96).

In contrast to its effects on the tumoricidal activity of macrophage cells, M-CSF-treated macrophages suppress IL-2 secretion and the proliferation of antigen- or mitogen-stimulated T lymphocytes (97). The failure of either catalase or indomethacin to inhibit this suppressive effect indicates that the effect is not due to the production of either prostaglandins or toxic oxygen radicals. M-CSF-treated macrophages fail to express cell-surface Ia antigens, maximally suggesting that treated macrophages may no longer present antigens to T lymphocytes effectively (96).

The antimicrobial activity of macrophages is increased after M-CSF stimulation. Resident peritoneal macrophages exhibit increased phagocytosis and overall killing of *L. monocytogenes* following treatment with M-CSF (98). M-CSF also stimulates the killing of *C. albicans* by both human monocytes (20) and elicited murine macrophages (99). Cells treated with M-CSF exhibit increased hydrogen peroxide and superoxide anion production, an elevated number of mannose receptors, and the enhanced uptake of yeast, suggesting several possible mechanisms by which the killing of *C. albicans* is increased (99, 100). In contrast to these studies, M-CSF-treated human macrophages are not stimulated to secrete H_2O_2 or to kill *Toxoplasma gondii* (56).

M-CSF also induces the resistance of murine macrophages to vesicular stomatitis virus infection (101). The resistance of M-CSF-treated cells is inhibited by antibody to IFN-α/β indicating the participation of IFN-α/β in the observed effect. In contrast, monocyte-derived macrophages may become permissive for the growth of HIV virus following M-CSF treatment (102).

Interleukins. In addition to the four classic CSF, there are a number of other soluble factors that influence colony formation either directly or indirectly. Among these factors are the interleukins, described originally for their effects on other biological systems.

IL-1. Interleukin 1, which is identical to hemopoietin 1, acts on a variety of cell types (103, 104). It has no proliferative effect on hemopoietic cells when acting alone. However, IL-1 has been shown to modulate the response of cells to CSF, possibly by enhancing the expression of cell surface receptors for these factors. Thus, IL-1 enhances the differentiation and survival of early progenitor cells that occur in response to IL-3. In addition, IL-1 stimulates the production of G-CSF and/or GM-CSF by T lymphocytes, macrophages, endothelial cells, and fibroblasts *in vitro* (25, 34–37).

IL-4. Like IL-1, interleukin 4 alone has no proliferative effect on normal hemopoietic progenitor cells. It does stimulate the formation of colonies in response to G-CSF and GM-CSF, while inhibiting their formation in response to IL-3 (12).

IL-5. Interleukin 5 selectively stimulates the proliferation of eosinophil precursors and the biological activity of mature eosinophils (63, 105). Activated T lymphocytes are the only known source of interleukin 5. This may account for the fact that the normal response of eosinophils to worm infestation is dependent upon the biological activity of T lymphocytes.

IL-6. Interleukin 6 exerts effects on a variety of cell types (106). It has no proliferative effect on human hemopoietic cells when acting alone (107). It does serve as a weak stimulus for the formation of granulocyte and macrophage colonies when added to cultures of murine bone marrow cells, however. In addition, IL-6 accelerates the formation of multilineage, granulocyte, and macrophage colonies induced by IL-3 (18).

CSF Production during Infection

Considering that an increase in white blood cell count is a hallmark of infection, changes in both the frequency of progenitor cells and the levels of CSF in the serum might be expected. Indeed, studies involving a number of infectious agents, i.e., *Salmonella typhimurium* (108), *Mycobacterium lepraemurium* (109), *Brucella abortus* (110), *S. mansoni* (111), and *S. japonicum* (112, 113), have reported elevated levels of CSF in the sera and increased numbers of progenitor cells within the bone marrow and spleen. Nonviable bacteria and bacterial products also modulate both the CSF levels and the progenitor cell number. Serum CSF levels and the number of progenitors are increased in mice following intraperitoneal injection of *Nocardia rubra* cell wall skeleton (114). Similarly, subcutaneous injection of heat-killed *Lac. casei* induces peak serum CSF activity 18 hr after injection (10). In the latter case, the number of progenitor cells in the bone marrow is elevated on Day 3, but is below normal on Day 10 after inoculation. Conversely, the number of progenitor cells in the spleen is elevated 3–10 days following inoculation. Mice injected with synthetic muramyl peptides also exhibit elevated serum CSF levels (11). Some of the same synthetic derivatives protect mice from infection by *Klebsiella pneumoniae.* All of the derivatives that are protective stimulate CSF production; not all of the derivatives capable of inducing CSF synthesis are protective, however.

Listeriosis in mice is an animal model used extensively to examine the role of cell-mediated immunity in host defenses to intracellular pathogens. Its study has provided insight into the changes in CSF and progenitor cells that occur during infection. Increased serum CSF levels are evident within 20 hr after infection with *L. monocytogenes* (115–116). These levels remain elevated for 7–10 days, gradually returning to normal as the infection is resolved. The number of progenitor cells in the bone marrow decreases during infection, attaining a minimum at 4–7 days after infection (106). The number of progenitors in the spleen, on the other hand, increases, reaching a maximum after 4–7 days (115). Analyses of sera indicate that the primary CSF produced during infection are M-CSF and G-CSF (115–117). Analyses of tissue homogenates indicate that the livers and spleens of infected animals contain the most M-CSF activity (118). Splenectomy prior to infection abrogates the increases in serum CSF levels observed (119).

Immune mice challenged with *Listeria* exhibit different kinetics of CSF production (120). The level of M-CSF in the sera of immune animals peaks at 12 hr after infection and nearly returns to a normal level by 48 hr. In contrast to nonimmune animals, the number of progenitor cells in the bone marrow of immune animals increases following infection. Furthermore, the number of progenitors in the spleens of immune mice increases only 2-fold in response to challenge, whereas the number in the spleens of nonimmune mice increases 6-fold (121). Nonimmune mice administered *Listeria*-immune, but not nonimmune, splenocytes produce CSF rapidly in response to *Listeria* challenge (121). The rapid production of CSF observed in adoptively immunized mice following infection is mediated by T lymphocytes; treatment of immune splenocytes with anti-Thy 1.2 and complement prior to transfer abrogates the phenomenon.

The production of CSF by spleen cells *in vitro* has been evaluated. Immune splenocytes incubated with heat-killed *Listeria* produce five times more CSF activity than do nonimmune splenocytes (122). Moreover, the supernatants obtained from cultures of immune and nonimmune splenocytes contain different CSF activities. The culture supernatants derived from immune cell populations induce granulocyte colonies, primarily; the supernatants from nonimmune cells give preferential rise to macrophage colonies.

$CD4^+$, *Listeria*-specific, T cell clones incubated with heat-killed antigen produce both IL-3 and GM-CSF (26, 123). Passive transfer of either the clones or their supernatants to nonimmune mice confers resistance to a lethal challenge with *Listeria*. Removal of GM-CSF from the supernatants reduces the level of protection conferred. Thus, GM-CSF synthesized and secreted by $CD4^+$ T lymphocytes during infection may play an important role in host defenses.

While T lymphocytes are a critical factor in the elevated production of CSF during secondary immunological responses, other cell types may play an important role in the production of CSF during the early stages of a primary response. It has been reported, for example, that heat-killed *Lac. casei* injected into nonimmune mice stimulates CSF production by macrophages (124). Consequently, there appears to be a number of mechanisms by which the production of CSF might be increased. T cell-independent mechanisms may be important during the initial stages of infection; T cell-dependent mechanisms may enhance CSF secretion and modulate the types of CSF secreted during the later stages.

Immunotherapy with the CSF

A potentially important role exists for hemopoietic growth factors in the restorative therapy of immunosuppressed patients, including cancer patients undergoing chemotherapy, bone marrow transplant patients, and patients with acquired immune deficiency syndrome, severe burns, or overwhelming infections (22). Isolation of the cDNA that encode the CSF has enabled investigators to synthesize the recombinant proteins in quantities sufficient to test their therapeutic potential. Clinical trials involving recombinant GM-CSF and G-CSF have been reported; recombinant M-CSF and IL-3 are currently being evaluated. The early results indicate that the effects of CSF *in vivo* are similar to the effects observed *in vitro*.

The number of white blood cells in the peripheral circulation increases dramatically following the administration of CSF. Neutrophils, monocytes, and eosinophils are elevated in mice given recombinant murine IL-3 (125). Similarly, nonhuman primates injected with human G-CSF or GM-CSF exhibit dose-dependent increases in the same cell populations (126, 127). Tests in humans confirm the effects of CSF on the peripheral white blood cell count. Increases in the numbers of circulating monocytes and neutrophils are observed in cancer patients or patients with bone marrow failure undergoing treatment with GM-CSF (128–130).

In addition to increasing the number of peripheral white blood cells, CSF can reduce the toxic effects of chemotherapy and irradiation on the bone marrow. The administration of GM-CSF promotes the recovery of neutrophils, eosinophils, and monocytes in cyclophosphamide-treated mice that normally exhibit myelosuppression (131). Similarly, human G-CSF increases the recovery of white blood cells in Syrian hamsters following cyclophosphamide treatment (132). Recombinant murine GM-CSF and G-CSF partially protect mice from the lethal effects of irradiation (133). GM-CSF and G-CSF given to human cancer patients reverse

the neutropenia induced by chemotherapy (134). GM-CSF treatment also increases the number of neutrophils, eosinophils, and monocytes found in the blood of patients with acquired immune deficiency syndrome (135). Finally, human M-CSF increases the neutrophil counts in children with chronic neutropenia (136) and in patients undergoing cytotoxic therapy (137) presumably by stimulating the production of other colony-stimulating factors by monocytes.

Mature phagocytes are activated *in vivo* by the administration of CSF. The primary immune response to sRBC is enhanced in mice treated with murine GM-CSF. Mice injected with GM-CSF exhibit a 2- to 3-fold increase in the production of antibody to a low dose of sRBC (42). The macrophages obtained from GM-CSF-treated mice exhibit increases in Ia density, Fc receptor expression, and IL-1 secretion, and inhibit the growth of *T. cruzi in vitro* (138, 139). Furthermore, the clearance of *S. typhimurium* is increased in mice following the administration of GM-CSF (138). G-CSF increases the resistance to infection by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Serratia marcescens*, or *C. albicans* in animals rendered neutropenic by thermal injury or cyclophosphamide treatment (132, 140, 141). Additionally, recombinant murine GM-CSF given to mice provides protection against listerial infections (26). Several studies indicate that human phagocytes are also activated by CSF *in vivo*. Monocytes obtained from patients following treatment with GM-CSF secrete more interferon and TNF-α, and exhibit a greater capacity for ADCC, than do monocytes obtained prior to treatment (128). Superoxide anion production in response to f-Met-Leu-Phe or phorbol myristate acetate is elevated in the neutrophils derived from cancer patients following the administration of GM-CSF (142, 143). Similarly, an increase is observed in the production of superoxide anions and the binding of f-Met-Leu-Phe by granulocytes obtained from hamsters administered G-CSF (132).

Although early clinical trials involving the CSF have been encouraging, several experiments indicate that in certain situations the CSF may have no effect on host defenses or, in fact, may be detrimental. It has been shown, for example, that treatment of mice with antibodies to GM-CSF and IL-3 dramatically decreases the incidence of neurological symptoms in cases of cerebral malaria (144). In a study of murine leishmaniasis, treatment of mice with GM-CSF actually led to an increase in the parasite burden (145). There is experimental evidence to suggest that the CSF may increase the replication of human immunodeficiency virus in monocytes (54, 98). It is apparent, therefore, that the use of CSF in the treatment of some infections could result in a susceptible population of phagocytes in which intracellular parasites proliferate more readily (146).

It is also conceivable that the administration of CSF clinically could exert adverse effects on the hemopoietic system. For example, CSF could provide a proliferative signal to cells in preleukemic patients and, thus, promote the onset of leukemia. Indeed, some malignant cells express CSF receptors (22). There is also experimental evidence to indicate that multiple injections of CSF may elicit antibody production (129) or a state of tolerance in which further administration of these cytokines is no longer effective (147). Fortunately, early clinical trials suggest that the CSF are relatively safe and have few side effects (22). In these trials, bone pain was the major complaint associated with the CSF used in immunotherapy; G-CSF seemed to be less toxic than GM-CSF.

Summary and Future Prospects

The role of CSF in the production of activated phagocytes during infection is summarized in Figure 2. Resident macrophages and immigrating granulocytes serve in the first line of host defenses against microbial invasion. The phagocytes kill some of the invading microorganisms and the macrophages process and present microbial antigens to antigen-specific T lymphocytes. IL-1 secreted by a number of cell types, including macrophages, promotes clonal expansion and the activation of sensitized T lymphocytes, which in turn produce a variety of cytokines, including CSF. These cytokines, in combination with the microbial products released during infection, stimulate the proliferation and differentiation of bone marrow progenitor cells and the increased formation of circulating white blood cells. In response to chemotactic factors, the phagocytes in the circulation adhere to endothelial cells adjacent to the inflammatory site, undergo diapedesis, and migrate to the site of infection. These phagocytes, activated by CSF and other cytokines, exhibit increased antimicro-

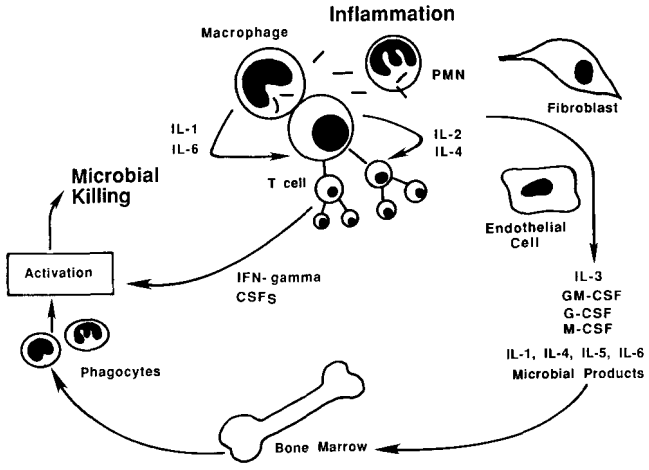

Figure 2. The role of CSF in the production of activated phagocytes during microbial infection.

bial activity. Thus, the CSF play a central role in phagocyte production, differentiation, and activation.

The genes encoding the CSF in humans and in mice have been cloned and expressed as recombinant material. The recombinant CSF exhibit essentially the same biological activity as do the native proteins. Moreover, many of the activities expressed *in vitro* have also been observed *in vivo*. The results of animal experiments and clinical trials confirm the role of CSF in phagocyte production and the augmentation of host defenses. While cytokines are currently being evaluated on an individual basis, therapies in the future may involve a combination of cytokines used in an effort to control host defenses precisely and to provide more effective treatment. It has been shown, for example, that murine IL-3 and M-CSF induce the cycling of progenitor cells when used together at doses that are individually ineffective (148). Additionally, a combination of either GM-CSF or G-CSF and IL-1 greatly increases the rate of survival from lethal irradiation (149). Furthermore, more effective treatments may utilize a combination of cytokines and conventional drugs. In one experiment, for example, gentamicin enhanced the anti-pseudomonal activity induced by G-CSF (150). In another experiment, tumor necrosis factor acted synergistically with amikacin and macrolides to inhibit the growth of *Mycobacterium avium* complex *in vitro* (151). Lastly, GM-CSF increased the antiviral activity of AZT against HIV (53, 55).

Clearly, the CSF represent an exciting field of medical biology, in which advances in the basic sciences are being applied rapidly in the clinic. Thus, a variety of patients, including those suffering from hemopoietic dysfunctions, malignancies, or overwhelming infections, may benefit from the therapeutic use of CSF and other cytokines in the near future.

1. Dexter TM, Moore M. Growth and development in the haemopoietic system: The role of lymphokines and their possible therapeutic potential in disease and malignancy. Carcinogenesis **7**:509–516, 1986.
2. Broxmeyer HE, Williams DE. The production of myeloid blood cells and their regulation during health and disease. CRC Crit Rev Oncol Hematol **8**:173–226, 1988.
3. Cannistra SA, Griffin JD. Regulation of the production and function of granulocytes and monocytes. Semin Hematol **25**:173–188, 1988.
4. Spangrude GJ. Enrichment of murine haemopoietic stem cells: Diverging roads. Immunol Today **10**:344–350, 1989.
5. Andreff M, Welte K. Hematopoietic colony-stimulating factors. Semin Oncol **16**:211–229, 1989.
6. Leary AG, Yang YC, Clark SC, Gasson JC, Golde DW, Ogawa M. Recombinant gibbon interleukin 3 supports formation of human multilineage colonies and blast cell colonies in culture: Comparison with recombinant human granulocyte-macrophage colony-stimulating factor. Blood **70**:1343–1348, 1987.
7. Broxmeyer HE, Williams DE. Actions of hematopoietic colony-stimulating factors *in vivo* and *in vitro*. Pathol Immunopathol Res **6**:207–220, 1987.
8. Wing EJ, Shadduck RK. Colony-stimulating factor. In: Torrence PF, Ed. Biological Response Modifiers: New Approaches to Disease Intervention. New York: Academic Press, p219–243, 1985.
9. Bender JG, Van Epps DE, Stewart CC. A model for the regulation of myelopoiesis by specific factors. J Leukocyte Biol **39**:101–111, 1986.
10. Yokokura T, Nomoto K, Shimizu T, Nomoto K. Enhancement of hematopoietic response of mice by subcutaneous administration of *Lactobacillus casei*. Infect Immun **52**:156–160, 1986.
11. Galelli A, Lefrancier P, Chedid L. Colony-stimulating activity by synthetic muramyl peptides: Variation with chemical structure and association with anti-infectious activity. Infect Immun **46**:495–500, 1984.
12. Bussolino F, Wang JM, Defilippi P, Turrin F, Sanavio F, Edgell C-JS, Aglietta M, Arese P, Manotovani A. Granulocyte- and granulocyte-macrophage colony stimulating factors induce human endothelial cells to migrate and proliferate. Nature **337**:471–473, 1989.
13. Dedhar S, Gaboury L, Galloway P, Eaves C. Human granulocyte-macrophage colony stimulating factor is a growth factor active on a variety of cell types of non-hematopoietic origin. Proc Natl Acad Sci USA **85**:9253–9257, 1988.
14. Nicola NA. Why do hemopoietic growth factor receptors interact with each other? Immunol Today **8**:134–140, 1987.
15. Farrar WL, Evans S, Harel-Bellan A, Ferris DK. Molecular events associated with the action of haemopoietic growth factors. J Cell Sci (suppl 10):243–255, 1988.
16. Pimentel E. Colony-stimulating factors. Ann Clin Lab Sci **20**:36–55, 1990.
17. Niemeyer CM, Sieff CA, Mathey-Prevot B, Bierer BE, Clark S, Nathan DG. Interleukin-3 (IL-3) is produced only by activated human T lymphocytes. Blood **70**(suppl 1):182a, 1987.
18. Leary AG, Idebuchi K, Hirai Y, Wong GG, Yang Y-C, Clark SC, Ogawa M. Synergism between interleukin-6 and interleukin-3 in supporting proliferation of human hematopoietic stem cells: Comparison with interleukin-1 alpha. Blood **71**:1759–1763, 1988.
19. Chen BD, Clark CR. Interleukin 3 (IL-3) regulates the *in vitro* proliferation of both blood monocytes and peritoneal macrophages: Synergism between a macrophage lineage-specific colony-stimulating factor (CSF-1) and IL-3. J Immunol **137**:563–570, 1986.
20. Wang M, Friedman H, Djeu JY. Enhancement of human monocyte function against *Candida albicans* by the colony-stimulating factors (CSF): IL-3, granulocyte-macrophage-CSF, and macrophage-CSF. J Immunol **143**:671–677, 1989.
21. Cannistra SA, Vellenga E, Groshek P, Rambaldi A, Griffin JD. Human granulocyte-monocyte colony-stimulating factor and interleukin 3 stimulate monocyte cytotoxicity through a tumor necrosis factor-dependent mechanism. Blood **71**:672–676, 1988.
22. Groopman JE, Molina JM, Scadden DT. Hematopoietic growth factors: Biology and clinical applications. N Engl J Med **321**:1449–1459, 1989.
23. Gordon MY, Riley GP, Watt SM, Greaves MF. Compartmentalization of a haematopoietic growth factor (GM-CSF) by glycosaminoglycans in a bone marrow microenvironment. Nature **326**:403–405, 1987.
24. Roberts R, Gallagher J, Spooncer E, Allen TD, Bloomfield F, Dexter TM. Heparin sulphate bound growth factors: A mechanism for stromal cell mediated haematopoiesis. Nature **332**:376–378, 1988.
25. Hermann F, Oster W, Muer SC, Lindemann A, Mertelsmann RH. Interleukin-1 stimulates T lymphocytes to produce granu-

locyte-monocyte colony-stimulating factor. J Clin Invest **81**:1415–1418, 1988.
26. Magee DM, Wing EJ. Secretion of colony-stimulating factors by T cell Clones: Role in adoptive protection against *Listeria monocytogenes*. J Immunol **143**:2336–2341, 1989.
27. Kelso A, Metcalf D, Gough NM. Independent regulation of granulocyte-macrophage colony-stimulating factor and multilineage colony-stimulating factor production in T lymphocyte clones. J Immunol **136**:1718–1725, 1986.
28. Van Damme J, Schaafsma MR, Fibbe WE, Falkenburg JH, Opdenakker G, Billiau A. Simultaneous production of interleukin 6, interferon-beta, and colony-stimulating activity by fibroblast after viral and bacterial infection. Eur J Immunol **19**:163–168, 1989.
29. Ernst TJ, Griffin JD. Regulation of colony-stimulating factor production by normal and leukemic human cells. Immunol Res **8**:202–214, 1989.
30. Ruef C, Coleman DL. Granulocyte-macrophage colony-stimulating factor: Pleiotropic cytokine with potential clinical usefulness. Rev Infect Dis **12**:41–62, 1990.
31. Thorens B, Mermod J-J, Vassalli P. Phagocytosis and inflammatory stimuli induce GM-CSF mRNA in macrophages through posttranscriptional regulation. Cell **48**:671–679, 1987.
32. Zucali JR, Broxmeyer HE, Gross MA, Dinarello CA. Recombinant human tumor necrosis factor alpha and beta stimulate fibroblasts to produce hemopoietic growth factors *in vitro*. J Immunol **140**:840–844, 1988.
33. Broudy VC, Kaushansky K, Segal GM, Harlan JM, Anderson L. Tumor necrosis factor type alpha stimulates human endothelial cells to produce granulocyte/macrophage colony-stimulating factor. Proc Natl Acad Sci USA **83**:7467–7471, 1986.
34. Segal GM, McCall E, Bagby GC. The erythroid burst promoting activity (BPA) produced by interleukin 1 stimulated endothelial cells is granulocyte-macrophage colony stimulating factor (GM-CSF). Blood **70**(suppl 1):184a, 1987.
35. Zucali JR, Dinarello EA, Oblon DJ, Gross MA, Anderson L, Weiner RS. Interleukin 1 stimulates fibroblasts to produce granulocyte-macrophage colony stimulating activity. J Clin Invest **77**:1857–1863, 1986.
36. Seelentag WK, Mermod JJ, Montesano R, Vassalli P. Additive effects of interleukin 1 and tumour necrosis factor-alpha on the accumulation of the three granulocyte and macrophage colony-stimulating factor mRNAs in human endothelial cells. EMBO J **6**:2261–2265, 1987.
37. Seelentag WK, Mermod JJ, Vassalli P. Interleukin 1 and tumor necrosis factor-alpha additively increase the levels of granulocyte-macrophage and granulocyte colony-stimulating factor (CSF) mRNA in human fibroblasts. Eur J Immunol **19**:209–212, 1989.
38. Metcalf D. The molecular biology and functions of the granulocyte-macrophage colony-stimulating factors. Blood **67**:257–267, 1986.
39. Sieff CA, Emerson SG, Donahue RE, Nathan DG, Wang EA, Wong GG, Clark SC. Human recombinant granulocyte-macrophage colony stimulating factor: A multilineage hemopoietin. Science **230**:1171–1174, 1985.
40. Koike K, Ogawa M, Ihle JN, Miyake T, Shimizu T, Miyajima A, Yokota T, Arai K-I. Recombinant murine granulocyte-macrophage (GM) colony-stimulating factor supports formation of GM and multipotential blast cell colonies in culture: Comparison with the effects of interleukin-3. J Cell Physiol **131**:458–464, 1987.
41. Wang JM, Colella S, Allavena P, Mantovani A. Chemotactic activity of human recombinant granulocyte-macrophage colony-stimulating factor. Immunology **60**:439–444, 1987.
42. Morrissey PJ, Bressler L, Park LS, Alpert A, Gillis S. Granulocyte-macrophage colony-stimulating factor augments the primary antibody response by enhancing the function of antigen presenting cells. J Immunol **139**:1113–1119, 1987.
43. Falk LA, Wahl LM. Vogel SN. Analysis of Ia antigen expression in macrophages derived from bone marrow cells cultured in granulocyte-macrophage colony-stimulating factor or macrophage colony-stimulating factor. J Immunol **140**:2652–2660, 1988.
44. Grabstein KH, Urdal DL, Tushinski RJ, Mochizuki DY, Price VL, Cantrell MA, Gillis MA, Gillis S, Conlon PJ. Induction of macrophage tumoricidal activity by granulocyte-macrophage colony-stimulating factor. Science **232**:506–508, 1986.
45. Wing EJ, Magee DM, Whiteside TL, Kaplan SS, Shadduck RK. Recombinant human granulocyte/macrophage colony-stimulating factor enhances monocyte cytotoxicity and secretion of tumor necrosis factor α and interferon in cancer patients. Blood **73**:643–646, 1989.
46. Cannistra SA, Rambaldi A, Spriggs DR, Hermann F, Kufe D, Griffin JD. Human granulocyte-macrophage colony-stimulating factor induces the expression of the tumor necrosis factor gene in the U937 cell line and by normal human monocytes. J Clin Invest **79**:1720–1728, 1987.
47. Belosevic M, Davis CE, Meltzer MS, Nacy CA. Regulation of activated macrophage antimicrobial activities: Identification of lymphokines that cooperate with gamma interferon for induction of resistance to infection. J Immunol **141**:890–896, 1988.
48. Weiser WY, Van Niel A, Clark SC, David JR, Remold HG. Recombinant human granulocyte/macrophage colony-stimulating factor activates intracellular killing of *Leishmania donovani* by human monocyte-derived macrophages. J Exp Med **166**:1436–1446, 1987.
49. Handmann E, Burgess AW. Stimulation by granulocyte-macrophage colony-stimulating factor of *Leishmania tropica* killing by macrophages. J Immunol **122**:1134–1137, 1979.
50. Reed SG, Nathan CF, Pihl DL, Rodricks P, Shanebeck K, Conlon PJ, Grabstein, KH. Recombinant granulocyte/macrophage colony-stimulating factor activates macrophages to inhibit *Trypanosoma cruzi* and release hydrogen peroxide: Comparison with interferon gamma. J Exp Med **166**:1734–1746, 1987.
51. Coleman DL, Chodakewitz JA, Bartiss AH, Mellors JW. Granulocyte-macrophage colony-stimulating factor enhances selective effector functions of tissue-derived macrophages. Blood **72**:573–578, 1988.
52. Hammer SM, Gillis JM, Groopman JE, Rose RM. *In vitro* modification of human immunodeficiency virus infection by granulocyte-macrophage colony-stimulating factor and gamma interferon. Proc Natl Acad Sci USA **83**:8734–8738, 1986.
53. Hammer SM, Gillis JM. Synergistic activity of granulocyte-macrophage colony-stimulating factor and 3′-azido-3′-deoxythymidine against human immunodeficiency virus *in vitro*. Antimicrob Agents Chemother **1987**:1046–1050, 1987.
54. Perno CF, Yarchoan R, Cooney DA, Hartman NR, Webb DS, Hao Z, Mitsuya H, Johns DG, Broder S. Replication of human immunodeficiency virus in monocytes: Granulocyte/macrophage colony-stimulating factor (GM-CSF) potentiates viral production yet enhances the antiviral effect mediated by 3′-azido-2′3′-dideoxythymidine (AZT) and other dideoxynucleoside congeners of thymidine. J Exp Med **169**:933–951, 1989.
55. Bhalla K, Birkhofer M, Grant S, Graham G. The effect of recombinant human granulocyte-macrophage colony-stimulating factor (rGM-CSF) on 3′-azido-3′deoxythymidine (AZT)-mediated biochemical and cytotoxic effects on normal human myeloid progenitor cells. Exp Hematol **17**:17–20, 1989.
56. Nathan CF, Prendergast TJ, Wiebe ME, Stanley ER, Platzer E, Remold HG. Activation of human macrophages: Comparison of other cytokines with interferon gamma. J Exp Med **160**:600–605, 1984.

57. Jensen WA, Rose RM, Burke RH, Anton K, Remold HG. Cytokine activation of antibacterial activity in human pulmonary macrophages: Comparison of recombinant interferon gamma and granulocyte-macrophage colony-stimulating factor. Cell Immunol **117**:369–377, 1988.
58. Wang JM, Rambaldi A, Biondi A, Chen ZG, Sanderson CJ, Mantovani A. Recombinant human interleukin-5 is a selective eosinophil chemoattractant. Eur J Immunol **19**:701–705, 1989.
59. Weisbart RH, Golde DW, Gasson JC. Biosynthetic human GM-CSF modulates the number and affinity of neutrophil f-Met-Leu-Phe receptors. J Immunol **137**:3584–3587, 1986.
60. Arnaout MA, Wang EA, Clark SC, Sieff CA. Human recombinant granulocyte-macrophage colony-stimulating factor increases cell-to-cell adhesion and surface expression of adhesion-promoting surface glycoproteins on mature granulocytes. J Clin Invest **78**:597–601, 1986.
61. Metcalf D, Begley CG, Johnson GR, Nicola NA, Vadas MA, Lopez AF, Williamson DJ, Wong GG, Clark SC, Wang EA. Biologic properties *in vitro* of a recombinant human granulocyte-macrophage colony-stimulating factor. Blood **67**:37–45, 1986.
62. Vadas M, Nicola NA, Metcalf D. Activation of antibody-dependent cell-mediated cytotoxicity of human neutrophils and eosinophils by separate colony-stimulating factors. J Immunol **130**:795–799, 1953.
63. Lopez AF, Sanderson CJ, Gamble JR, Campbell HD, Young IG, Vadas MA. Recombinant human interleukin 5 is a selective activator of human eosinophil function. J Exp Med **167**:219–224, 1988.
64. Perussia B, Kobayashi M, Rossi ME, Anegon I, Trinchieri G. Immune interferon enhances functional properties of human granulocytes: Role of Fc receptors and effect of lymphotoxin, tumor necrosis factor, and granulocyte-macrophage colony-stimulating factor. J Immunol **138**:765–774, 1987.
65. Lopes AF, Williamson DJ, Gamble JR, Begley CG, Harlan JM, Klebanoff SJ, Waltersdorph A, Wong G, Clark SC, Vadas MA. Recombinant human granulocyte-macrophage colony-stimulating factor stimulates *in vitro* mature human neutrophil and eosinophil function, surface receptor expression, and survival. J Clin Invest **78**:1220, 1986.
66. Liesveld JL, Abboud AN, Looney RJ, Ryan DH, Brennan JK. Expression of IgG Fc receptors in myeloid leukemic cell lines: Effect of colony-stimulating factors and cytokines. J Immunol **140**:1527–1533, 1988.
67. Sullivan R, Fredette JP, Leavitt JL, Gadenne AS, Griffin JD, Simons ER. Effects of recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSFrh) on transmembrane potentials in granulocytes: Relationship between enhancement of ligand-mediated depolarization and augmentation of superoxide anion (O_2-) production. J Cell Physiol **139**:361–369, 1989.
68. Lindemann A, Riedel D, Oster W, Meuer SC, Blohm D, Mertelsmann RH. Granulocyte/macrophage colony-stimulating factor induces interleukin 1 production by human polymorphonuclear neutrophils. J Immunol **140**:837–839, 1988.
69. Fleischmann J, Golde DW, Weisbart RH, Gasson JC. Granulocyte-macrophage colony-stimulating factor enhances phagocytosis of bacteria by human neutrophils. Blood **68**:706–711, 1986.
70. Villalta F, Kierszenbaum F. Effects of human colony-stimulating factor on the uptake and destruction of a pathogenic parasite (*Trypanosoma cruzi*) by human neutrophils. J Immunol **137**:1703–1707, 1986.
71. Silberstein DS, Owen WF, Gasson JC, DiPersio JF, Golde DW, Bina JC. Enhancement of human eosinophil cytotoxicity and leukotriene synthesis by biosynthetic (recombinant) granulocyte-macrophage colony-stimulating factor. J Immunol **137**:3290–3294, 1986.
72. Watari K, Asano S, Shirafuji N, Kodo H, Ozawa K, Takaku F, Kamachi S. Serum granulocyte colony-stimulating factor levels in healthy volunteers and patients with various disorders as estimated by enzyme immunoassay. Blood **73**:117–122, 1989.
73. Fibbe WE, van Damme J, Billau A, Goselink HM, Voogt PJ, van Eeden G, Ralph P, Altrock BW, Falkenburg JHF. Interleukin-1 induces human marrow stromal cells in long-term culture to produce granulocyte-macrophage colony-stimulating factor and macrophage colony-stimulating factor. Blood **71**:430–435, 1988.
74. Yang Y-C, Tsai S, Wong GG, Clark SC. Interleukin-1 regulation of hematopoietic growth factor production by human stromal fibroblasts. J Cell Physiol **134**:292–296, 1988.
75. Vellenga E, Rambaldi A, Ernst TJ, Ostapovicz D, Griffin JD. Independent regulation of M-CSF and G-CSF gene expression in human monocytes. Blood **71**:1529–1532, 1988.
76. Valtieri M, Tweardy DJ, Caracciolo D, Johnson K, Mavilio F, Altmann S, Santoli D, Rovera G. Cytokine-dependent granulocytic differentiation. Regulation of proliferative and differentiative responses in a murine progenitor cell line. J Immunol **138**:3829–3835, 1987.
77. Souza LM, Boone TC, Gabrilove J, Lai PH, Zsebo KM, Moudock DC, Chazin VR, Bruszewski J, Lu H, Chen KK, Barendt J, Platzer E, Moore MAS, Mertelsmann R, Welte K. Recombinant human granulocyte colony-stimulating factor: Effects on normal and leukemic myeloid cells. Science **232**:61, 1986.
78. Nicola NA, Metcalf D, Matsumoto M, Johnson GR. Purification of a factor inducing differentiation in murine myelomonocytic leukemia cells: Identification as granulocyte colony-stimulating factor. J Biol Chem **258**:9017–9023, 1983.
79. Wang JM, Chen ZG, Colella S, Bonilla MA, Welte K, Bordignon C, Mantovani A. Chemotactic activity of recombinant human granulocyte colony-stimulating factor. Blood **72**:1456–1460, 1988.
80. Weisbart RH, Kacena A, Schuh A, Golde DW. GM-CSF induces human neutrophil IgA-mediated phagocytosis by an IgA Fc receptor activation mechanism. Nature **332**:647–648, 1988.
81. Kitagawa S, Yuo A, Souza LM, Saito M, Miura Y, and Takaku F. Recombinant human granulocyte colony-stimulating factor enhances superoxide release in human granulocytes stimulated by the chemotactic peptide. Biochem Biophys Res Commun **144**:1143–1146, 1987.
82. Sieff CA, Niemeyer CM, Mentzer SJ, Faller DV. Interleukin-1, tumor necrosis factor, and the production of colony-stimulating factors by cultured mesenchymal cells. Blood **72**:1316–1323, 1988.
83. Troutt AB, Lee F. Tissue distribution of murine hemopoietic growth factor mRNA production. J Cell Physiol **138**:38–44, 1989.
84. Sieff CA. Hemopoietic growth factors. J Clin Invest **79**:1549–1557, 1987
85. Kawasaki ES, Ladner MB, Wang AM, Van Arsdell J, Warren MK, Coyne MY, Schweickart VL, Lee M-T, Wilson KJ, Boosman A, Stanley ER, Ralph P, Mark DF. Molecular cloning of a complementary DNA encoding human macrophage-specific colony-stimulating factor (CSF-1). Science **230**:291, 1985.
86. Wong GG, Temple PA, Leary AC, Witek-Giannotti JS, Yang Y-C, Ciarletta AB, Chung M, Murtha P, Kriz R, Kaufman RJ, Ferenz CR, Sibley BS, Turner KJ, Hewick RM, Clark SC, Yanai N, Yokota H, Yamada M, Saito M, Motoyoshi K, Takaku F. Human CSF-1: Molecular cloning and expression of 4-kb cDNA encoding the human urinary protein. Science **235**:1504, 1987.
87. Caracciolo D, Shirsat N, Wong GG, Lange B, Clark S, Rovera G. Recombinant human macrophage colony stimulating factor (M-CSF) requires subliminal concentrations of granulocyte/

macrophage (GM-CSF) for optimal stimulation of human macrophage colony formation *in vitro*. J Exp Med **166**:1851–1860, 1987.
88. Broxmeyer HE, Cooper S, Lu L, Miller ME, Langefeld CD, Ralph P. Enhanced stimulation of human bone marrow macrophage colony formation in vitro by recombinant human macrophage colony-stimulating factor in agarose medium and at low oxygen tension. Blood **76**:323–329, 1990.
89. Ampel NM, Wing EJ, Waheed A, Shadduck RK. Stimulatory effects of purified macrophage colony-stimulating factor on murine resident peritoneal macrophages. Cell Immunol **97**:344–356, 1986.
90. Wang JM, Griffin JD, Rambaldi A, Chen ZG, Mantovani A. Induction of monocyte migration by recombinant macrophage colony-stimulating factor. J Immunol **141**:575–579, 1988.
91. Wing EJ, Waheed A, Shadduck RK, Nagle LS. Effect of colony-stimulating factor on murine macrophages: Induction of antitumor activity. J Clin Invest **69**:270–276, 1982.
92. Ralph P, Nektons I. Stimulation of macrophage tumoricidal activity by the growth and differentiation factor CSF-1. Cell Immunol **105**:270–279, 1987.
93. Nakoinz I, Ralph P. Stimulation of macrophage antibody-dependent killing of tumor targets by recombinant lymphokine factors and M-CSF. Cell Immunol **116**:331–340, 1988.
94. Warren MK, Ralph P. Macrophage growth factor CSF-1 stimulates human monocyte production of interferon, tumor necrosis factor, and colony stimulating activity. J Immunol **137**:2281–2285, 1986.
95. Magee DM, Wing EJ, Ampel NM, Waheed A, Shadduck RK. Macrophage colony-stimulating factor (M-CSF) enhances the expression of Fc receptors on murine peritoneal macrophages. Immunology **62**:373–378, 1987.
96. Clark SC, Kamen R. The human hematopoietic colony-stimulating factors. Science **236**:1229–1237, 1986.
97. Wing EJ, Magee DM, Pearson AC, Waheed A, Shadduck RK. Peritoneal macrophages exposed to purified macrophage colony-stimulating factor (M-CSF) suppress mitogen- and antigen-stimulated lymphocyte proliferation. J Immunol **137**:2768–2773, 1986.
98. Cheers C, Hill M, Haigh AM, Stanley ER. Stimulation of macrophage phagocytic but not bactericidal activity by colony-stimulating factor 1. Infect Immun **57**:1512–1516, 1989.
99. Karbassi A, Becker JM, Foster JS, Moore RN. Enhanced killing of *Candida albicans* by murine macrophages treated with macrophage colony-stimulating factor: Evidence for augmented expression of mannose receptors. Infect Immun **139**:417–421, 1987.
100. Wing EJ, Ampel NM, Waheed A, Shadduck RS. Macrophage colony-stimulating factor (M-CSF) enhances the capacity of murine macrophages to secrete oxygen reduction products. J Immunol **135**:2052–2056, 1985.
101. Lee M, Warren MK. CSF-1-induced resistance to viral infection in murine macrophages. J Immunol **138**:3019–3022, 1987.
102. Gendelman HE, Orenstein JM, Martin MA, Ferrua C, Mitra R, Phipps T, Wahl LA, Lane HC, Fauci AS, Burke DS. Efficient isolation and propagation of human immunodeficiency virus on recombinant colony-stimulating factor 1-treated monocytes. J Exp Med **167**:1428–1441, 1988.
103. Stanley ER, Bartocci A, Patinkin D, Rosendall M, Bradley TR. Regulation of very primitive, multipotent, hemopoietic cells by hemopoietin-1. Cell **45**:667–674, 1986.
104. Warren D, Moore MAS. Synergy of interleukin 1 and granulocyte colony-stimulating factor: *In vivo* stimulation of stem-cell recovery and hematopoietic regeneration following 5-fluorouracil treatment of mice. Proc Natl Acad Sci USA **84**:7134–7138, 1987.
105. Sanderson CJ, Warren DJ, Strath M. Identification of a lymphokine that stimulates eosinophil differentiation *in vitro*. Its relationship to interleukin-3 and functional properties of eosinophils produced in culture. J Exp Med **162**:60–74, 1985.
106. Wong GG, Clark SC. Multiple actions of interleukin 6 within a cytokine network. Immunol Today **9**:137–139, 1988.
107. Wong GG, Witek-Giannotti JS, Temple PA, Kriz R, Ferenz C, Hewick RM, Clark SC, Ikebuchi K, Ogawa M. Stimulation of murine hemopoietic colony formation by human IL-6. J Immunol **140**:3040, 1988.
108. Trudgett A, McNeill TA, Killen M. Granulocyte-macrophage precursor cell and colony-stimulating factor responses of mice infected with *Salmonella typhimurium*. Infect Immun **8**:450–455, 1973.
109. Resnick M, Fibach E, Lebastard M, Levy L, Bercovier H. Response of the murine hematopoietic system to chronic infection with *Mycobacterium lepraemurium*. Infect Immun **56**:3145–3151, 1988.
110. Cheers C, Young AM. Serum colony-stimulating activity and colony forming cells in murine brucellosis: Relationship to immunopathology. Microb Pathog **3**:185–194, 1987.
111. Clark CR, Chen BD-M, Boros DL. Macrophage progenitor cell and colony-stimulating factor production during granulomatous schistosomiasis mansoni in mice. Infect Immun **56**:2680–2685, 1988.
112. Owhashi M, Nawa Y. Granulocyte-macrophage colony-stimulating factor produced by splenic T lymphocytes of mice infected with *Schistosoma japonicum*. Infect Immun **51**:213–217, 1986.
113. Owhashi M, Nawa Y. Granulocyte-macrophage colony-stimulating factor in the sera of *Schistosoma japonicum*-infected mice. Infect Immun **49**:533–537, 1985.
114. Hayashi S, Masuno T, Hosoe S, Kawase I, Sakatani M, Ogura T, Kishimoto S, Yamamura Y. Augmented production of colony-stimulating factor in C3H/HeN mice immunized with *Nocardia rubra* cell wall skeleton. Infect Immun **52**:128–133, 1986.
115. Wing EJ, Waheed A, Shadduck RK. Changes in serum colony-stimulating factor and monocytic progenitor cells during *Listeria monocytogenes* infection in mice. Infect Immun **45**:180–184, 1984.
116. Young AM, Cheers C. Colony-forming cells and colony-stimulating activity during listeriosis in genetically resistant and susceptible mice. Cell Immunol **97**:227–237, 1986.
117. Cheers C, Stanley ER. Macrophage production during murine listeriosis: Colony-stimulating factor 1 (CSF-1) and CSF-1-binding cells in genetically resistant and susceptible mice. Infect Immun **56**:2972–2978, 1988.
118. Cheers C, Haigh AM, Kelso A, Metcalf D, Stanley ER, Young AM. Production of colony-stimulating factors (CSFs) during infection: Separate determinations of macrophage-, granulocyte-, granulocyte-macrophage-, and multi-CSFs. Infect Immun **56**:247–251, 1988.
119. Wood PR, Young AM, McKimm-Breschkin JL, Cheers C. Effect of splenectomy on production of colony-stimulating factor in *Listeria monocytogenes*-infected mice. Infect Immun **46**:860–861, 1987.
120. Wing EJ, Barczynski LK, Waheed A, Shadduck RK. Effect of *Listeria monocytogenes* on serum levels of colony-stimulating factor and number of progenitor cells in immune and nonimmune mice. Infect Immun **49**:325–328, 1985.
121. Wing EJ, Magee DM, Barczynski LK. Analysis of colony-stimulating factors and macrophage progenitors in mice immunized against *Listeria monocytogenes* by adoptive transfer. Infect Immun **55**:1843–1847, 1987.
122. Magee DM, Wing EJ. Antigen specific production of colony-stimulating factors by *Listeria monocytogenes*-immune L3T4+ lymphocytes. J Infect Dis **157**:941–949, 1988.
123. Magee DM, Wing EJ. Cloned L3T4+ T lymphocytes protect

mice against *Listeria monocytogenes* by secreting interferon-gamma. J Immunol **141**:3203–3207, 1988.
124. Nanno M, Shimizu T, Mike A, Ohwaki M, Mutai M. Role of macrophages in serum colony-stimulating factor induction by *Lactobacillus casei* in mice. Infect Immun **56**:357–362, 1988.
125. Metcalf D, Begley CG, Johnson GR, Nicola NA, Lopez AF, Williamson DJ. Effects of purified bacterially synthesized murine multi-CSF (IL-3) on hematopoiesis in normal adult mice. Blood **68**:46–57, 1986.
126. Mayer P, Lam C, Obenaus H, Liehl E, Besemer J. Recombinant human GM-CSF induces leukocytosis and activates peripheral blood polymorphonuclear neutrophils in nonhuman primates. Blood **70**:206–213, 1987.
127. Welte K, Bonilla MA, Gillio AP, Boone TC, Potter GK, Gabrilove JL, Moore MA, O'Reilly RJ, Souza LM. Recombinant human granulocyte colony-stimulating factor: Effects on hematopoiesis in normal and cyclophosphamide-treated primates. J Exp Med **165**:941–948, 1987.
128. Wing EJ, Magee DM, Whiteside TL, Kaplan SS, Shadduck RK. Recombinant human granulocyte/macrophage colony-stimulating factor enhances monocyte cytotoxicity and secretion of tumor necrosis factor alpha and interferon in cancer patients. Blood **73**:643–646, 1989.
129. Vadhan-Raj S, Hittelman WN, Broxmeyer HE, Keating M, Urdal D, Gutterman JU. *In vivo* biological activities of recombinant human granulocyte-macrophage colony-stimulating factor. Ann NY Acad Sci **554**:231–240, 1989.
130. Broxmeyer HE, Vadhan-Raj S. Preclinical and clinical studies with the hematopoietic colony-stimulating factors and related interleukins. Immunol Res **8**:185–201, 1989.
131. Tanka T, Okamura S, Okada K, Suda A, Shimono N, Ohhara N, Hirota Y, Sawae Y, Niho Y. Protective effect of recombinant murine granulocyte-macrophage colony-stimulating factor against *Pseudomonas aeruginosa* infection in leukocytopenic mice. Infect Immun **57**:1792–1799, 1989.
132. Cohen AM, Hines DK, Korach ES, Ratzkin BJ. *In vivo* activation of neutrophil function in hamsters by recombinant human granulocyte colony-stimulating factor. Infect Immun **56**:2861–2865, 1988.
133. Talmadge JE, Tribble H, Pennington R, Bowersox O, Schneider MA, Castelli P, Black PL, Abe F. Protective, restorative, and therapeutic properties of recombinant colony-stimulating factors. Blood **73**:2093–2103, 1989.
134. Morstyn G, Lieschke GJ, Sheridan W, Layton J, Cebon J, Fox RM. Clinical experience with recombinant human granulocyte colony-stimulating factor and granulocyte macrophage colony-stimulating factor. Semin Hematol **26**:9–13, 1989.
135. Groopman JE, Mitsuyasu RT, DeLeo MJ, Oette DH, Golde DW. Effect of recombinant human granulocyte-macrophage colony-stimulating factor on myelopoiesis in the acquired immunodeficiency syndrome. N Engl J Med **317**:593–598, 1987.
136. Komiyama A, Ishiguro A, Kubo T, Matsuoka T, Yasukohchi S, Yasui K, Yanagisawa M, Yamada S, Yamazaki M, Akabane T. Increases in neutrophil counts by purified human urinary colony-stimulating factor in chronic neutropenia of childhood. Blood **71**:41–45, 1988.
137. Glaspy JA, Golde DW. Clinical applications of the myeloid growth factors. Semin Hematol **26**:14–17, 1989.
138. Morrissey PJ, Grabstein KH, Reed SG, Conlon PJ. Granulocyte/macrophage colony-stimulating factor: A potent activation signal for mature macrophages and monocytes. Int Arch Allergy Appl Immunol **88**:40–45, 1989.
139. Morrissey P, Bressler L, Charrier K, Alpert A. Response of resident murine macrophages to *in vivo* administration of granulocyte-macrophage colony-stimulating factor. J Immunol **140**:1910–1915, 1988.
140. Mooney DP, Gameli RL, O'Reilly M, Hebert JC. Recombinant human granulocyte colony-stimulating factor and *Pseudomonas* burn wound sepsis. Arch Surg **123**:1353–1357, 1988.
141. Matsumoto M, Matsubara S, Matsuno T, Tamura M, Hattori K, Nomura H, Ono M, Yokota T. Protective effect of human granulocyte colony-stimulating factor on microbial infection in neutropenic mice. Infect Immun **55**:2715–2720, 1987.
142. Sullivan R, Fredette JP, Socinski M, Elias A, Antman K, Schnipper L, Griffin JD. Enhancement of superoxide anion release by granulocytes harvested from patients receiving granulocyte-macrophage colony-stimulating factor. Br J Haematol **71**:475–479, 1989.
143. Kaplan SS, Basford RE, Wing EJ, Shadduck RK. The effect of recombinant human granulocyte macrophage colony-stimulating factor on neutrophil activation in patients with refractory carcinoma. Blood **73**:636–638, 1989.
144. Grau GE, Kindler J, Piguet PF, Lambert PH, Vassali P. Prevention of experimental cerebral malaria by anticytokine antibodies: IL-3 and granulocyte-macrophage colony-stimulating factor are intermediates in increased tumor necrosis factor production and macrophage accumulation. J Exp Med **168**:1499–1504, 1988.
145. Greil J, Bodendorfer B, Rollinghoff M, Solbach R. Application of recombinant granulocyte-macrophage colony-stimulating factor has a detrimental effect in experimental murine leishmaniasis. Eur J Immunol **18**:1527–1533, 1988.
146. Mirkovich AM, Galelli A, Allison AC, Modabber FZ. Increased myelopoiesis during *Leishmania major* infection in mice: Generation of "safe targets," a possible way to evade the effector immune mechanism. Clin Exp Immunol **64**:1–7, 1986.
147. Chikkappa G, Broxmeyer HE, Cooper S, Williams DE, Hangoc G, Greenberg ML, Waheed A, Shadduck RK. Effect *in vivo* of multiple injections of purified murine and recombinant human macrophage colony-stimulating factor to mice. Cancer Res **49**:3558–3561, 1989.
148. Williams DE, Hangoc G, Cooper S, Boswell HS, Shadduck RK, Gillis S, Waheed A, Urdal D, Broxmeyer HE. The effects of purified recombinant interleukin-3 and/or purified natural murine CSF-1 on the proliferation of murine high- and low-proliferative potential colony-forming cells: Demonstration of *in vivo* synergism. Blood **70**:401–403, 1987.
149. Neta R, Oppenheim JJ, Douches SD. Interdependence of the radioprotective effects of human recombinant interleukin 1 alpha, tumor necrosis factor alpha, granulocyte colony-stimulating factor, and murine recombinant granulocyte-macrophage colony-stimulating factor. J Immunol **140**:108–111, 1988.
150. Silver GM, Gamelli RL, O'Reilly M. The beneficial effect of granulocyte colony-stimulating factor (G-CSF) in combination with gentamicin on survival after *Pseudomonas* burn wound infection. Surgery **106**:452–456, 1989.
151. Bermudez LEM, Young LS. Activities of amikacin, roxithromycin, and axithromycin alone or in combination with tumor necrosis factor against *Mycobacterium avium* complex. Antimicrob Agents Chemother **32**:1149–1153, 1988.