

THE JOURNAL OF
ALLERGY
AND
CLINICAL IMMUNOLOGY

VOLUME 101

NUMBER 1, PART 1

Updates on cells and cytokines

The therapeutic use of hematopoietic growth factors

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The discovery, isolation, and characterization of colony-stimulating factors radically altered the ability of scientists to manipulate experimental models of hematopoiesis in vitro and provided the basis for tremendous gains in our understanding of the molecular mechanisms regulating this most vital aspect of homeostasis. Within the last decade, the cloning and large-scale manufacture of hematopoietic growth factors (HGFs) by the pharmaceutical and biotechnology industries has permitted the insights gained in these in vitro systems to be applied to the use of cytokines as novel therapeutics in vivo in human beings.

The majority of the clinical trials of HGFs have been conducted in the context of therapy of hematologic and oncologic malignancies. Sufficient data from human clinical trials have now accrued to permit publication of evidence-based practice parameters by an expert panel of the American Society of Clinical Oncology.¹

As the indications for the therapeutic use of HGFs become more well-defined and their application expands to include even normal healthy blood donors,^{2,3} the number of patients receiving these drugs will increase significantly. A concomitant increase in the incidence of cytokine-related adverse events should not be unex-

Abbreviations used

BMT:	Bone marrow transplant
HGF:	Hematopoietic growth factor
SCF:	Stem cell factor
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
G-CSF:	Granulocyte colony-stimulating factor
PBPC:	Peripheral blood progenitor cell

pected. It is likely that the allergy/immunology specialist will be more frequently asked to determine if an adverse event or a constellation of symptoms found in a patient receiving HGF therapy represent an allergic reaction. The specialist may also be asked to comment on the safety of continued therapy with the suspect drug or drugs.

As the chemotherapeutic regimens used in the treatment of malignancies become more aggressive and thus more myelosuppressive, the ability to utilize HGFs often represents an integral component of potentially life-saving therapy. Therefore it is imperative that growth factor-related adverse events not be labeled as *allergic* without substantial justification. In this regard, it is essential that allergy/immunology specialists possess sufficient knowledge of the biology, clinical applications, and toxicity of HGFs to permit prudent evaluation of suspected allergic drug reactions and effective communication of these opinions to the other members of the patient care team.

BACKGROUND

HGFs, also termed colony-stimulating factors, are a family of glycoproteins that are required for the survival of hematopoietic progenitors. Hematopoiesis may be defined as the orderly progression of pluripotent stem

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Supported in part by United States Public Health Service grants, the Beth Israel Deaconess Hospital Pathology Foundation, Inc., and AMGEN, Inc.

Received for publication Oct. 7, 1997; accepted for publication Oct. 7, 1997.

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J Allergy Clin Immunol 1998;101:1-6.

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0091-6749/98 \$5.00 + 0 1/1/865781/1/86578

cells, which are both self-replicating and a source of progeny, to become nonreplicative mature cells that circulate in the peripheral blood.⁴ HGFs regulate hematopoiesis through interactions with specific receptors on primitive stem cells by stimulating their proliferation and differentiation toward committed lineages. Currently, the pluripotent CD34⁺ stem cell represents the most primitive hematopoietic progenitor identifiable by immunotyping. These cells represent approximately 0.5% to 2% of the mononuclear cells in the bone marrow and less than 0.1% of the mononuclear cells in the peripheral blood.⁵

At present, HGFs have been subdivided into three groups on the basis of the maturity of the hematopoietic progenitors upon which they act. Early acting cytokines include stem cell factor (SCF), the flk-2/flt-3 ligand, and IL-6. IL-3, IL-4, and granulocyte-macrophage colony-stimulating factor (GM-CSF) are considered intermediate acting agents; IL-5, erythropoietin, thrombopoietin, and granulocyte colony-stimulating factor (G-CSF) represent late acting growth factors.⁶ G-CSF is unique in that it also has some effects on very early progenitors.

In addition to stimulating the proliferation and maturation of bone marrow progenitors, HGFs also may regulate the functional activation of mature myeloid elements. Such effects can augment the effector function or functions of these cells, such as priming of the oxidative response and enhanced phagocytosis.

The major endogenous sources of HGFs include fibroblasts, endothelial cells, lymphocytes, monocytes, and macrophages.⁴

PREPARATIONS AND BIOLOGIC PROPERTIES OF HGFs

All of the HGFs available for clinical use as therapeutic agents have been produced by using recombinant DNA technology. When a bacterial expression system (usually *Escherichia coli*) is used to manufacture these products, the resulting human protein contains an additional amino acid (N-terminal methionine) that is not present in the native form of the HGF. In addition, *E. coli*-derived HGFs are not glycosylated. Because endogenous human HGFs are glycoproteins, other manufacturers have utilized yeast or mammalian cell-expression systems to generate a glycosylated product.

Although the available data are not conclusive, whether the HGF is glycosylated may have important therapeutic implications. In addition to possible differences in pharmacokinetics, glycosylation results in a higher molecular weight with a potential loss of specific activity. Because these drugs are dosed on a microgram per kilogram or microgram per square meter basis, different preparations may not have equivalent bioactivity. Finally, several *in vitro* studies have suggested that glycosylated HGFs may have less potency than the same nonglycosylated cytokine.⁷ In contrast, more recent studies have raised the possibility that the glycosylated forms of HGFs might have greater clinical efficacy *in vivo*. There is one report

showing glycosylated GM-CSF to have a longer serum half-life, greater neutrophil-stimulating activity, less leukotriene production, and fewer side effects than a nonglycosylated GM-CSF preparation.⁸ In a separate study, glycosylated G-CSF was found to produce superior mobilization of peripheral blood progenitor cells (PBPCs) compared with an identical dose of nonglycosylated G-CSF.⁹

G-CSF

To date, filgrastim is the only commercially available preparation of G-CSF available in the United States. It is an *E. coli*-derived recombinant protein, and it is nonglycosylated. Lenograstim is a glycosylated form of G-CSF produced in Chinese hamster ovary cell lines that has been studied primarily in other countries.

In adults the recommended dose of filgrastim is 5 to 10 $\mu\text{g/kg/day}$ (approximately 200 to 400 $\mu\text{g/m}^2/\text{day}$) administered subcutaneously or intravenously.¹ The dose is usually rounded to the nearest vial size to enhance patient convenience and reduce costs.

The presumed target cells for G-CSF include a late precursor committed to the neutrophil lineage and the mature neutrophil. G-CSF is a lineage-specific growth factor, the principal biologic effect of which is to increase the proliferation and differentiation of neutrophils from committed progenitors.⁴ G-CSF also potentiates the survival and function of mature neutrophils, including increasing phagocytic activity, antimicrobial killing, and antibody-dependent cell-mediated cytotoxicity.¹⁰ G-CSF was approved for clinical use to reduce the incidence of febrile neutropenia.¹¹

GM-CSF

Sargramostim was the first preparation of GM-CSF to receive Food and Drug Administration (FDA) approval. It is a yeast-derived (*Saccharomyces cerevisiae*) recombinant protein that is glycosylated. Molgramostim, which is still in clinical development in the United States, is *E. coli*-derived and nonglycosylated. Other forms of GM-CSF not yet commercially available include regmogramostim (Chinese hamster ovary cell-derived, glycosylated) and ecogramostim (*E. coli*-derived, nonglycosylated).

In adults, the recommended dose of sargramostim is 250 $\mu\text{g/m}^2/\text{day}$ (approximately 5 $\mu\text{g/kg/day}$) administered subcutaneously or intravenously, with the dose rounded to the nearest vial size.¹

GM-CSF has multilineage stimulatory effects extending from immature hematopoietic progenitors to differentiated lineage-restricted neutrophils, monocytes, and eosinophils. Although its predominant effect *in vivo* is to increase the production of mature neutrophils and macrophages,⁴ when used at doses of 3 $\mu\text{g/kg/day}$ for 10 days peripheral eosinophilia was a major component of the leukocytosis (5% compared with 1% before treatment).⁷ At higher doses (20 $\mu\text{g/kg/d}$), an 18-fold increase in circulating eosinophils was observed. There were no consistent effects on basophil levels.⁷ GM-CSF also enhances the function of mature macrophages and neu-

trophils, including the ability to increase microbial killing through increased phagocytosis and superoxide production.⁴

GM-CSF was approved for clinical use to accelerate myeloid recovery in patients with lymphoid malignancies undergoing autologous bone marrow transplant (BMT).¹¹

SCF

Recombinant methionyl human SCF is a noncovalent, dimeric protein produced in *E. Coli* (nonglycosylated). Human clinical trials with SCF began in 1991, and the Food and Drug Administration is currently in the process of considering its approval for noninvestigational clinical use. Definitive dosing recommendations have not yet been established, but clinical trials have primarily used 5 to 20 $\mu\text{g/kg/day}$.

The anticipated clinical usefulness of SCF is based on its unique ability to synergize with other lineage-specific hematopoietic growth factors to stimulate primitive, undifferentiated blast cells. In vitro studies of colony-stimulating activity with SCF in combination with other HGFs (e.g., G-CSF, GM-CSF, IL-3, and erythropoietin) have demonstrated an increased number of colonies, as well as an increased number of cells per colony when compared with that produced by each of the growth factors alone.⁵ In adult humans, SCF is present both as a circulating serum protein and in a membrane-bound form. It is now believed that the membrane-bound form of SCF is the form most important in hematopoiesis. Because membrane-bound SCF is produced by and present on bone marrow stromal cells, it functions within the marrow microenvironment to support the proliferation and survival of progenitor cells. Here it may also synergize with other HGFs to induce lineage commitment.¹²

In addition to hematopoietic progenitor cells, the receptor for SCF is expressed on various cell types, including mast cells, melanocytes, neurons, and germ cells.⁵ In vitro studies have shown that SCF can promote the development of mast cells from human hematopoietic progenitor cells.¹³ Recent clinical trials have confirmed the ability of SCF to produce mast cell hyperplasia in vivo in humans. In a Phase I study of SCF given subcutaneously to patients with advanced breast carcinoma (5 to 50 $\mu\text{g/kg/day}$ for 14 days), a significant 60% increase in the numbers of skin mast cells was observed at cutaneous sites that had not been directly injected with the drug.¹⁴ In addition, the patients exhibited increased urinary levels of the major histamine metabolite methyl-histamine and markedly increased serum levels of mast cell α -tryptase as detected by an assay that can measure both the α and β forms of this protease.¹⁴ In this regard, it is noteworthy that SCF can induce direct mediator release in vitro from rat or mouse peritoneal mast cells and from human skin mast cells.¹³ At even lower concentrations in vitro, SCF can augment IgE-dependent activation of human lung or skin mast cells.¹³

CLINICAL APPLICATIONS AND EFFICACY OF HGFs

Primary administration

Neutropenia and infection are the major dose-limiting side effects of chemotherapy. The primary administration of HGFs is defined as use of an HGF in the first cycle of treatment before any occurrence of neutropenia or febrile neutropenia (temperature = 38.5° C or greater; absolute neutrophil count < $0.5 \times 10^9/\text{L}$) that may result from chemotherapy.¹ Primary administration of HGFs is recommended for patients receiving myelosuppressive chemotherapy regimens associated with a greater than 40% expected incidence of febrile neutropenia.¹

Several prospective, randomized, placebo-controlled clinical trials have demonstrated that G-CSF significantly reduced the incidence of febrile neutropenia in treatment regimens in which 40% or more of the placebo population developed febrile neutropenia.^{1, 10} Randomized trials of GM-CSF have yielded similar benefits, although the results have been less consistent. These positive effects of G-CSF and GM-CSF were not associated with any improvements in infectious mortality, treatment response rates, or survival rates.

Interestingly, available data do not clearly support the use of HGFs in conjunction with antibiotics for treatment of afebrile or febrile patients with neutropenia.¹¹ Although some studies of HGFs used in this clinical setting have shown benefit in some intermediate endpoints (e.g., median number of days of neutropenia) or time to resolution of febrile neutropenia, a corresponding improvement in other more clinically relevant endpoints (e.g., days of fever, use of antibiotics, and median number of days of hospitalization) has not been documented.^{15, 16}

Primary administration of HGFs is also recommended for patients receiving less myelosuppressive chemotherapy regimens but who have risk factors that might increase the likelihood of developing febrile neutropenia or infection. These risk factors include bone marrow compromise from disease involvement, prior chemotherapy or radiation therapy, active tissue infection, or a history of febrile neutropenia with previous courses of chemotherapy.¹¹

Secondary administration

Secondary administration is defined as use of an HGF to protect against new episodes of febrile neutropenia or chemotherapy dose modifications in subsequent cycles of treatment in a patient who has experienced one of these complications in an earlier course of that chemotherapy.¹ The use of HGFs may also be considered if prolonged neutropenia is causing excessive dose reduction or delay in chemotherapy. In one randomized trial of G-CSF after chemotherapy, patients who had developed febrile neutropenia were crossed over to receive open-label G-CSF in subsequent cycles. These patients had both a shorter duration of neutropenia and a 77% reduction in the rate of fever with neutropenia.¹⁰

HGFs as adjuncts to progenitor cell transplantation

Historically, autologous BMT has been used to rescue patients from lethal marrow injury after high-dose myeloablative chemotherapeutic regimens. Unfortunately, the 2 to 4 weeks of pancytopenia that predictably accompany these myeloablative chemotherapies result in major complications, including infection, the need for RBC and platelet transfusions, delayed or incomplete engraftment, and a prolonged and expensive hospitalization. Numerous clinical trials have provided sufficient data to firmly establish that HGFs can shorten the neutropenic period and reduce infectious complications after autologous BMT.¹ In addition, these studies also suggest beneficial effects of HGFs on other end points such as duration of fever, antibiotic use, hospitalization, and therapeutic costs.¹ No improvement in response rate or survival has been observed. Both G-CSF and GM-CSF have proven efficacious in this setting, and at the present time there is insufficient evidence to favor one agent over the other. Although there are less supporting data available, HGFs appear to provide similar beneficial effects in patients receiving allogeneic BMTs. There has been no evident increase in complications of graft-versus-host disease, graft rejection, or relapse associated with the use of HGFs in randomized studies of autologous BMTs.¹ It should be noted that the beneficial effects of HGFs in BMTs do not result from earlier engraftment but rather from accelerated myelopoiesis once the engraftment is initially established.¹⁶

In recent years, transplantation of PBPCs has been widely used as an alternative approach to autologous BMTs. PBPC mobilization for harvesting for transplant can be augmented in several ways, including use of chemotherapy, HGFs alone or in combination, or both. In contrast to autologous BMT, PBPCs are easily collected by leukapheresis without the need for anesthesia or surgery. A number of studies have demonstrated a several-fold increase in the number of hematopoietic progenitors that can be collected by leukapheresis when HGFs are used to enhance mobilization of PBPCs.¹ Additional data suggest that reinfusion of HGF-mobilized PBPCs after high-dose chemotherapy results in more rapid neutrophil and platelet recovery, faster overall recovery, and less clinical toxicity (e.g., transfusions, mucositis, and antibiotic use).¹ Although initial studies of PBPC mobilization and reinfusion used the patient as the source of the PBPCs, recent reports indicate that excellent yields of CD34⁺ stem cells can be obtained from normal healthy donors given HGF for 4 to 5 days.^{2,3} The ability to collect adequate numbers of CD34⁺ cells appears critical to the success of the subsequent transplant because clinical studies have shown that it is the CD34⁺ cells that have the capability of long-term engraftment.⁵

Early clinical results suggest that use of SCF in combination with another cytokine (i.e., G-CSF) may significantly increase the yield of hematopoietic progenitors as compared with PBPC mobilization with HGF

alone. One recent study showed a dose response to SCF used for the mobilization of long-term culture-initiating cells, with a 5.8-fold increase in long-term culture-initiating cell mobilization in those patients receiving chemotherapy, G-CSF, and SCF compared with patients receiving chemotherapy and G-CSF alone.¹⁷ A similar but separate study found that in patients who had received extensive prior therapy with stem-cell toxic chemotherapeutic agents, the use of SCF plus G-CSF mobilized higher numbers of CD34⁺ cells and resulted in a decreased time to an untransfused platelet count of $20 \times 10^9/L$ after transfusion.¹⁸ Phase III multicenter clinical trials are underway to document the effect of SCF in decreasing the time and number of leukapheresis procedures needed to obtain an adequate yield of PBPCs for transplantation.¹² Very preliminary results have shown that patients receiving the combination of SCF plus G-CSF had higher CD34⁺ cell yields sustained throughout the period of leukapheresis compared with those receiving G-CSF alone.¹⁹ This was associated with a statistically significant reduction in the number of leukapheresis procedures required to reach a target yield of CD34⁺ cells. Thus the use of SCF in combination with other HGFs may result in more successful and less costly methods for PBPC mobilization.

HGF-ASSOCIATED TOXICITY G-CSF

Medullary bone pain is the most frequent side effect observed in patients receiving G-CSF, with 15% to 39% of recipients complaining of this symptom.¹ Other adverse effects have included exacerbation of preexisting inflammatory disorders such as eczema, psoriasis, or vasculitis.¹ Rashes and injection site reactions are rarely seen. Increased serum levels of alkaline phosphatase and lactate dehydrogenase are not uncommon and are thought to reflect the release of enzymes from high numbers of leukocytes rather than liver or muscle toxicity.² To date, the development of anti-G-CSF antibodies has not been reported.¹ There have been one or two reports of anaphylactic-like reactions that appeared to be temporally associated with G-CSF administration, but causality could not be established.²⁰

In a series of four patients referred to the Allergy Clinic at the Beth Israel Deaconess Medical Center for evaluation of suspected anaphylactic-like reactions to G-CSF (or GM-CSF), we found it possible to perform both skin prick and intradermal skin tests using the undiluted growth factor without occurrence of an irritant skin response (J. Costa, unpublished results, 1997). Three of four patients who did not show any cutaneous skin test reactivity went on to receive cytokine therapy without untoward effects. For these patients, the need to eliminate an IgE-mediated hypersensitivity to G-CSF or GM-CSF was essential because the therapeutic options available for the treatment of their malignancy would have been otherwise unnecessarily restricted. One of four patients developed a clear-cut wheal and flare response within 10 minutes after intradermal skin testing

with a 1:100 dilution of growth factor, and she did not receive any further cytokine therapy.

GM-CSF

The most common adverse effects observed in patients receiving GM-CSF include fever, nausea, fatigue, headache, bone pain, chills, myalgias, and injection site reactions.¹ Route of administration may be an important variable in the incidence of side effects, with intravenous dosing more frequently accompanied by generalized rash and *first dose reactions*, whereas subcutaneous administration has been associated with local reactions at injection sites.⁷ A so-called first dose reaction has been observed in occasional patients. It is characterized by transient flushing, dyspnea, transient hypoxia, tachycardia, and hypotension.^{1,7} This adverse event appears to occur more frequently with the *E. coli*-derived, nonglycosylated form of GM-CSF than the yeast-derived product.

Antibodies to GM-CSF have been identified in approximately 4% of those tested.¹ Although the full clinical significance of such antibodies is uncertain, there is one report of several patients who developed antibodies directed against epitopes on the native protein backbone. In the endogenous form of GM-CSF found in vivo, these sites are normally protected by O-linked glycosylation. However, in the recombinant forms of GM-CSF produced in yeast and *E. coli*, they are exposed and thus available to act as novel antigens.²¹ Some of these patients with antibodies to GM-CSF had lower serum levels of GM-CSF on day 7 or 8 of their infusion, as compared with steady-state levels measured on the first day of the infusion. However, no evidence was found in marrow culture assays to suggest that the antibodies had a neutralizing effect.²¹

SCF

Virtually all patients who have been given SCF have a pruritic wheal and flare response at the injection site approximately 90 to 120 minutes after subcutaneous injection.¹⁴ Examination of these injection site reactions by transmission electron microscopy has revealed evidence of extensive, anaphylactic-type degranulation of dermal mast cells.¹⁴ These data support the in vitro observations of SCF-induced degranulation and suggest that in vivo administration of SCF results in a localized area of cutaneous mast cell activation and mediator release. In addition, 13 of 56 patients in Phase I trials exhibited moderate to severe anaphylactic-like reactions, which included respiratory symptoms (e.g., cough, sore throat, throat tightness, and/or dyspnea) with or without generalized urticaria.¹⁸ All of these reactions were transient and reversible. These reactions are believed to be mast cell-mediated and dose related, occurring primarily in those patients who received more than 25 µg/kg/day of SCF. These observations suggest that it will not be possible to use skin tests in the evaluation of suspected SCF-induced adverse events.

Practitioners also should be aware that as a result of these early clinical toxicities, the subsequent SCF clinical

trials have specifically avoided enrolling patients with asthma or other significant IgE-mediated hypersensitivity disorders and have required participants to use a standard prophylactic medication regimen consisting of both H1 and H2 antihistamines and inhaled albuterol.⁵ These drugs are taken for the duration of SCF therapy. With these modifications, SCF-induced adverse events have been less frequently observed in Phase II and III clinical trials, although the potential toxicity in the absence of prophylactic medications or in atopic individuals is unknown at this time.

SUMMARY

Biotechnology has provided today's physician with an ever expanding arsenal of potent immunopharmaceuticals, aptly termed *biologic response modifiers*. Molecular biology and cloning technology have created the opportunity for modern medicine to exploit the body's own natural regulatory mechanisms and now enable us to manipulate fundamental aspects of homeostasis. Thus although hematopoietic growth factors may have been the logical first choice for the clinical application of cytokine-based therapeutics, the extension of such approaches to other vital aspects of human biology that are controlled by cytokines (e.g., host defense, response to injury, inflammation, and immune regulation) is not far off. Indeed, many such novel therapeutics designed to take advantage of cytokine-mediated processes are already in the pharmaceutical pipeline.

Not only can we anticipate an increase in the quantity and variety of cytokine-based therapeutic agents, but as clinical experience with these drugs grows, so will the list of clinical settings in which their beneficial effects can be appreciated. Already the use of HGFs has expanded beyond the realm of cancer therapy. In a very recent randomized, placebo-controlled trial of G-CSF in cases of diabetic foot infection, G-CSF therapy was shown to be associated with earlier eradication of pathogens from the infected ulcer, quicker resolution of cellulitis, shorter hospital stay, and shorter duration of intravenous antibiotic treatment.²² The allergy/immunology specialist should be prepared to assist in the evaluation and management of patients receiving cytokine-based therapeutic agents, as well as to help explore potential new clinical applications of these drugs in the treatment of allergic diseases.

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