Hemopoietic Growth Factors: A Review

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Abstract

Several hemopoietic growth factors have now been purified, cloned, and produced in bacteria. Granulocyte colony stimulating factor and granulocyte-macrophage colony stimulating factor are already being used in clinical trials. Within 12 months two more hemopoietic growth factors, macrophage colony stimulating factor (also called colony stimulating factor 1) and interleukin 3 (also called multi-colony stimulating factor) will be used for patient treatment. This review discusses the recent advances in our knowledge of the molecular properties and biological specificities of these factors. It is now clear that these molecules are able to modulate selectively the activity of mature blood cells as well as stimulating the production of specific lineages of blood cells. The availability of recombinant hemopoietic growth factors purified from animal or yeast cell conditioned medium or bacteria has facilitated in vivo experiments, as well as the clinical trials. Each of the growth factors has a unique spectrum of biological activities and it appears that the growth factors will enhance the recovery and function of circulating white blood cells after cancer therapy or bone marrow transplantation.

Introduction

With the present enthusiasm for the use of biological response modifiers for cancer therapy, one could be forgiven for thinking that the activation of blood cells is a new paradigm in molecular medicine. However, Elias Metchnikoff (1) and Almoth Wright (2) eloquently noted the same possibilities many years ago, but our incomplete knowledge of the production, activation and biology of leukocytes has delayed the effective testing of this hypothesis for almost a century. During the last 5 years there has been a major change in our understanding of the molecules which control both the production and activation of leukocytes (3-10). It is already evident that some of these molecules are likely to have important roles in the development of new therapies for cancer patients (11), for the treatment of patients with life-threatening infections, for wound healing, and perhaps even for the prophylactic boosting of our cellular defense systems in aged or immunocompromised patients.

In this review we will discuss the structure, biology, and likely therapeutic indications of some of the hemopoietic growth factors. Since many properties of both murine and human hemopoietic growth factors have been tabulated in a number of recent reviews (8, 10, 12-14), we will concentrate on the more recent publications, attempting to highlight the properties of the molecules which may have relevance as therapeutic agents. We will address some of the broader concepts indicated by this work as well as summarize some of the recent progress which has been made on the genetics, synthesis, and use of the hemopoietic growth factors.

Compared to other biological response modifiers, some of which have already been tested in patients, the biological and chemical properties of the hemopoietic growth factors have been studied in depth and the clinical indications for these factors can be predicted with some degree of confidence. For the first time we are confronted with biological reagents which should selectively “Stimulate the phagocytes” (Shaw, 1906) (15). Our current knowledge suggests that these reagents should have immediate applications for patients with impaired phagocyte function.

Hemopoiesis

Hemopoietic Cells. The mature cells in the hemopoietic system are lymphocytes, monocytes, erythrocytes, neutrophils, eosinophils, basophils, and platelets. Each of these cells has a limited life span and only lymphocytes and monocytes are capable of cell proliferation. All of these cells are produced as a result of the balance between self-renewal and commitment of hemopoietic stem cells. In special situations where there is increased loss of cells, the stem and progenitor cell compartments respond in an appropriate way by increasing the levels of mature cells. These changes appear predominantly to be achieved by alterations in the rate of production of mature cells and by the shifting of cells from the progenitor compartments to the differentiating compartments. There appears to be little control over the rate of cell destruction during normal hemopoiesis.

There is clear evidence (Fig. 1) that the organization of the hemopoietic system is hierarchical, with multipotential stem cells leading to mature nonproliferating cells (3). The nomenclature for progenitor cells is based on the mature cells (3) which they produce. Thus, the progenitor cells are designated as colony forming unit erythroid, BFU-e, GM-CFC, eosinophil colony forming cell, and megakaryocyte colony forming cell. All of the progenitor cells (except perhaps the earliest stem cells) can be detected by in vitro culture and their proliferation is totally dependent on specific growth factors (16). The evidence for distinct subsets of progenitor cells is that they can be partially separated from each other by velocity sedimentation, isopyknic centrifugation, and on the basis of surface markers by flow cytometry (17-22).

It is not clear whether the direction of differentiation of the stem cells is determined stochastically or whether growth regulators influence it. The studies of Koike et al. (23) and Metcalf and Burgess (24) suggest that the direction of differentiation is influenced by the presence and concentration of specific hemopoietic growth factors.

Progenitor cells recently identified by in vitro techniques are the high proliferative potential colony forming cells and can be detected by stimulation in vitro with M-CSF (also called CSF-2).

Received 12/30/87; revised 6/14/88; accepted 7/6/88.

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HEMPOIETIC GROWTH FACTORS

1) and hemopoietin 1 (25–29). Nakahata et al. (30) have suggested that there is also another group of multipotential, self-renewing cells which appear to enter randomly into cycle from G0 and which can then be stimulated to produce blast cell colonies by multi-CSF (IL-3). Perhaps hemopoietin 1 is responsible for the initial stimulation of these cells from G0 to G1 (31–33). Recently it has been shown that IL-4 acts on these early cells (34).

Subpopulations of Granulocyte Progenitor Cells. There appears to be a hierarchy of human granulocyte-macrophage progenitor cells with two separate growth factors stimulating different subsets of the GM-CFC. These have been operationally called Day 14 GM-CFC and Day 7 GM-CFC. There is evidence that some of the Day 14 GM-CFC are precursors of Day 7 GM-CFC (35). Morstyn et al. (36) were able to partially purify these two subpopulations using lectin binding and cell sorting. The Day 14 population was enriched for myeloblasts and the Day 7 population for promyelocytes and myelocytes. Begley et al. (37), using a monoclonal antibody to mature cells, have confirmed that myelocytes and promyelocytes are a distinct subset of progenitor cells. The distinction between the Day 7 and Day 14 granulocyte progenitor cells is important because they are preferentially stimulated, by G-CSF (originally called CSFβ) and GM-CFC (formerly called CSFα) (36). A further complication is that Francis et al. (38) have shown that as cells differentiate in the GM pathway they become more sensitive to conditioned media containing CSFs, i.e., to GM-CSF and G-CSF.

Hemopoietic Growth Factors

Biological Specificity. Since the discovery of the method for growing blood cells in culture (39, 40) four "granulocyte-macrophage" CSFs have been isolated. The first hemopoietic growth factor was detected because of its ability to stimulate the production of granulocytes and macrophages from murine spleen and marrow cells. The same assay system has been used to detect the other hemopoietic growth factors (16). When marrow cells are dispersed in agar at approximately 10^7 cells/ml, unless a hemopoietic growth factor is present, all of the cells die. However, if one of the hemopoietic growth factors is present, the early myeloid cells continue to proliferate and differentiate, producing a colony of mature granulocytes and/or macrophages. When human marrow is used, the colonies require 7 to 14 days to develop between 50 and 5000 mature cells and the number and type of differentiated cells depend on the concentration and species of the hemopoietic growth factor. Two names were given to the first hemopoietic growth factor: macrophage-granulocyte inducer (40) and CSF (39). These names proved to be inadequate as another growth factor for murine marrow cells was also discovered (41). This molecule stimulated the production of macrophage colonies in preference to granulocyte colonies. Since this second hemopoietic growth factor is also a colony stimulating factor, confusion between the two molecules was inevitable. Two sets of nomenclature were used to distinguish between these two molecules: GM-CSF by one group (42) and CSF-2 by other groups (43); the other molecule was renamed CSF-1 or M-CSF. Most commonly, GM-CSF and CSF-1 are used to distinguish between these two molecules (44).

A third hemopoietic growth factor specific for granulocytic cells was discovered in the conditioned medium from human placenta (45) and in the serum of mice treated with endotoxin (46). This molecule is widely known as G-CSF (47).

The fourth hemopoietic growth factor is also capable of stimulating the production of granulocytes and macrophages. This molecule was identified by several laboratories working on the different properties of the conditioned medium at lectin stimulated murine spleen cells (48) and the conditioned medium from a "myeloid" cell line WEHI3B(D~) (49, 50). Initial observations suggested that this conditioned medium induced T-lymphocyte differentiation; therefore the component responsible for this effect was named interleukin 3 (51). Subsequent investigations have shown that the apparent effects on T-lymphocytes were actually associated with the production of neutrophils and macrophages. However, it was known that this conditioned medium stimulated the production of eosinophils, megakaryocytes, and erythroid cells as well as granulocytes and macrophages. While chromotographic investigations indicated that pokeweed mitogen stimulated spleen cell conditioned medium contained a GM-CSF, the purification data were also compatible with the presence of another molecule capable of stimulating the production of all mature hemopoietic cells (48). Thus the molecule was also called multi-CSF. Indeed, it was subsequently shown that the conditioned medium from WEHI3B(D~) cells did not contain GM-CSF and that the hemopoietic growth factor activity was all associated with multi-CSF (IL-3) (50, 52). Murine multi-CSF (IL-3) was the first hemopoietic growth factor to be molecularly cloned (53) and the human equivalent of multi-CSF has been cloned recently (54). Its range of biological activities has not been explored in detail; however, the cDNA for gibbon IL-3 has also been cloned (54) and this molecule supports the growth of erythroid and myeloid precursors in human marrow cells.

Thus, the production of granulocytes and macrophages from the progenitor cells of mouse marrow can be stimulated by any one of four distinct molecules, multi-CSF (IL-3), GM-CSF, M-CSF (CSF-1), and G-CSF. There are human equivalents of all four molecules and three of these, multi-CSF, GM-CSF, and G-CSF, have been shown to stimulate the proliferation of leukemic progenitor cells (54–56). Other terms have been used to describe mixtures (or impure forms) of these hemopoietic growth factors, e.g., CSFα, CSFβ, pluripoietin, pluripoietin α,
and burst promoting activity (8). Usually the properties of these conditioned media can be adequately accounted for by the properties of one of the four CSFs. Other molecules are able to stimulate (or modify) the lineage specific proliferation and/or differentiation of marrow progenitor cells, e.g., interleukin 1 (57), hemopoietin 1 (25), eosinophil differentiation factor (58–60), erythropoietin (61); interleukin 2 (62, 63), interleukin 4 (64) and interleukin 6. We have not attempted to include descriptions of the biology or chemistry of these molecules in this review.

GM-CSF is known to stimulate eosinophil production (45, 65) and the initial divisions of progenitor cells in the erythroid and megakaryocyte lineages (32, 66). In the mouse high concentrations of G-CSF are also able to stimulate macrophage production; however, only the initial proliferation of the other hemopoietic progenitors is effected by G-CSF. M-CSF predominantly produces macrophages, but at high concentrations some granulocytes are produced (67). M-CSF does not seem to effect even the initial divisions of the progenitor cells in the other hemopoietic lineages.

Another class of hemopoietic cell “response modulators” needs to be mentioned, i.e., those molecules that appear to influence hemopoiesis either indirectly or in the presence of one of the CSFs. Molecules such as IL-1 stimulate monocytes to produce hemopoietic growth factors (68). Although IL-1 may not act directly on myelopoiesis, activation of the synthesis of M-CSF and GM-CSF will stimulate the production of granulocytes and macrophages. Indeed, depending on the number, distribution, and state of activation of the tissue monocytes (or macrophages) molecules such as IL-1 could initiate a cascade which leads to high concentrations of the hemopoietic growth factors and consequently the large scale activation, accumulation, and production of leukocytes.

At least two other mechanisms of amplifying the effects of the hemopoietic growth factors have been proposed. The overall production of mature cells depends on the number of available progenitor cells. By increasing the production of progenitor cells, the production of mature cells can be accelerated. For example, hemopoietin 1 stimulates the production of macrophage progenitors (25) and in the presence of both hemopoietin 1 and M-CSF, monocyte production can be increased considerably. Other molecules appear to act synergistically with hemopoietic growth factors, stimulating the rate of proliferation of progenitor cells in response to multi-CSF; e.g., interleukin 4 stimulates the rate of multi-CSF dependent mast cell proliferation (64).

Granulocyte-Macrophage Colony Stimulating Factor. The molecule known as GM-CSF has been purified to homogeneity from a human cell line (69) and murine lung conditioned medium (70). To some extent the native GM-CSF has been used to define the range of biological activities of GM-CSF. However, the purified proteins also provided amino acid sequence information which led to the identification and sequencing of cDNAs corresponding to both the human and murine GM-CSFs. It has been possible to express recombinant GM-CSF for both species in COS cells (55, 71), yeast (72, 73), and Escherichia coli (74, 75). Indeed biologically active, recombinant GM-CSFs have been purified from each of these sources and these have been used to define some of the activities of this molecule in vivo (11, 76).

The availability of purified GM-CSF, whether natural or recombinant, will allow progress toward an understanding of its structure and function. At present our knowledge of the structure of GM-CSF is essentially limited to the amino acid sequence. From a full length cDNA it has been possible to deduce the complete amino acid sequence of pro-GM-CSF (71) and a putative prepro-GM-CSF (77). The hypothetical prepro-GM-CSF would have a structure similar to that of a transmembrane molecule; however, in situations where this molecule would be expected to be synthesized, only the secreted form of the protein has been detected. Initial amino acid sequencing on murine GM-CSF from lung conditioned medium yielded NH2-terminal X-Thr-Val-Glu-Arg-Pro-Trp-Lys-His ... (78); i.e., the NH2 terminus commenced six residues after the expected cleavage site (71), between Ser39 and Ala30. The Ser–Ala cleavage site is used for GM-CSF derived murine T-lymphocyte stimulating by concanavalin A. It is clear that limited NH2-terminal truncations can be tolerated without influencing the biological activity. Since E. coli synthesized human and murine GM-CSFs are fully biologically active in vitro we know that the carbohydrate moieties are not necessary for either receptor binding or activation. Studies on the serum half-life of bacterially synthesized murine GM-CSF indicate that the serum half-life (6–10 min) is similar for the native molecule.

Analysis of the structure of the GM-CSF mRNA has revealed a segment of nucleotide sequence in the 3'-untranslated section of the mRNA which is important for controlling the breakdown of the mRNA (79). The GM-CSF gene is inducible and mRNA turnover is rapid. It is as though short bursts of synthesis rather than chronic production are used to control GM-CSF levels. It may be possible to manipulate the 3'-untranslated section of the cDNA to produce a GM-CSF mRNA with a longer half-life. This would lead to increased mRNA levels and presumably a more efficient animal cell production system for GM-CSF to be developed.

There has been considerable interest in the range of biological activities associated with the different hemopoietic growth factors (80, 81). The availability of large amounts of recombinant GM-CSF is allowing the biological specificity to be explored in more detail both in vitro and in vivo. Purified murine GM-CSF stimulates a single cell to proliferate and differentiate to produce neutrophils, eosinophils, granulocytes, or macrophages (65, 82). The low frequency of eosinophil progenitor cells initially masked the detection of the eosinophils produced by murine GM-CSF. Although it had not been purified, it was suggested that human GM-CSF could also stimulate eosinophil progenitors (83). The purification and cloning of sufficient quantities of both murine (32, 66) and human GM-CSF confirmed that these three cell lineages are all stimulated by GM-CSF (75, 80, 84).

The stimulation of isolated hemopoietic progenitor cells indicates that GM-CSF can initiate proliferation in both the erythroid and megakaryocytic lineages, but other molecules are necessary to complete the production of mature cells in these lineages (32). The discovery of murine multi-CSF established the concept that a single molecule could stimulate the production of all hemopoietic cells (48). Although earlier studies did not detect megakaryocyte production in response to GM-CSF, it is now apparent that a proportion of megakaryocyte progenitors can be stimulated with either murine (66) or human (84, 85) GM-CSF.

The search for an equivalent molecule produced by human cells initially led to the detection of mixtures of molecules which were thought to be pluripoietins and multilineage growth factors. In most cases these activities have proved to be mixtures of GM-CSF and G-CSF.

The biological specificity of human GM-CSF has proved to be more difficult to determine than the corresponding murine...
molecule. One major reason for this problem is the ability of human bone marrow cells to produce growth factors which interact in the colony stimulating assays. Even small numbers of contaminating monocytes, T-cells, endothelial cells, or stromal cells are likely to produce significant levels of molecules which can synergize with and modulate the activity of human GM-CSF (86-88). Thus, even a population of BFU-e which is 50% pure may contain cells which will respond to GM-CSF to produce molecules able to effect BFU-e proliferation. It is difficult to ascertain whether a proportion of BFU-e is able to respond directly to GM-CSF or whether the GM-CSF "induces" the production of another molecule which acts on some or all of the BFU-e.

It should be emphasized that this "biological" specificity has generally been determined by in vitro responses. Indeed, the analytical efforts to determine which cells proliferate in direct response to GM-CSF rather than those which proliferate as a result of indirect stimulation need to be considered in the appropriate context. When GM-CSF is released in vivo, its full range of effects will be manifest (i.e., direct and indirect), and proliferative or differentiative responses can be expected to occur in a wide range of cell lineages.

Although the discovery of GM-CSF occurred because of its in vitro proliferative effects on hemopoietic progenitor cells in the marrow and spleen, even in the first reports, it was recognized that this proliferation was associated with differentiation. During recent years there has been an increasing awareness of the action of GM-CSF on mature neutrophils and macrophages. It is now apparent that in the presence of initiator molecules, GM-CSF is capable of activating both neutrophils and eosinophils (65, 75, 80).

Granulocyte-Colony Stimulating Factor. The conditioned media from mouse L-cells and human urine stimulate almost exclusively macrophage progenitors (89), whereas mouse lung conditioned medium stimulates both granulocyte and macrophage progenitors (66) and mitogen-stimulated spleen cells produce growth "factors" which produce granulocytes, macrophages, erythroid, eosinophil, and megakaryocyte precursors (90). The CSFs in each of these preparations are heterogeneous and proved to be difficult to purify, but there were some indications that several distinctly different molecules could stimulate granulocyte (91, 92) and macrophage progenitors. Two potent sources of GM-CSF for human bone marrow cells, human leukocytes stimulated by phytohemagglutinin and human placental conditioned medium, appear to contain several CSFs. Phytohemagglutinin-stimulated human leukocytes were separated into a component capable of stimulating murine macrophage progenitors and the "GM-CSF" which stimulated human progenitor cells (93). Using phenyl-Sepharose chromatography, human placental conditioned medium was separated into two distinct colony stimulating factors, α and β (45): CSFα stimulated granulocyte, macrophage and eosinophil progenitors; whereas CSFβ stimulated only granulocyte and macrophage progenitors (45). When used at low concentrations the CSFα preparation selectively stimulated macrophage progenitors, whereas at low concentrations CSFβ preferentially stimulated granulocyte progenitors. The human CSFα activity had properties similar to those of murine GM-CSF. At low concentrations the human CSFβ activity mimicked the biological activity of the granulocytic specific murine G-CSF.

Most hemopoietic growth factor preparations are produced from tissues or cells cultured in vitro. Indeed, the concentration of these molecules in fresh tissue specimens and body fluids is extremely low and it is difficult to detect and characterize these molecules. There is a particular exception to this situation; when mice are given injections of endotoxin, several CSFs can be detected in the blood. Two groups initiated studies designed to characterize the molecule(s) in endotoxin serum which was responsible for inducing leukemic cell differentiation (46, 94). Both groups discovered that most of the colony stimulating activity in endotoxin serum could be neutralized by antisera raised against partially purified M-CSF (CSF-1) prepared from L-cell conditioned medium (95). However, the ability of endotoxin serum to induce WEHI3B(D⁺) cells was not diminished by this antiserum. Lotem et al. (94) did not detect any effect of the partially purified endotoxin serum differentiation factor (macrophage-granulocyte inducer 2) on normal cells. This group has developed a novel hypothesis concerning the link between proliferation and differentiation, they propose that hemopoietic regulatory molecules either induce differentiation or stimulate proliferation (96). In contrast Burgess and Metcalf (46) found that the endotoxin serum differentiation factor for WEHI3B(D⁺) cells was associated with some colony stimulating activity. All of the colonies stimulated by this CSF were composed of granulocytes. Thus, they suggested that a G-CSF was responsible for the differentiation of the WEHI3B(D⁺) cells. Although other differentiation factors may exist (97), it is likely that the ability of endotoxin serum to induce leukemic cell differentiation is due to the G-CSF. This molecule was purified from mouse lung conditioned medium (47) and the purified molecule stimulates the proliferation of normal granulocytic progenitor cells as well as the differentiation of WEHI3B(D⁺) cells.

Molecular cloning and receptor competition studies have made it clear that many conditioned media are likely to contain mixtures of GM-CSF, G-CSF, and M-CSF. A particular example is the identity of pluriprotein with G-CSF and pluriprotein α with GM-CSF (98). Although GM-CSF and G-CSF are becoming generally accepted terms, it is important to note that the cellular specificity of these two hemopoietic growth factors is considerably broader than their names imply. Both GM-CSF and G-CSF are capable of stimulating a limited number of divisions early in the erythroid, megakaryocyte, and eosinophil lineages (99). However, it is currently a matter of controversy whether either molecule can stimulate the production of mature cells in the erythroid and megakaryocyte lineages (100). In vitro and in vivo both GM-CSF and G-CSF can stimulate cell production in other hemopoietic lineages. However, these effects may be indirect (i.e., by inducing the production of other growth factors or modulators) (11, 85). This phenomenon is still being investigated in detail.

Human G-CSF has been synthesized by recombinant DNA methods using bacteria and appears to be active on its relevant target cells (101, 102). Two mRNAs have been detected for human G-CSF; one of these mRNAs encodes 3 extra residues (103). It is likely that the larger molecule arises from a splicing artifact inasmuch as the shorter G-CSF is considerably more active in the bioassay (102). There appear to be no detectable difference between the natural G-CSF (56) and the bacterially synthesized G-CSF in vivo. Thus the carbohydrate moiety does not appear to influence the interaction between G-CSF and its target cell. Apart from erythropoietin, there is no definitive information which gives us a clue to the function of the carbohydrate moiety attached to the hemopoietic growth factors. Apart from G-CSF, there is no definitive information which gives us a clue to the function of the carbohydrate moiety attached to the hemopoietic growth factors. Apart from erythropoietin, there is no definitive information which gives us a clue to the function of the carbohydrate moiety attached to the hemopoietic growth factors. Apart from erythropoietin, there is no definitive information which gives us a clue to the function of the carbohydrate moiety attached to the hemopoietic growth factors.
At present there is insufficient structural information to indicate which parts of the G-CSF molecule are required for its biological activity. There is a single free sulfhydryl near the NH₂ terminus (101) but this does not seem to be important for the activity of the molecule. Peptide analogues may help to define the binding domain by allowing the epitope for neutralizing antibodies to be defined. Once location of the binding site can be identified, analogues incorporating these residues can be prepared to study the linkage between binding and mitogenesis.

Macrophage-Colony Stimulating Factor. There is a high molecular weight hemopoietic growth factor, M-CSF [also called CSF-1 by the group which achieved the first purification of this molecule (89)], which has a propensity to stimulate the proliferation of macrophage progenitors. M-CSF has been detected in a wide range of human and murine tissues where it is present as a glycosylated homodimer (105). M-CSF has a variable amount of carbohydrate; human urinary M-CSF has an apparent molecular weight between 45,000 and 60,000 (43) and the molecular weight of the murine M-CSF homodimer varies from 60,000 to 80,000 (43). M-CSF is active only in the dimer form; when it is dissociated by urea-mercaptoethanol or acidic acetonitrile the monomer is no longer active (67, 105).

M-CSF has been available only in microgram amounts and the characterization of the important structural features has been quite difficult. However, in 1985 human M-CSF was molecularly cloned (106). Interestingly, the open reading frame for the M-CSF cDNA predicts a polypeptide 224 amino acids long (equivalent to a molecular weight of 26,000). There is a putative leader sequence of 32 amino acids in front of the NH₂-terminal sequence determined from the purified human urinary M-CSF. Since the size of the M-CSF polypeptide chain appears to be 14,500, some posttranslational processing of the polypeptide must occur.

Attempts to isolate an equivalent cDNA for murine M-CSF have proved to be even more difficult. However, one clone has been isolated (107) and there is considerable homology at the level of amino acid sequence.

It is important to consider the range of biological activities of M-CSFs on mice and human cells. Human urinary M-CSF does not stimulate human colony formation directly (108), but it is able to stimulate mouse marrow colony formation. Human urinary M-CSF stimulates granulocytic clusters to form in unfraccionated human marrow. This occurs by the stimulation of monocytes to produce G-CSF. Thus, the human urinary M-CSF seems to function as a stimulus for the final stages of granulocytic development in humans and a stimulator of the early monocyte progenitor cells in mouse marrow. Murine M-CSF stimulates murine marrow cells to form macrophages, but does not stimulate human marrow cells. Even though murine M-CSF is predominantly a stimulator of murine macrophage and monocyte progenitors, there is still some stimulation of neutrophil formation. Indeed, in serum-free cultures of individual hemopoietic progenitor cells, murine M-CSF appears to be an excellent stimulus for neutrophil-macroage progenitors (23). M-CSF is also a powerful stimulus for mononuclear phagocytes (41) and murine monocytes require the presence of either GM-CSF or M-CSF for their survival (109).

Both human and murine M-CSF can induce proliferation of murine mononuclear phagocytes; however, although human mononuclear phagocytes can be functionally activated by M-CSF, as yet human monocytes have not responded mitogenically to any agent.

It will be interesting to study the biological effects of M-CSF in vivo. There are strongly synergistic interactions between M-CSF and other hemopoietic growth factors in vitro (23, 25, 110). Many of these interactions should be manifest in vivo and even small concentrations of Multi-CSF or G-CSF could be expected to synergize with the effects of M-CSF on both progenitor cells and mature cells.

Multi-Colony Stimulating Factor (Multi-CSF, IL-3). The discovery of GM-CSF and M-CSF encouraged a search for other molecules responsible for stem cell proliferation and production of eosinophils, megakaryocytes, and erythroid cells. Numerous sources of GM-CSF and M-CSF were discovered, but the other colony types were difficult to produce in vitro. It was known that the conditioned medium from mitogen stimulated mononuclear blood cells, together with erythropoietin, was able to stimulate granulocyte, macrophage, and erythroid colonies (111). Parker and Metcalf (112) produced PWM-SCM which supported the proliferation of progenitors for natural killer cells, eosinophils, megakaryocytes, and erythroid cells. The only other source of growth factors for these cells was the conditioned medium from a murine myelomonocytic leukemia WEHI3B(D-) (49). Since GM-CSF was a distinct molecular entity, it was initially assumed that PWM-SCM and WEHI3B(D-) conditioned medium also contained distinct eosinophil CSF, megakaryocyte CSF, and erythroid CSFs. However, attempts to separate these molecules from PWM-SCM invariably yielded two classes of CSF: GM-CSF; and another biologically active pool which contained a molecule(s) capable of stimulating all of the other progenitors (48, 90). Murine multi-CSF (IL-3) was the first of the homopoietic growth factors to be molecularly cloned (53, 113). Factor dependent mast cell clones (114, 115, 116) were used to assay products secreted by Xenopus oocytes previously treated with injections of subfractions of WEHI3B(D-) mRNA. The enriched mRNA was used to prepare a cDNA library and the appropriate cDNA pools from the library screened in the oocyte assay using hybrid selection and elution. The availability of a full length cDNA and NH₂-terminal sequences for multi-CSF indicated that considerable heterogeneity could occur at the NH₂ terminus (presumably due to differential processing). Despite some limited homology at the signal peptide cleavage site (117), no other significant homologies have been detected between the predicted amino acid sequences for murine multi-CSF, GM-CSF, G-CSF, or M-CSF peptide chains.

Attempts to locate the human equivalent of multi-CSF using the murine multi-CSF cDNA were not successful. There is no overlap in biological activity between murine and human IL-3. Even the rat multi-CSF (118) amino acid sequence is significantly different from murine multi-CSF.

Further analysis of the murine GM-CSF and multi-CSF cDNA clones indicated that as well as the secreted forms, the longer clones could encode pre-CSF molecules with a number of charged residues at the NH₂ terminus. This NH₂-terminal arrangement of these longer molecules would generate a "transmembrane-like" domain allowing the GM-CSF or multi-CSF to anchor in the plasma membrane. At present neither the mRNA nor the protein corresponding to the transmembrane form of these two molecules has been detected.

Interactions between Hemopoietic Growth Factors. The HGFs can act synergistically; i.e., combinations of two or more of the HGFs can induce proliferation and differentiation more effectively than the individual growth factors. Until each of the HGFs had been purified it was difficult to study these synergistic effects definitively and even now it is important to note that there are many other potent modulators of hemopoiesis in vitro, e.g., fetal calf serum (23, 119), hemopoietin 1 (25), and IL-4
The molecular basis for these synergistic effects is poorly understood. The situation will be even more complex in vivo where the high density of cells in the marrow and spleen can be expected to produce significant concentrations of other modulating agents (120–124).

It is simple to show the interactions between the different growth factors. When murine marrow cells are stimulated by M-CSF (CSF-1) in medium containing fetal calf serum most of the colonies contain only monocytes/macrophages. Using concentrations of M-CSF which maximally stimulate marrow colony formation, 95% of the colonies are composed of monocytes. However, when M-CSF is mixed with GM-CSF many granulocytic and mixed granulocyte/macrophage colonies develop (110). Indeed, even when the GM-CSF is used at low concentration, as long as there is maximal stimulation by M-CSF, granulocytic colonies will form. Thus, as long as there is a strong proliferative stimulus, even a weak differentiation signal can influence the pattern of hemopoietic differentiation.

In serum free cultures a combination of multi-CSF and M-CSF increased the size (i.e., number of cells) of both granulocyte and macrophage colonies (23). Furthermore, the number of GM-progenitor cells responding to the combination of multi-CSF and M-CSF is greater than the sum of the number of colonies stimulated by the individual factors.

Hemopoietin 1 is a molecule which appears to stimulate the initial proliferation of multipotential hemopoietic cells and early macrophage progenitors (25), but it is not able to induce complete maturation. Mature cells are produced only in the presence of the other hemopoietic regulators. There also appears to be a synergistic interaction between hemopoietin 1 and multi-CSF (23). In the presence of both hemopoietin 1 and multi-CSF the number of colonies formed by multipotential progenitors increases almost 30-fold and many of the colonies still contain multipotential progenitors.

The molecular basis of this synergism has not been studied in detail, although in other cellular systems (fibroblast and epithelial) synergistic interactions have been reported to occur among epidermal growth factor, platelet derived growth factor (125), and human transforming growth factor β (126). There is some evidence that the synergistic interactions are modulated at the level of the cell surface receptor via an hierarchical network (127). Thus it has been proposed that the activated multi-CSF and GM-CSF receptors transmodulate the M-CSF and G-CSF receptors and that it is these interactions which are responsible for the production of granulocytes and macrophages in the presence of Multi- or GM-CSF. By extrapolation, the generation of eosinophils, megakaryocytes, and erythroid cells by multi-CSF and eosinophils by GM-CSF might be expected to be mediated by the transmodulation of the receptors for eosinophil (59, 128, 129) megakaryocyte (130, 131), or erythroid (132) growth regulatory proteins. Receptor transmodulation could be a vital link between proliferation and differentiation. Normal progenitor cells stimulated by multi-CSF would be expected to proliferate and those which acquire G-CSF or M-CSF receptors would then be induced to differentiate (even in the absence of G-CSF or M-CSF). Multi-CSF and GM-CSF might be capable of producing granulocytes and macrophages at sites not normally endowed with sufficient concentrations of M-CSF or G-CSF.

The interrelationship among three phases of hemopoietic cell production (i.e., proliferation, differentiation, and activation) needs to be considered when analyzing leukemic cell populations. Most of the hemopoietic growth factors are probably capable of influencing these different phases of hemopoiesis, but there will be a different potency for each growth factor at each phase. For example multi-CSF and GM-CSF appear to be two of the preferred stimuli for expanding the early proliferation steps. Although both are also capable of inducing differentiation and activating end cell function, they may mediate these effects through the transmodulation pathways via the M-CSF and G-CSF receptors.

In contrast, while G-CSF may influence the early proliferative events in hemopoiesis, this regulator appears to be a more potent activator of neutrophil function than multi-CSF (133). In order to manipulate the hemopoietic system we will need to learn how to use these regulators (or their antagonists) to stimulate (or inhibit) the different phases of hemopoiesis. The linkages between these three aspects of hemopoiesis are required to maintain the number of mature blood cells. In the murine system several myeloid cell lines have been isolated which are still dependent on growth factor but appear to be blocked at different stages of differentiation. When this block occurs before the acquisition of G-CSF or M-CSF receptor, the cells will not differentiate, even in the presence of these regulators. However, in other cell lines such as WEHI3B(D⁺), GM-CSF and G-CSF receptors coexist but do not appear to be linked (127), i.e., occupancy of the GM-CSF receptor fails to transmodulate the G-CSF receptor. Thus, even though GM-CSF stimulates the proliferation rate of WEHI3B(D⁺) it is not a potent inducer of differentiation. These cells can still differentiate if they are treated directly with G-CSF or actinomycin D. A similar situation may occur in many primary leukemias and it is imperative that we learn more about the biochemistry of receptor transmodulation in normal and neoplastic cells. At present our knowledge of the receptors for the hemopoietic growth factors limits our ability to understand the biochemical interactions which mediate the receptor signal (72, 134–136).
cytopenia. The effects of GM-CSF were not restricted to the circulating blood WBC counts. They also reported that GM-CSF produced a significant increase in the numbers of granulocytes and monocytes, and eosinophils and a decrease in lymphocytes. The basis for the accumulation of GM-CSF is short (<5 min), multiple (up to 6) injections were in the range 6-200 ng/animal. Since the serum half-life is not clear whether during infections the low numbers of neutrophils are maximally activated or whether additional GM-CSF might cause further activation and additional antibacterial effect. There are also reports that patients being treated with chemotherapy may exhibit diminished neutrophil function (148). This functional inhibition may be overcome by GM-CSF. This possibility is under investigation. Another question relates to the functional status of neutrophils in the elderly and whether GM-CSF might prove a useful activator.

There is an important distinction between GM-CSF and G-CSF; GM-CSF activates both mature neutrophils and eosinophils (140, 149), whereas G-CSF activates only neutrophils. MCSF activates mature monocytes and, in particular, causes the production of interferon (150) and plasminogen activator (151, 152). These actions of M-CSF may have clinical applications in the treatment of infections.

In Vivo Effects of CSFs

Granulocyte-Macrophage Colony Stimulating Factor. Until recently insufficient amounts of GM-CSF were available for definitive in vivo studies. The availability of all of the CSFs in gram quantities is now allowing the pharmacology and physiology of each to be studied in detail. Further, many laboratories are testing multiple combinations of the CSFs with other modulators such as the interferons and interleukins.

Murine Studies. Metcalf et al. (76) have studied the effects of bacterially synthesized murine GM-CSF in vivo. The doses used were in the range 6-200 ng/animal. Since the serum half-life of GM-CSF is short (<5 min), multiple (up to 6) injections were used. These injections resulted in elevated peripheral blood levels of granulocytes and eosinophils. Administration i.p. produced a significant increase in the peritoneal neutrophils, monocytes, and eosinophils and a decrease in lymphocytes. These experiments were controlled carefully to avoid effects due to contaminating endotoxin. A careful analysis of various organs revealed an increased number of granulocytes and monocytes in the liver (76). There was also an increased number of granulocytes in the lung (76). The basis for the accumulation of mature hemopoietic cells in the peritoneal cavity, liver, and lung is not apparent because these are not normally sites of hemopoiesis.

Simian Studies. Donahue et al. (11) reported on the effects of GM-CSF (1-2 x 10^7 units/mg) infused continuously into 7 macaque monkeys. They found significant elevations of peripheral blood WBC counts. They also reported that GM-CSF stimulated hemopoiesis in a monkey with a virally induced cytopenia. The effects of GM-CSF were not restricted to the myeloid system as much as a reticuloendothelial system was also affected after the GM-CSF treatment. One animal apparently developed antibodies which recognized the GM-CSF; these antibodies, however, did not interfere with hemopoiesis. A major problem with this study was that the human GM-CSF was only partially purified from COS cell conditioned medium. Nevertheless assuming that the findings are reproducible with pure GM-CSF one can speculate that GM-CSF may prove useful in conditions such as acquired immunodeficiency syndrome in which there is a neutropenia perhaps secondary to a deficiency of lymphokines produced by lymphocytes.

Multi-Colony Stimulating Factor. Kindler et al. (153) have reported the effects of multi-CSF in mice. Similar results have been obtained by Metcalf et al. (154) and Lord et al. (155). Kindler et al. (153) used doses of 0.03-0.1 µg/24 h for 7 days. The total number of progenitor cells in normal mice was increased 2-fold and, in particular, there was a rise in the erythroid and myeloid progenitor cells in the spleen. If mice were irradiated prior to therapy there was a 10-fold increase in progenitor cell levels compared to control animals. Multi-CSF injected into the peritoneal cavity produced increased numbers of monocytes (154) and these monocytes appeared to function as more effective phagocytes. The human equivalent of murine IL-3 has been cloned recently but its activity in vivo is not yet known.

Granulocyte Colony Stimulating Factor. One group (156) has reported on the in vivo activity of purified rG-CSF. The rG-CSF was administered s.c. Following a single injection there was a rise in neutrophil counts within 2 h. After 15 days of treatment there was an 8-fold rise in neutrophil levels. When rG-CSF was withdrawn the blood neutrophil levels returned to normal within 24 h (Fig. 2). Repeated s.c. injections of 2.5 µg/mouse/day resulted in sustained elevated neutrophil counts for more than 3 weeks. Daily single injections of 10 µg of rG-CSF for 14 days resulted in a 20-fold increase in neutrophil levels.
over the basal level. In the mouse the effects were restricted to
the myeloid system and there were no consistent changes in the
levels of lymphocytes, monocytes, platelets, or RBC. In the
mouse a transient effect on lymphocytes was apparent. G-CSF
also increased the number of CFU-spleen in the spleen. It is
likely that this is an indirect effect, perhaps representing recrui-
tment, since G-CSF has no in vitro effect on multipotential
progenitor cells.

When purified G-CSF was given after an alkylating drug such
cascyclophosphamide, the neutropenia produced by this agent
was completely abrogated (156). Administration of G-CSF can
preempt the death of mice which have been infected with a
lethal dose of Gram negative microorganisms (Pseudomonas
(157). Pseudomonas is a common cause of infections and deaths
in cancer patients being treated with cytotoxic chemotherapy.

Hemopoietic Growth Factors and Leukemia

The acute myeloid leukemias are subdivided into 7 categories
by the French-American-British classification system (158).
Each leukemia is characterized according to the degree of
proliferation of the leukemic clone in the marrow. The variants
of myeloid leukemia are further distinguished by the morpho-
logical and biochemical properties of the leukemic cells (158).

A feature of all cases of acute myeloid leukemia is that there
appears to be a block to the normal maturation of blast cells.
This block may be associated with a disturbance in the biochem-
ical pathways associated with receptor cross-modulation (126)
or with the control of expression of the genes encoding differ-
etiation factors (96). Therapy of acute myeloid leukemias
usually leads to the eradication of the leukemic clone and the
recovery of normal hemopoiesis (159), although in some cases
normal differentiation and maturation of the leukemic cells are
achieved (159).

Are the CSFs involved in the development or maintenance
of the myeloid leukemias? Would it be possible by manipulating
the levels of one or more of the CSFs to improve the therapy
of leukemia patients? It will be important to determine the
basis of the suppression of normal hemopoiesis by the leukemic
cells and the levels of each of the CSFs in the tissues and serum
of patients with AML. Further, an understanding of the de-
pendence of the proliferation and differentiation of AML cells
on the normal CSFs and other biological response modifiers
should help define the conditions to create for attacking the
leukemic cells with cytotoxic drugs.

Both the autocrine and autonomous models for leukemic cell
population predict factor independent myeloid leukemias.
However, in vitro studies suggest that at the time of diagnosis
most myeloid leukemias require exogenous CSF to proliferate
(160). There are a few leukemias which do not require CSF
(161). In some cases the leukemic cells may produce CSF (162).
It has also been found that the dose-response curves for CSF
stimulation of normal and leukemic cells are similar (38, 163).
The number of G-CSF receptors on normal and leukemic cells
is similar at the equivalent stages of development. Some cases
of AML, particularly during the later phases of the disease,
may not be dependent on an exogenous source of CSF but this
has not been extensively tested. However, it should be noted
that many leukemias in blast crisis do not proliferate in vitro,
even in the presence of the CSFs. Even those that do not grow
in vitro obviously proliferate in vivo, suggesting there are he-
mopoietic growth factors still to be identified. It is not clear
which growth factor is stimulating these leukemias in vivo. It
may be that the leukemia models developed by Lang et al. (164)
represent the later stages of some cases of myeloid leukemia.
However, their direct relevance to spontaneously arising leu-
kemias is yet to be established.

Morstyn et al. (165) demonstrated that normal promyelocytes
and myelocytes were highly responsive to partially purified
human G-CSF. Recently, human G-CSF has been shown to be
very similar to murine G-CSF and 125I-labeled murine G-CSF
can be used to study human G-CSF receptors on leukemic cells
(104); 14 patients with myeloid leukemia had near normal G-
CSF receptor levels. The data for human GM receptors (166)
also demonstrate that leukemic myeloid cells have near normal
receptor levels. In many ways this evidence suggests that these
leukemias are not driven by autocrine stimulation.

Leukemogenesis. Murine marrow cells can be forced to adopt
the leukemic phenotype in several different ways. Continuous
cell lines have been derived from apparently normal long-term
marrow culture. These cell lines are invariably dependent on
multi-CSF (115, 167, 168). However, cell lines dependent on
G-CSF (169) and M-CSF (170) have also been obtained.

These cell lines which are absolutely dependent on the
hemopoietic growth factor for proliferation are blocked at an early
stage of differentiation but are not leukemogenic in syngeneic
recipients. They can be made independent of CSF by sponta-
neous mutation (168), by transformation with the Abelson
murine leukemia virus (171, 172), or by infection with a recombin-
ant retrovirus that encodes and expresses GM-CSF (164). The
molecular lesions leading to leukemia in the case of inser-
tion of the GM-CSF gene and the spontaneous mutations are
associated with the autocrine secretion of GM-CSF or multi-
CSF. The v-abl gene appears to circumvent the requirements
for GM-CSF and probably does this by producing a tyrosine
kinase which mimics the intracellular events normally triggered
by the GM-CSF-GM-CSF receptor complex. Dunn3 has re-
cently found that after the transfection of factor dependent cells
with a retrovirus which encodes and expresses the polyoma
middle T-antigen one class of cells becomes leukemic but re-
mains dependent on GM-CSF for growth in vitro. These cells
appear to resemble human leukemic cells.

Acute Myelogenous Leukemia. It has been shown recently
that the genes for M-CSF, GM-CSF, multi-CSF, interleukin 4,
and M-CSF receptor are all clustered on chro-
mosome 5 (173, 174). There is a clinical syndrome called the
5q– syndrome that involves a deletion of the long arm of
chromosome 5 (174). It is intriguing to speculate that the
disorder in these patients is due to activation of a hemopoietic
growth factor or receptor gene. However, this has not yet been
investigated.

Promyelocytic leukemia has a 17/15 translocation and the
G-CSF gene is on chromosome 17. The abnormality in this
situation may be the absence of a differentiation stimulus (G-
CSF) leading to a maturation block at the level of the promye-
locyte (175).

Production and Storage of GM-CSF

Most organs can produce the granulocyte-macrophage colony
stimulating factors. The organs that have been shown to pro-
duce GM-CSF using bioassays (176) include salivary gland,
lung, thymus, kidney, spleen, lymph node, pancreas, brain,
heart, small intestine, testis, skeletal muscle, liver, and skin.
Nicola et al. (177) have demonstrated that GM-CSF synthe-
sized by different organs had very similar biochemical proper-

3 A. Dunn, personal communication.
ties. These studies indicate either that GM-CSF is made by cells present in all tissues such as monocytes, endothelial cells (177, 178), or fibroblasts or that cells of many different types make GM-CSF. This has not been clarified directly as yet. Endotoxin and tumor necrosis factor appear to stimulate CSF release (87, 179). The presence of GM-CSF in serum is still a matter of some contention. Undoubtedly serum contains hemopoietic growth factors, but whether GM-CSF, multi-CSF, G-CSF, M-CSF, or IL-1 is responsible for the biological responses has not been studied with the appropriate reagents.

A recent study (180) has demonstrated GM-CSF production by endothelial cells, smooth muscle, and fibroblasts and that these can be stimulated to produce CSF by tumor necrosis factor. Broudy et al. (180) also showed that protein synthesis was required. Koury and Pragnell (181) demonstrated CSF production by fibroblasts. The in vivo importance of these observations is unclear. The kinetics of release in vivo and the failure of cycloheximide to reduce accumulation of CSFs in endotoxin serum suggest that at least one of the CSFs exists in a stored form (e.g., in membrane bound vesicles, in granules, or even on the surface of cells). The initial release of these growth factors is probably connected with their ability to activate mature cells rather than to increase cell production.

Another approach to identifying the tissues and cell types that produce GM-CSF is to identify the cells and tissues where GM-CSF mRNA is present. Recently, Chan et al. (182) used full length GM-CSF cDNA as a probe and screened, by dot blot hybridization analysis, 64 tissue samples including liver, colon, esophagus, rectum, stomach, breast, lung, skin, and various solid tumors and leukemias. There was no constitutive production of detectable levels of GM-CSF mRNA in any sample studied. Since smooth muscle, endothelial cells, and fibroblasts would have been present in these tissues and other techniques suggest they synthesize GM-CSF the approach may have been too insensitive. However, it is also possible that the GM-CSF mRNA only appears 8–12 hours after the tissues have been stimulated. Chan et al. (182) did confirm that mRNA for GM-CSF is present in activated T-lymphocytes and were able to identify the region of the GM-CSF gene involved in regulating the transcription of the GM-CSF mRNA.

Clinical Disorders and Abnormal GM-CSF Levels

There is both direct and indirect evidence that CSFs can control hemopoiesis in vivo. This evidence includes a correlation between urine CSF levels and WBC levels (183), a demonstration that tumors that produce CSF are associated with elevated WBC and that the in vivo administration of purified CSFs produces high WBC. Table 2 contains a list of clinical conditions that are associated with altered levels of CSF. In each case CSF was measured by the marrow bioassay. All of these studies need to be reassessed because the bioassay does not distinguish among multi-CSF, GM-CSF, or mixtures of M-CSF and G-CSF. Also some studies (184, 188) on the distribution of human CSFs were undertaken with murine cells as targets. The latter assays presumably detected human G-CSF but not human GM-CSF since only human G-CSF acts on murine targets. It is also possible that M-CSF which acts indirectly to stimulate G-CSF release may have been detected in some of these studies. In cases such as those associated with infections where levels of endotoxin may have been high, indirect effects of endotoxin in the bioassay rather than GM-CSF levels may also interfere with the assay. More direct assays (e.g., a radioimmunoassay) are required to characterize the distribution of each of the CSFs in various diseases. Studies of CSF levels in normal and disease states are now under way and an enzyme-linked immunosorbent assay is available for monitoring exogenous bacterially synthesized GM-CSF administered to patients (199). This assay also detects native GM-CSF in serum.

Early Clinical Trials with G-CSF

The potential clinical applications of the CSFs are listed in Table 3. One possible application of the CSFs is to prevent or reverse the neutropenia that follows cytotoxic chemotherapy and thus reduce the incidence or duration of infections. Mostyn et al. (200) have reported a Phase I/II study of G-CSF in patients receiving i.v. melphalan. The patients received G-CSF for 5 days prior to chemotherapy to assess the effect of G-CSF. The major findings were that G-CSF caused an immediate transient fall in circulating neutrophils followed by a rise which was dependent on the dose of G-CSF administered. Circulating levels of neutrophils over 70,000/mm³ were achieved. The neutrophils exhibited granulation and Döhle bodies. The major change in the marrow was an increase in the proportion of promyelocytes to over 30%. These changes would appear to reflect initial margination or sequestration of neutrophils followed by release and then synthesis of neutrophils after 4–5 days. Following melphalan administration the period of neutropenia was reduced from 8 days to 0 days in patients who had not received prior cytotoxic therapy. Patients responded less well to G-CSF if they had been extensively pretreated with chemotherapy or radiotherapy. Bronchud et al. (201) have reported in a study of 12 patients a reduction in the neutropenia caused by combination chemotherapy in small cell lung cancer, and Gabrilove et al. (202) have reported a reduction in neutropenia in patients receiving combination chemotherapy for bladder cancer. These studies involved the administration of G-CSF during one cycle of chemotherapy. Studies are now required of G-CSF administration for longer periods of time and randomized studies are required to confirm that G-CSF causes not only a reduction in the period of neutropenia but also protection against bacterial and fungal infections. The pharmacokinetics

### Table 2 Relationship between CSF levels and clinical states

<table>
<thead>
<tr>
<th>Clinical state</th>
<th>Serum CSF level</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclic neutropenia</td>
<td>CSF levels cycle and precede rise in WBC</td>
<td>183</td>
</tr>
<tr>
<td>Aplastic anemia</td>
<td>Elevated CSF in urine</td>
<td>184</td>
</tr>
<tr>
<td>Congenital neutropenia</td>
<td>Diminished serum CSF level</td>
<td>185</td>
</tr>
<tr>
<td>Endotoxin administration</td>
<td>Elevated serum and urine</td>
<td>186, 187</td>
</tr>
<tr>
<td>Infection</td>
<td>Elevated serum and urine CSF</td>
<td>188, 189</td>
</tr>
<tr>
<td>Infectious mononucleosis</td>
<td>Low levels of CSF</td>
<td>190</td>
</tr>
<tr>
<td>Acute neutropenia</td>
<td>Elevated CSF levels</td>
<td>191</td>
</tr>
<tr>
<td>Chronic neutropenia without infection</td>
<td>CSF levels normal</td>
<td>192</td>
</tr>
<tr>
<td>Chemotherapy for lymphoma</td>
<td>Elevated CSF levels</td>
<td>193</td>
</tr>
<tr>
<td>Therapy with anti-neutrophil antibody</td>
<td>Elevated CSF levels</td>
<td>194</td>
</tr>
<tr>
<td>Human cancers associated with neutrophilia</td>
<td>Elevated CSF levels</td>
<td>195–198</td>
</tr>
</tbody>
</table>

### Table 3 Potential clinical applications of G-CSF and GM-CSF

1. Mitigation of chemotherapy induced neutropenia.
2. Improvement of host defense.
4. Recovery following marrow transplantation.
5. Expansion of progenitor cells prior to transplantation.
6. Marrow failure.
7. Improvement of granulocyte function.
8. Anticancer effects.

The major findings were that G-CSF caused an immediate transient fall in circulating neutrophils followed by a rise which was dependent on the dose of G-CSF administered. Circulating levels of neutrophils over 70,000/mm³ were achieved. The neutrophils exhibited granulation and Döhle bodies. The major change in the marrow was an increase in the proportion of promyelocytes to over 30%. These changes would appear to reflect initial margination or sequestration of neutrophils followed by release and then synthesis of neutrophils after 4–5 days. Following melphalan administration the period of neutropenia was reduced from 8 days to 0 days in patients who had not received prior cytotoxic therapy. Patients responded less well to G-CSF if they had been extensively pretreated with chemotherapy or radiotherapy. Bronchud et al. (201) have reported in a study of 12 patients a reduction in the neutropenia caused by combination chemotherapy in small cell lung cancer, and Gabrilove et al. (202) have reported a reduction in neutropenia in patients receiving combination chemotherapy for bladder cancer. These studies involved the administration of G-CSF during one cycle of chemotherapy. Studies are now required of G-CSF administration for longer periods of time and randomized studies are required to confirm that G-CSF causes not only a reduction in the period of neutropenia but also protection against bacterial and fungal infections. The pharmacokinetics
of G-CSF following i.v. administration reveals at least one phase of elimination of about 110 min. Morstyn et al. (200) also reported a brief first phase lasting for 3 to 8 minutes by bioassay. The best route of administration of G-CSF has not yet been identified; however, we have given 10-day continuous s.c. infusion with good effect and no local reactions. G-CSF is well tolerated although bone pain has been reported (200). A maximum tolerated dose has not been identified and studies have stopped because it was considered that WBC levels above 75,000/mm³ were undesirable.

Early Clinical Studies with GM-CSF

Three forms of GM-CSF are in clinical trials: mammalian derived material (Sandoz); material produced by yeast (Immunex); and bacterially synthesized material (Schering Plough). Like G-CSF, GM-CSF causes elevations in WBC levels. At low doses the predominant rise is in neutrophils; however, at higher doses a rise in monocytes and eosinophils is also seen. The immediate effect of GM-CSF is a transient fall in the WBC level (203) which occurs after both i.v. and s.c. administration and which is followed by a rise. The best route of administration of GM-CSF has not been identified. GM-CSF has been shown to reduce the period of neutropenia after autologous marrow transplantation from 16 to 10 days (204). It has been shown to cause elevations in the WBC levels in patients with acquired immunodeficiency syndrome. Cebon et al. (199) have studied the pharmacokinetics of GM-CSF and found that after a single s.c. dose of GM-CSF of 10 μg/kg the serum level of GM-CSF is sustained above 1 ng/ml for more than 12 h. There would therefore appear to be no advantage to continuous i.v. infusions of GM-CSF. This is particularly important because GM-CSF is a chemotactant and might cause leukostasis and thrombosis at the catheter tip. The s.c. route of administration, however, has been associated with local rashes in some patients. Vadhan-Raj et al. (205) have reported a beneficial effect in patients with myelodysplasia. However, Mertelsmann (206) reported at a recent meeting that in some cases where the proportion of blasts in the marrow is greater than 20% GM-CSF causes a worsening of the leukemia. An apparent difference between the early clinical studies using G-CSF (200–202) and GM-CSF (199, 203–205) is that no maximum tolerated dose of G-CSF has been identified, whereas there is clearly a maximum tolerated dose of GM-CSF. The dose limiting toxicity of GM-CSF may reflect direct effects or indirect effects perhaps as a consequence of monocyte activation and release of other cytokines. The toxicities of GM-CSF include bone pain, fever, edema, rashes, and serositis, including pericarditis.

Future Directions

Anticancer Effects of GM-CSF. GM-CSF can function as a macrophage activating factor and consequently can promote the ability of macrophages to recognize and destroy human melanoma cells. GM-CSF also activates antibody dependent cell cytotoxicity (140). Administered systemically, the CSFs are unlikely to have anticancer effects. However, there are clinical situations, e.g., ovarian cancer, in which the malignancy is confined to the peritoneal cavity for a significant period of time. In this situation the i.p. infusion of GM-CSF and tumor directed antibodies may be able to stimulate an anticancer effect via the activation of antibody mediated monocyte cytotoxicity.

Effects of Colony Stimulating Factors on Nonhemopoietic Cells. Studies on G-CSF receptors (133) and GM-CSF receptors (134) indicate that both are restricted to cells of the hemopoietic series. However, Ruff et al. (207) have reported that at high concentrations GM-CSF inhibits the proliferation of small cell lung cancer cells. It suggests either that malignant cells may express GM-CSF receptor aberrantly or that these tumors are closely related to hemopoietic cells. Baldwin et al. (218) have reported G- and GM-CSF receptors on small cell lung cancers. We do not know what effect the CSFs will have on such tumors or whether we will lose the selective protection of the marrow.

Potential Side Effects of CSF Therapy. The direct effects of the colony stimulating factors will be confined to cells that contain CSF receptors; however, the stimulated cells may be induced to secrete other biological response modifiers which could influence the action of these molecules. These could include IL-1 and tumor necrosis factor. Accumulation of granulocytes and/or monocytes in the lungs, liver, pericardium, and peritoneum is possible. Such infiltrates have been observed in preclinical studies (76).

Another potential side effect is the development of antibodies to CSF. Such antibodies are theoretically more likely to develop in response to glycosylated than to nonglycosylated CSF; however, even nonglycosylated CSF may prove antigenic due to minor differences in the three dimensional structure or its state of aggregation.

Antibodies to hGM-CSF were detected in some of the monkeys treated with the COS cell derived human GM-CSF (11). However, in this situation there was a species difference between the GM-CSF and the animals and the carbohydrate moiety would be quite different. The antibodies were not neutralizing and appeared to be of little pathological significance.

Role of Combinations of Hemopoietic Growth Factors. Many combinations of biological response modifiers will be used in an attempt to modify hemopoiesis and phagocyte function and it is a little too early to predict the best combinations of hemopoietic growth factors, modulators, and cytotoxic drugs. A considerable effort will be required to define the in vivo effects of the hemopoietic growth factors so that appropriate baseline data are available from which their use can be optimized. The actions of GM-CSF and G-CSF are synergistic (209, 210). However, the testing of the combinations of these growth factors should be delayed until we have adequate baseline data on the individual molecules.

The hemopoietic growth factors have been characterized in detail and we have a considerable knowledge of their biology in vitro. The exquisite specificity of these growth factors makes them potentially powerful modulators of blood cell production and function. At present we can only hope that this potential can be combined with new or current therapies to help transplant or cancer patients. The results of the initial animal studies have been so encouraging that it is tempting to suggest that the hemopoietic growth factors will lead to entirely new approaches in oncology. Now that neutrophil levels can be restored more quickly, it is essential to develop similar strategies for the elevation of platelets. However, it is also clear that the control of hemopoiesis is complex and depends on the interaction of many factors. We do not fully understand this network and much work remains before we can ensure that the hemopoietic growth factors can be used for the maximum benefit of the patient.

Acknowledgments

The authors wish to thank Jean Kingett for her care and patience in the preparation of this manuscript. We are also grateful to Henrike
Heckman for translating Metchnikoff's paper (1) from German into English.

References


HEMOPOIETIC GROWTH FACTORS


HEMOPOIETIC GROWTH FACTORS


Hemopoietic Growth Factors: A Review

George Morstyn and Antony W. Burgess