The potential role of recombinant hematopoietic colony-stimulating factors in preventing infections in the immunocompromised host

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J RUSTHOVEN. The potential role of recombinant hematopoietic colony-stimulating factors in preventing infections in the immunocompromised host. Can J Infect Dis 1991;2(2):74-88. Hematopoietic colony-stimulating factors coordinate the proliferation and maturation of bone marrow and peripheral blood cells during normal hematopoiesis. Most of these factors are now available as recombinant human colony-stimulating factors, and preclinical and clinical testing is proceeding rapidly. Granulocyte and granulocyte/macrophage colony-stimulating factors have been the most extensively studied to date. In human clinical trials, granulocyte colony-stimulating factor improves neutrophil counts and function, reduces episodes of febrile neutropenia, improves neutrophil recovery after disease- or treatment-induced myelosuppression, and reduces the number of serious infections in several neutropenic disease states. Granulocyte/macrophage colony-stimulating factor has similar biological properties but may also improve eosinophil proliferation and function, and platelet cell recovery after myelotoxic bone marrow injury. Interleukin-1 boosts the effects of granulocyte colony-stimulating factor and granulocyte/macrophage colony-stimulating factor, but also may promote the resolution of established infections in conjunction with antibiotics. The therapeutic realities and future therapeutic implications of these agents for the therapy of infections, cancer and hemopoietic disorders are discussed.

Key Words: Colony-stimulating factors, Febrile neutropenia, Hematopoiesis, Neutropenia

Rôle potentiel des facteurs recombinants stimulant les colonies hématopoïétiques dans la prévention des infections chez l'hôte immunodéprimé

RESUME: Les facteurs stimulant les colonies hématopoïétiques coordonnent la prolifération et la maturation des cellules de la moelle osseuse et du sang périphérique au cours de l'hématopoïèse normale. La plupart de ces facteurs connus sous le nom de "facteurs stimulant les colonies" font actuellement l'objet d'évaluations précliniques et cliniques qui progressent

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rapidement. Les facteurs stimulant les colonies de granulocytes et de granulocytes macrophages ont été les plus étudiés jusqu'à présent. Au cours des études chez l'homme, le facteur stimulant les colonies de granulocytes augmente le taux et la fonction des polynucléaires neutrophiles, réduit les épisodes de neutropénie fébrile, accélère le retour à la normale des polynucléaires abaissés par la maladie ou une thérapie myélosuppressive, et réduit le nombre des infections graves dans plusieurs affections qui s'accompagnent de neutropénie. Le facteur stimulant les colonies de granulocytes macrophages a des propriétés biologiques similaires mais il pourrait aussi améliorer la prolifération et la fonction des éosinophiles et le retour à la normale des plaquettes après des lésions myélotoxiques de la moelle épinière. L'interleukine-1 majore les effets du facteur stimulant les colonies de granulocytes et ceux du facteur stimulant les colonies de granulocytes macrophages, mais il pourrait aussi promouvoir la résolution des infections établies de concert avec les antibiotiques. Les réalités thérapeutiques et les implications thérapeutiques futures de ces agents dans le traitement des infections, du cancer et des troubles hémopoïétiques sont examinées.

THE 1990s PROMISE TO BE EXCITING YEARS IN THE clinical development of recombinant biological products. The medical disciplines on which these agents will likely have the greatest impact are hematology, oncology and infectious diseases. The clinical questions which are already being addressed through early clinical trials include those affecting the immunocompromised host. Of these promising new agents, the hematopoietic colony-stimulating factors (CSFs) have thus far been the most widely tested in this clinical setting and will be the subject of this review. Reference will be made to Canadian strategies and clinical trials for the development of these agents.

REGULATION OF NORMAL HEMATOPOIESIS BY CSFs

Under normal conditions in the human bone marrow, the CSFs regulate the expansion of a given hematopoietic cell lineage from the stem cell to the functionally mature peripheral blood cell (1). While some CSFs function primarily by stimulating the clonal proliferation of uncommitted progenitors (eg, interleukin-3), others induce both proliferation and differentiation along a functionally committed pathway (eg, granulocyte-CSF [G-CSF] and erythropoietin). Furthermore, CSFs interact to induce synergistic hematopoietic responses, as well as to down-regulate each other's cell surface receptors (2,3).

The term 'colony-stimulating factor' originated from early in vitro studies. Assays were developed which allowed for controlled growth of cell colonies derived from normal bone marrow. Each colony evolved from a single cell known as a 'colony-forming unit' (CFU). Different constituents or factors in the media were found to promote the growth of different colony types (4,5). Thus, by manipulating these factors, different assays were developed whereby lineage-specific colonies could be studied. For example, the CFU-granulocyte/macrophage assay promotes the growth of CFUs

committed to granulocyte/macrophage maturation and function. In the CFU-mega assay, CFUs proliferate to form megakaryocyte colonies. Through painstaking purification procedures, each factor necessary to produce distinct types of colonies was identified in the 1970s. One of the last assays to be developed was that which detected a cell which could differentiate into erythrocytes, granulocytes, monocytes and megakaryocytes. However, this cell (the CFU-multiprogenitor cell) has minimal potential for self renewal. While the factors responsible for CFU proliferation can mimic hematopoiesis in vitro, the maintenance of prolonged hematopoiesis in vitro requires the addition of a stromal layer which produces yet other factors (4).

In addition to their effects on hematopoiesis, some CSFs have a broad range of immune functions. For example, granulocyte/macrophage-CSF (GM-CSF) induces the production of interleukin-1 and tumour necrosis factor (TNF) by peripheral blood mononuclear cells, and through the latter may stimulate monocyte cytotoxicity against tumour targets (6). GM-CSF also augments the monocyte/macrophage-mediated inhibition of intracellular *Trypanosoma cruzi* replication (7), and stimulates the killing of *Leishmania tropica* within murine macrophages (8).

While diversified in their effects on hematopoiesis, human CSFs have some striking similarities. All are glycoproteins, with the carbohydrate portion having little or no effect on biological function. The genes for CSFs are found close either to other CSFs or to other genes related to neutrophil function. Interleukin-3, GM-CSF, macrophage-CSF (M-CSF) and the receptor for M-CSF all lie on a restricted portion of chromosome 5. Deletion of this region is associated with the myelopoietic disorder known as the 5q-deletion syndrome (9). G-CSF, on the other hand, lies near the gene for myeloperoxidase located on chromosome 17 (10). Finally, all CSFs are produced from a variety of

TABLE 1
Characteristics of human colony-stimulating factors (CSFs)

Factor	Targets of action	Gene locus	Biological activities	References
G-CSF	Neutrophil	17q	Stimulates growth, maturation and activation of neutrophil colonies and their cells in vitro Augments neutrophil-mediated cytotoxicity Induces differentiation of leukemia cell lines in vitro Stimulates leukemic progenitor proliferation Supports target cell survival	1,10,18, 27-30, 35-39
GM-CSF	Neutrophil Eosinophil Macrophage Basophil T lymphocyte Endothelial cell Keratinocyte	5q	Stimulates growth of neutrophilic, eosinophilic, monocytic, erythrocytic and megakaryocytic precursor cells Activates mature neutrophils, eosinophils and monocytes Augments neutrophil- and eosinophil-mediated cytotoxicity against microorganisms and/or tumour cells May stimulate leukemic progenitor proliferation Supports hematopoietic target cell survival	7-9, 17, 20-27, 31, 33, 34
M-CSF	Macrophage	5q	Supports monocyte proliferation, maturation and survival in vitro Augments monocyte-mediated anti-tumour cytotoxicity Supports target cell survival	1, 2
IL-3	Early progenitors	5q	Stimulates growth of early progenitors Supports basophil (mast cell) growth Induces proliferation of nonlymphocytic leukemic blasts	15, 16
IL-1	Early hematopoietic progenitors also responsible to other CSFs	2q	Sensitizes early progenitors to effects of other CSFs Induces CSF release by accessory cells Improves survival during lethal Gram-negative infection in mice	22, 46, 47
Erythropoietin	Erythroid precursor cells	7q	Induces hemoglobin formation Stimulates committed erythroid progenitor proliferation	13, 14

G-CSF Granulocyte-CSF; GM-CSF Granulocyte/macrophage-CSF; IL Interleukin; M-CSF Macrophage-CSF

extramedullary sources. Some are produced from lymphocytes and are therefore true lymphokines or interleukins (eg, GM-CSF and multi-CSF or interleukin-3), while others are produced by fibroblasts and epithelial cells (G-CSF, M-CSF, GM-CSF and interleukin-6) or liver cells (erythropoietin), to name a few (11,12). It is also clear that some CSFs may also have nonhematopoietic targets, as illustrated by the interaction of GM-CSF with its receptor on keratinocytes and endothelial cells. Table 1 summarizes the distinguishing characteristics of six natural CSFs for which recombinant counterparts are now available.

HUMAN CSFs

To date, most human clinical trials of recombinant human CSFs have focused on their potential application to the treatment of congenital neutropenias, cancer and hematopoietic disorders. Recombinant erythropoietin has been shown to be effective in reducing anemia of renal insufficiency, and may become useful in cancer patients whose natural erythropoietin is reduced (13,14). A gene regulating megakaryocytopoiesis has been identified, and a recombinant product will likely be available soon for testing. However, the four recombinant CSFs which will have the greatest impact on preventing neutropenia and infection

when myelopoiesis is abnormal or disrupted are G-CSF, GM-CSF, interleukin-1 and interleukin-3 (M-CSF may have restricted usefulness for reducing specific infections or boosting anti-tumour responses, but only very preliminary clinical testing has occurred to date).

These CSFs are distinct in ways that may be conducive to sequential administration in the future. Interleukin-3 stimulates the clonal proliferation of very early, multipotent progenitors in normal bone marrow cell populations as well as in those previously treated with chemotherapy (15,16). GM-CSF targets later progenitors for proliferation and promotes neutrophil and macrophage differentiation and maturation (17). G-CSF acts exclusively on cells committed to the neutrophilic granulocytic lineage to promote neutrophil proliferation and terminal maturation (18). All three CSFs also enhance target cell survival by suppressing apoptosis, the normal physiological process by which cells actively self-destruct and die (19). Different sequential combinations of these CSFs have resulted in additive or synergistic increases in proliferative and maturational capabilities, suggesting future schedules which may allow for reduced side effects and cost of individual CSFs while maintaining optimal clinical efficacy. Table 2 lists the types of conditions for which these CSFs have been tested thus far. Recombi-

TABLE 2
Clinical response to granulocyte colony-stimulating factor (G-CSF) and granulocyte/macrophage colony-stimulating factor (GM-CSF) in humans

Disease state	rhuCSFs	Results	References
Congenital or acquired severe neutropenia			
Kostmann's syndrome	G-CSF	Improved absolute neutrophil count Accelerated improvement in infection recovery	58, 59
	GM-CSF	Variable improvement in absolute neutrophil count Augmentation of eosinophilic response Healing of infections	60, 61
Idiopathic	GM-CSF	Improvement in absolute neutrophil count	61
Cyclic (collie dogs)	G-CSF	Persistent neutrophilia maintained	62
	GM-CSF	No effect	62
Disease-induced neutropenia			
Hairy cell leukemia	G-CSF	Improvement in disease- and treatment-induced neutropenia	66
Acquired immune deficiency syndrome	GM-CSF	Improvement in disease-induced neutropenia	67
Myelodysplastic syndromes	G-CSF	Enhancement of bone marrow myeloid maturation Augmented neutrophil function	63
	GM-CSF	Improved but unsustained neutrophil counts Multiorgan neutrophilic infiltration (one patient)	64, 65
Treatment-induced neutropenia			
Previously treated cancer patients	G-CSF	Dose-dependent rise in absolute neutrophil count Preservation of neutrophil function	68
	G-CSF	Improved absolute neutrophil count nadir; reduced febrile neutropenia, antibiotic requirements and hospitalization Reduced incidence mucositis	71-73
Cancer patients receiving chemotherapy	GM-CSF	Improved absolute neutrophil count nadir; reduced febrile neutropenia, reduced neutropenic days and fewer infectious complications	77-79
	G-CSF	Fewer febrile days, fewer neutropenic days, fewer days on antibiotics and fewer infections	74, 75
Autologous bone marrow transplantation	G-CSF	As with G-CSF but also more rapid platelet recovery Improved graft survival	80-83

rhuCSFs Recombinant human colony-stimulating factors

nant human GM-CSF and recombinant human G-CSF have been the most extensively studied CSFs and their application to reducing the risk of infection in various clinical situations will be discussed in detail. Clinical trials using interleukin-1 have not been completed, but the promise of this agent in the control or eradication of infection as illustrated in animals will be highlighted. The potential role of other CSFs or interleukins through their interactions with recombinant human GM-CSF and recombinant human G-CSF will also be reviewed (eg, interleukin-1). Early clinical trials of recombinant human interleukin-3 have just begun and data regarding its effect on clinical outcomes such as infection are not yet available.

DIFFERENTIAL EFFECTS OF G-CSF AND GM-CSF ON NEUTROPHIL FUNCTION

Both recombinant human G-CSF and recombinant human GM-CSF augment most aspects of neutrophil function. While neither agent can trig-

ger a respiratory burst in neutrophils under conventional assay conditions, they do prime neutrophils to generate an enhanced burst in response to formylated peptides such as *n*-formylmethionyl-leucylphenylalanine (FMLP) (20,21). Since they do not prime or trigger the respiratory burst of monocytes and macrophages which can endogenously secrete both agents, it appears that macrophages could provoke bursts in neutrophils in vivo (22,23). Whether or not these CSFs enhance the burst initiated by contact with infecting organisms is not yet known. Other common effects on neutrophils include the modulation of FMLP and immunoglobulin A receptors, an increase in the adhesion properties of neutrophils, enhancement of phagocytosis, and an augmentation of neutrophil-mediated cytotoxic activity against some tumour cell lines and cells infected with human immunodeficiency virus (24-27). While recombinant human G-CSF is a chemotactic signal for neutrophils, the data on recombinant human GM-CSF is conflicting, with one assay showing migration inhibition and the other showing chemotaxis (28). One study also reported the inhibition of

neutrophil migration during continuous intravenous infusion of recombinant human GM-CSF after autologous bone marrow transplantation (29). Recombinant human G-CSF stimulates the secretion of alpha-interferon from mature neutrophils. Since the latter is a suppressant of neutropoiesis, it may play a role in mediating negative feedback following neutrophil stimulation by recombinant human G-CSF (30). Besides restoring the defect in superoxide release of neutrophils inherent in myelodysplastic syndromes, recombinant human G-CSF can enhance the FMLP-stimulated superoxide release of neutrophils generated in vivo during recovery after cytotoxic chemotherapy. Similarly, recombinant human GM-CSF can enhance superoxide production in patients with refractory cancer (31). Thus, these CSFs appear qualitatively and quantitatively to support neutrophil recovery after myelosuppressive therapy.

Eosinophils are also stimulated by recombinant human GM-CSF, the effect of which may exceed that seen for granulocyte neutrophils. Functional augmentation occurs for superoxide production, protein synthesis, antibody-dependent cytotoxicity against tumour cells, and adherence to and killing of *Schistosoma mansoni* (32,33). These effects could be exploited to boost host defences against this and other parasitic diseases.

STUDIES IN ANIMALS

Recombinant human G-CSF: Several murine models have been established for the study of G-CSF. In response to early concerns that exogenously administered CSFs could lead to leukemic transformation or the development of myelodysplastic disorders, Chang et al (34) transplanted murine bone marrow cells, which were transfected with a G-CSF cDNA-bearing retroviral vector, into lethally irradiated donors. These mice produced very high levels of G-CSF, with marked neutrophilic granulocytosis in hematopoietic tissues and granulocytic infiltration of the liver and lung. However, no tumours occurred despite chronic G-CSF secretion, suggesting that chronic G-CSF administration does not lead to neoplastic transformation of normal hematopoietic cells. Cheers et al (35) studied two strains of mice infected with *Listeria monocytogenes*, one genetically resistant (C57 BL/10) and one susceptible (BALB/cJ) to *L monocytogenes* infection. They correlated the release of serum CSFs with the cause of infection and the colony-forming cell response in the bone marrow to infection (previous studies suggested that higher colony-forming cell levels in the marrow predicted for better survival from listeria infection). As predicted, colony-forming cell levels were

lower in the sensitive strain, but serum M-CSF and G-CSF levels were higher in this strain. This suggested a genetically determined resistance of colony-forming cells to respond to elevated levels of these CSFs and thus may contribute to the inherent sensitivity of this strain to listeria infection.

Matsumoto et al (36) infected mice intraperitoneally with *Pseudomonas aeruginosa*, *Serratia marcescens*, *Escherichia coli*, *Staphylococcus aureus* or *Candida albicans* four days after receiving 200 mg/kg of cyclophosphamide intraperitoneally. This routinely resulted in severe neutropenia and death from infection within four days. When human G-CSF was administered as a daily subcutaneous injection for four days beginning the day after infection, mice were protected against neutropenia and infection in a dose-dependent manner. At the 1.0 or 2.5 µg doses, complete protection from infection was achieved for all species except *E coli*. These clinical findings were associated with the generation of highly active oxygen radicals in peritoneal exudate cells compared to controls. The human G-CSF itself did not inhibit bacterial growth in vitro. Recently, Welte et al (37) developed a primate model of cyclophosphamide-induced neutropenia. As in the murine model, recombinant human G-CSF prevented the development of severe and prolonged neutropenia compared to controls.

In a recent study by Fushiki et al (38), recombinant human G-CSF protected mice against whole body irradiation-induced neutropenia after they received 0.5 or 1.0 Gray by a single dose, or 0.5 Gray for five consecutive days. Neutrophil nadirs were improved and neutrophil recovery was facilitated. Collectively, these data suggested that: clinical states may exist in which inherent resistance to G-CSF results in an increased susceptibility to infection; chronic administration of recombinant human G-CSF is not associated with malignant transformation; and recombinant human G-CSF may be useful in overcoming chemotherapy- or radiotherapy-induced neutropenia, and may result in increased protection against common pathogens. The myeloprotective effects may be synergistically augmented when interleukin-1 is given with G-CSF. Interleukin-1 induces endogenous CSFs, may sensitize progenitors to stimulation by G-CSF, and protects progenitors from the effects of radiation (3).

Recombinant human GM-CSF: Chronic recombinant human GM-CSF stimulation in a transgenic mouse model resulted in multiorgan infiltration of not only neutrophils but also eosinophils and macrophages (39,40). Chronic activation of these inflammatory cells was more devastating than the increased hemopoietic production, resulting in

retinal blindness and a wasting syndrome which reduced survival. Donahue et al (41) and Mayers et al (42) showed that by continuous intravenous infusion or subcutaneous injection, recombinant human GM-CSF produced marked (three- to six-fold) increases in circulating granulocytes, eosinophils, monocytes and lymphocytes, but not in platelets or erythrocytes in primates. Neutrophil function was also enhanced. In autologous bone marrow transplantation models of mice and primates, recombinant human GM-CSF significantly shortened the period of neutropenia and thrombocytopenia compared to controls (43,44). These studies led quickly to human trials of recombinant human G-CSF and recombinant human GM-CSF in various immunocompromised states.

Recombinant human interleukin-1: Recombinant human interleukin-1 has been primarily investigated in combination with other CSFs. This is due to its ability to induce the release of other CSFs (such as G-CSF) by other cells and to sensitize cells to the effects of other CSFs. However, recent data suggest a potential role for interleukin-1 in favorably altering the outcome of established infections. Encouraged by reports of a protective effect in non-neutropenic mice infected with *Ps aeruginosa* or *Klebsiella pneumoniae*, van der Meer et al (45) infected mice suffering from cyclophosphamide-induced neutropenia with *Ps aeruginosa* by intramuscular injection.

Mice receiving recombinant human interleukin-1 as a single intraperitoneal injection 24 h before infection were compared with mice which did not receive recombinant human interleukin-1. Animals receiving recombinant human interleukin-1 benefited whether or not they also received gentamicin. Survival was significantly improved (98% versus 71% at 24 h; and 61% versus 11% at 48 h; $P < 0.001$) despite no difference in peritoneal macrophage function or the number of bacteria cultured from various organs. While the reason for this striking result is not clear, further study is warranted to understand the potential role of interleukin-1 in anti-infection therapy. In a more recent study, the same group reported that recombinant human interleukin-1 given as a single low dose accelerated neutrophil recovery following cyclophosphamide-induced neutropenia (46). The mechanism of this observation may be the endogenous release of G-CSF and/or GM-CSF by interleukin-1 targets such as endothelial cells or bone marrow stromal cells (3). These studies suggest a future role for interleukin-1 as an adjunct to antibiotics in the treatment and prevention of infections in immunocompromised and noncompromised patients.

HUMAN CLINICAL TRIALS

Treatment schedules and toxicity: Recombinant human G-CSF was originally cloned and expressed through an *E coli* vector in 1985. The biochemical aspects of this agent are reviewed elsewhere (18), and no significant biological difference has been detected compared to the natural product. No significant toxicity has been reported at maximally therapeutic doses. Bone pain is relatively frequent, and prevented or treated with acetaminophen. Low grade fevers, flu-like symptoms, blood pressure changes and liver function test abnormalities have been reported but are infrequent at most doses (less than 40 µg/kg) (47). The neutrophil response is dose-dependent, and subcutaneous bolus, continuous subcutaneous infusion and intravenous infusion appear to be equally effective (48).

Initial phase I testing of recombinant human GM-CSF showed a dose-response for circulating neutrophils at doses ranging from 0.3 to 60 µg/kg. All routes of administration tested were effective (intravenous 30 mins to continuous infusion, subcutaneous bolus, or subcutaneous continuous infusion) except the intravenous bolus route (49). However, dose-limiting toxicity occurred at dosages above 20 µg/kg as hypotension, severe myalgias, pulmonary toxicity or pericarditis (50). Long term, low dosages (0.25 to 4 µg/kg) as a daily subcutaneous bolus for up to six months, resulted in sustained efficacy and mild, mainly cutaneous, toxicity (51). Pharmacokinetic data suggested that a daily subcutaneous injection of 10 µg/kg gave adequate daily serum levels of greater than 1 ng/mL for more than 12 h (52). In a study comparing intravenous and subcutaneous dosing, Lieschke et al (49) recommended that dosages from 3 to 15 µg/kg/day given intravenously or subcutaneously be used in phase II trials. The subcutaneous route was preferable because of a lower incidence of generalized rash, fever and 'first dose reactions'. Such reactions included flushing, tachycardia, hypotension, muscle and bone pain, dyspnea, nausea and vomiting, and/or syncope, and can occur at first injection but not subsequent injections of the same chemotherapy cycle. These reactions have been more frequent in patients with small cell lung cancer (53).

Toxicity of recombinant human GM-CSF is generally greater than that with recombinant human G-CSF at recommended doses. Generalized rash is infrequent, but local rashes at the injection site occur in 40% of patients receiving subcutaneous injection (54). Other common side effects include low grade fever, bone pain and lethargy; the latter can be profound. While prophylactic acetaminophen and antihistamine usually control or prevent

most of these effects, the author has found that a nonsteroidal anti-inflammatory drug (NSAID) at low doses may be required to control severe bone pain, fatigue and fever (55,56).

The lineage-specific nature of these agents and their ability to promote both proliferation and functional maturation of neutrophil progenitors has led to clinical trials involving a diversity of disease states: cyclic and congenital neutropenia; myelodysplastic syndromes; disease-induced neutropenia (hairy-cell leukemia); and therapy-induced neutropenia (chemotherapy and bone marrow transplantation).

Congenital, idiopathic and cyclic neutropenia:

Severe congenital neutropenia (Kostmann's syndrome) is characterized by severe neutropenia due to maturation arrest at the promyelocytic level. Absolute neutrophil counts are less than $0.2 \times 10^9/L$, and patients experience frequent episodes of fever and systemic infection, often resulting in death despite antibiotics during infancy. Welte et al (57) treated five patients with Kostmann's syndrome with daily recombinant human G-CSF or GM-CSF (intravenous). In only one patient treated with GM-CSF did a significant increase in neutrophils occur; the other four patients responded with marked eosinophilia. By contrast, when treated with recombinant human G-CSF, all five patients had an increase in neutrophil counts above 1000/mL. One patient showed resolution of an antibiotic-resistant pulmonary infiltrate caused by peptostreptococcus. Since Welte et al have shown normal or elevated G-CSF levels in these patients, it is likely that late neutrophil precursors have a defective response to physiological levels of G-CSF. Bonilla et al (58) have reported similar results in severe congenital neutrophilia patients also treated with recombinant human G-CSF. Since chronic G-CSF administration has not been linked to the appearance of normal cell transformation or clinical tumours, these patients may be treatable with regular dosing of this agent.

Vadhan-Raj et al (59) recently reported a clinical response to recombinant human GM-CSF in a patient with severe congenital neutrophilia with healing of chronic infected and inflammatory sites. They speculated that the resulting eosinophilic (but not neutrophilic) granulocytosis may have mediated this response. The authors also reported that patients with idiopathic neutropenia responded dramatically to GM-CSF, suggesting a different defect of CSF responsiveness in this disease. Ganster et al (60) reported a good response with normal healing in a patient with severe congenital neutrophilia after either continuous or bolus subcutaneous recombinant human GM-CSF.

This group also reported therapeutic improvement in the absolute neutrophil count of patients with chronic severe neutropenia of varying causes, including previous anticancer therapy, autoimmune disease and idiopathic severe neutropenia.

Cyclic neutropenia presents as regular oscillations of the number of neutrophils in the peripheral blood. Other blood cells are affected to a lesser degree. The periodicity of neutropenia is 20 to 24 days in humans and 12 to 14 days in dogs. Lathrop et al (61) treated grey collie dogs with recombinant human G-CSF or recombinant human GM-CSF. While recombinant human G-CSF eliminated the cyclic neutropenia and maintained a persistent neutrophilia, recombinant human GM-CSF had no effect. However, cyclic neutropenia recurred over time despite continued administration; this correlated with neutralizing antibodies in the blood and is not likely to occur in humans given this preparation.

Therefore, it appears that in a small group of patients with neutrophil dysmyelopoiesis, recombinant human G-CSF will likely become the standard treatment to prevent life threatening infections due to protracted neutropenia. However, some patients may require a trial of both agents if the underlying etiology is uncertain.

Myelodysplastic syndromes: Both recombinant human G-CSF and GM-CSF enhance the differentiation of leukemic and myelodysplastic myeloid cells. Therefore, caution must be taken when myelodysplastic patients are treated. Early studies, however, have demonstrated improved peripheral blood neutrophil counts in patients with myelodysplastic syndrome receiving recombinant human G-CSF. Negrin et al (62) showed that neutrophil function was preserved, bone marrow myeloid maturation enhanced, and erythrocyte transfusion frequency reduced. No conversion to leukemia occurred over a short follow-up period. Only 12 patients were treated and, as with other similar studies to date, no clinical data on reduction of febrile episodes or documented infection were reported.

Two recent trials used recombinant human GM-CSF by different routes in myelodysplastic syndrome patients. Thompson et al (63) treated 16 patients by daily subcutaneous bolus injection. Eleven of 13 patients receiving greater than or equal to $1.0 \mu\text{g/kg/day}$ had dose-related increases of two- to 194-fold of circulating neutrophils. However, in all but two patients, the counts fell to baseline after discontinuation of recombinant human GM-CSF. Chronic administration at $3 \mu\text{g/kg/day}$ for up to nine weeks did not sustain the increase in four of five patients. Vadhan-Raj et al (64) reported similar results when giving recombi-

nant human GM-CSF by continuous intravenous infusion for 28 days as two 14 day cycles with a two week rest interval. One patient in the first study progressed to acute leukemia after two doses of recombinant human GM-CSF. Interestingly, one patient in the second study died of sepsis despite recombinant human GM-CSF treatment, and the post mortem examination revealed multiorgan infiltration with immature myeloid cells, suggesting granulocytic sarcoma similar to findings in the murine models receiving chronic recombinant human GM-CSF. Elevations of platelet counts occurred in several patients in each study.

At this time, the limited success of chronic administration to maintain normal myelopoiesis in most patients and the concern about conversion to acute leukemia makes the future role of the CSFs in myelodysplastic syndrome uncertain. One report, however, suggested that recombinant human GM-CSF may have suppressed the abnormal clone as a mechanism of restoring normal hematopoiesis in a patient with a myelodysplastic syndrome (65).

Disease-induced neutropenia: Chronic lymphoid malignancies characterized by frequent infections include chronic lymphocytic leukemia and hairy cell leukemia. In the latter, neutropenia can occur as a consequence of disease or treatment. Alpha-interferon and pentostatin are now standard treatments for this disease, and induce substantial improvements in neutrophil counts in 90% of patients. However, two to six months of treatment may be required to achieve this; furthermore, a reduction in neutrophil counts may occur during the first month of therapy with alpha-interferon. Glaspy et al (66) recently treated four patients with hairy cell leukemia who had severe neutropenia. Each patient received increasing doses of recombinant human G-CSF for five to six weeks by daily subcutaneous injection. All three evaluable patients responded with increases in neutrophil counts from $0.9 \times 10^9/L$ to greater than $4.0 \times 10^9/L$ within the first two weeks; two patients experienced resolution of ongoing infection including periorbital skin infection in one patient and a perirectal abscess with sepsis despite broad spectrum antibiotics in the second patient. One patient developed neutrophilic dermatosis (Sweet's disease), which may be a rare dose-limiting side effect of recombinant human G-CSF. This report suggests that recombinant human G-CSF may be a useful adjunct to standard therapy for this disease by reducing the risk of life threatening infection before therapeutic response.

Patients with the acquired immune deficiency syndrome (AIDS) have numerous abnormalities of

their host defences, not the least of which is leukopenia. This may be due to the disease itself or to drugs used in its treatment (eg, azidothymidine). Groopman et al (67) treated 16 patients with AIDS with good performance status and leukopenia. Circulating neutrophils and monocytes were dramatically increased after 14 days of low dose daily continuous intravenous infusion of recombinant human GM-CSF. The increase was gradual and sustained over the treatment period. No life threatening toxicity occurred, and only a few patients had mild symptoms controlled with symptomatic measures. Thus, recombinant human GM-CSF may become a useful adjunct to the treatment of patients with AIDS. Trials of subcutaneous dosing, however, are needed to allow practical outpatient therapy. In addition, these patients may benefit from the previously mentioned augmenting effects of recombinant human GM-CSF on neutrophil toxicity against human immunodeficiency virus (HIV) infected cells (27).

Treatment-induced neutropenia with recombinant human G-CSF: A growing number of therapeutic interventions are subjecting an increasing number of cancer patients to life threatening infections. These interventions generally involve the intensification of chemotherapy delivery by administration of drugs at higher doses, at more frequent intervals, or both. The impetus for developing these strategies has been provided by the advent of autologous bone marrow transplantation techniques and the growing acceptance that increasing the doses of established agents will yield higher response rates. Whether this increased risk of morbidity or mortality to the patient will result in significant improvements in survival, however, is yet to be determined.

The number of clinical trials in this area has grown rapidly in the last two to three years. While most trials have focused on the optimal dose and schedule which will prevent therapy-induced neutropenia, relatively few have tried to demonstrate conclusive improvements in clinical parameters such as reduction in febrile neutropenic episodes and infection rates and, in the case of autologous bone marrow transplantation, reduced hospital stays. In addition, most studies remain ongoing and have only been reported in abstract form. This review will examine conclusions reached by published and completed studies.

The earliest phase I toxicity trials showed that cancer patients tolerated recombinant human G-CSF very well (68). Furthermore, recombinant human G-CSF increased peripheral neutrophil counts in a dose-dependent fashion even in patients with previous chemotherapy, and neutrophil function was normal by *in vitro* testing.

Platelet counts, on the other hand, were unchanged or fell during recombinant human G-CSF therapy. Side effects were generally restricted to bone pain rarely requiring analgesia, and no augmentation of tumour growth has been reported in vivo. However, reports of in vitro enhancement of nonhematopoietic tumours by recombinant human GM-CSF has stimulated continued monitoring of tumour responses in clinical trials (69,70).

With such an impressive therapeutic index, some studies have examined the optimal dose and schedule to reduce or abrogate chemotherapy-induced neutropenia. Morstyn et al (48) studied patients with extensive cancer, nearly two-thirds of whom received prior chemotherapy. All patients received melphalan 25 mg/m² intravenously, and recombinant human G-CSF was given subcutaneously by bolus or continuous infusion in a wide variety of schedules, one of which included pre-chemotherapy recombinant human G-CSF. Dose-dependent protection against severe neutropenia occurred; the absolute neutrophil count fell to less than 1.0x10⁹/L in six of 12 patients receiving 3 µg/kg or less of recombinant human G-CSF, whereas this occurred in only three of 19 receiving doses in excess of 10 µg/kg. The authors concluded that 10 µg/kg/day by subcutaneous infusion provided the most reliable protection against neutropenia in this regimen.

Several studies have now been published reporting the effects of recombinant human G-CSF on clinical outcomes. Gabrilove et al (71) treated patients at various stages of transitional cell carcinoma of the urothelium with methotrexate, vinblastine, doxorubicin and cisplatin. The doses used are routinely highly myelotoxic; patients received recombinant human G-CSF by short intravenous infusion for six daily doses one week prior to chemotherapy, and another eight day course from days 4 to 11 after chemotherapy was begun. Cohorts of three to five patients received varying dosages of recombinant human G-CSF from 1 to 60 µg/kg/day. Recombinant human G-CSF was given to 18 patients in cycle 1 and withheld during cycle 2. Six patients received no recombinant human G-CSF during cycle 1 and served as controls. A dose-dependent increase in neutrophil counts was evident in all patients during recombinant human G-CSF treatment. There were 92% fewer days in which the absolute neutrophil count was less than 0.5x10⁹/L. A febrile neutropenic episode (ie, absolute neutrophil count less than 1.0x10⁹/L) occurred in four of the 24 cycles (17%) without recombinant human G-CSF, and in only one of 18 cycles (6%) where recombinant human G-CSF was given. Antibiotics were required for four to 15 days in the first group, and for only one

day in patients receiving recombinant human G-CSF. Again, the main side effect of recombinant human G-CSF was mild to moderate bone pain in 72% of patients. Interestingly, less chemotherapy-induced mucositis was noted in the cycles receiving recombinant human G-CSF. This study showed, therefore, that recombinant human G-CSF can reduce both the risk of infection as measured by days of neutropenia at less than 0.5x10⁹/L, and the frequency of febrile neutropenic episodes in patients receiving routinely myelotoxic combination chemotherapy.

Bronchud et al (48,72) has reported on several phase I/II studies of patients receiving intensive chemotherapy for small cell lung cancer, breast cancer and ovarian cancer. In the first study, patients with small cell lung cancer received ifosfamide, mesna, doxorubicin and etoposide every three weeks for six cycles. At entry into the trial, sequential pairs of patients received 1 to 40 µg/kg/day of recombinant human G-CSF by continuous intravenous infusion daily for 14 days, beginning 24 h after completion of chemotherapy. Patients were assigned to receive recombinant human G-CSF sequentially on odd or even cycles. Nine of 12 patients completed two cycles; in the six evaluable patients, there was a significant improvement in absolute neutrophil count on day 15 for cycles with recombinant human G-CSF compared to those without. The most striking difference occurred in the 5 to 40 µg/kg range. More importantly, six patients experienced episodes of febrile neutropenia requiring hospitalization and intravenous antibiotics during cycles without recombinant human G-CSF, whereas no such episodes occurred during cycles with recombinant human G-CSF (P=0.012). No clinical toxicity was seen, and neutrophil mobility and bactericidal activity were normal before and after the nadir. Therefore, it appeared that recombinant human G-CSF administration resulted in less febrile neutropenia, hospitalization and antibiotic therapy.

In their second study, Bronchud et al (72) used a fixed dosage schedule of continuous intravenous recombinant human G-CSF (10 µg/kg/day, days 2 to 8; and 5 µg/kg/day, days 9 to 12), and escalated the dose of doxorubicin given every three weeks to successive patient cohorts with advanced breast or ovarian cancer. A 2.2- to 4.5-fold increase in the dosage intensity (in mg/m²/week) of doxorubicin could be given with recombinant human G-CSF, compared to the historical experience without it. A controlled trial using very dose-intensive chemotherapy without autologous bone marrow transplantation for a variety of advanced cancers has been reported

recently by Neidhart et al (73). All patients received etoposide and cyclophosphamide at about five times the doses given in standard treatment protocols, and cisplatin at about twice the standard dose. Therapy was given every five weeks for up to four cycles. Doses were not changed for the subsequent cycle unless either the absolute neutrophil count was less than $0.1 \times 10^9/L$ for more than 14 days or the platelet count fell below $10 \times 10^9/L$.

The study compared patients receiving escalating dosages of recombinant human G-CSF (at 20, 40 and 60 $\mu g/kg/day$) ($n=10$) to those receiving no recombinant human G-CSF in cycle 1 as a control group ($n=11$) and eight patients who received recombinant human G-CSF only after cycle 1. Recombinant human G-CSF was given as a 30 min daily intravenous infusion for 21 days beginning three days after chemotherapy or until the absolute neutrophil count rose above $15.0 \times 10^9/L$ for five days. The results revealed that, compared to controls, patients receiving recombinant human G-CSF had a significantly shorter median duration of severe granulocytopenia (less than $0.1 \times 10^9/L$) ($P<0.03$). At the higher dosages, the time to recovery of the absolute neutrophil count above $0.5 \times 10^9/L$ was significantly shorter (six versus 12 days, $P<0.02$), and patients required less antibiotic therapy and less hospitalization compared to the lowest dose or controls. Seven of eight patients who received recombinant human G-CSF after cycle 1 had a shorter period of neutropenia on recombinant human G-CSF compared to cycle 1. The only toxicity related to recombinant human G-CSF was bone marrow pain requiring morphine in two patients.

Taylor et al (74) used a similarly intensive chemotherapy regimen (high dose carmustine, cyclophosphamide and etoposide) but with autologous bone marrow transplantation support in 18 patients with relapsed Hodgkin's disease. Recombinant human G-CSF was given as in the Neidhart et al study (71) using their highest dosage (60 $\mu g/kg$) but for a maximum of 28 instead of 21 days beginning on the day of transplantation. This group was compared with a cohort of 58 historical controls treated with the same regimen minus recombinant human G-CSF. Results showed that absolute neutrophil count recovery to $0.1 \times 10^9/L$, $0.5 \times 10^9/L$, and $1.0 \times 10^9/L$ were all highly significantly more rapid using recombinant human G-CSF (ie, median 13 versus 22 days to reach $0.5 \times 10^9/L$). Infectious complications were markedly reduced as well. Parameters included fewer febrile days (median three versus seven in controls) and fewer bacteremic episodes (17 versus 36%). These data are similar to those reported by

Sheridan et al (75) in a trial where 15 patients with solid tumours or acute lymphoblastic leukemia were treated with high dose chemotherapy and autologous bone marrow transplantation. Compared to 18 matched controls, recombinant human G-CSF given at 20 $\mu g/kg$ as a daily continuous subcutaneous infusion resulted in significant acceleration in neutrophil recovery, fewer days on intravenous antibiotics, and less time in reverse isolation. From these data one can conclude that: recombinant human G-CSF can be reliably and safely administered by continuous or short infusion intravenous or subcutaneous routes; a dose-response effect occurs at up to 60 $\mu g/kg$ or higher with a minimal increase in toxicity; more intensive chemotherapy regimens may require higher doses of recombinant human G-CSF for maximal myeloprotection; and clinical outcomes of infection (including reduced severity and duration of neutropenia, fewer severe infections, and fewer days of antibiotics and hospitalization) are improved using recombinant human G-CSF during high dose chemotherapy and in at least some disease states in which chronic severe neutropenia exists.

Treatment-induced neutropenia with recombinant human GM-CSF: The use of CSFs to prevent treatment-induced myelosuppression requires appropriate timing in order to optimize protection. Talmadge et al (76) found that mice given lethal doses of irradiation required CSF immediately after, whereas those receiving alkylating agents were best protected when CSF was begun 48 h after drug administration. This observation suggests that time must pass to allow for the clearance of the effects of alkylating agents from the bone marrow, to prevent greater early progenitor depletion by the cytotoxic effects of lingering alkylating drug during recombinant human GM-CSF stimulation. The duration of therapy is also important: cycles of chemotherapy should be as short as possible, and recombinant human GM-CSF should be stopped prior to the next cycle dose to reduce stimulation of precursors before exposure to chemotherapy. Morstyn et al (77) found that seven days of daily subcutaneous recombinant human GM-CSF beginning 24 h after completion of chemotherapy were sufficient to prevent major neutropenia in patients receiving etoposide and carboplatin every three to four weeks.

While numerous preliminary reports have been published in abstract form on the effects of recombinant human GM-CSF on chemotherapy-induced myelosuppression, only a few have been fully published in peer review journals. Antman et al (78) reported that recombinant human GM-CSF given by continuous intravenous infusion during

cycle 1 for patients receiving highly myelosuppressive therapy for sarcomas significantly reduced the severity and duration of neutropenia compared to cycle 2, where recombinant human GM-CSF was not given. Twelve patients completed both cycles of chemotherapy. Interestingly, platelet nadirs were also markedly improved. No dose-response correlation occurred over dosages of 4 to 64 µg/kg, and dose-limiting toxicity occurred above 32 µg/kg. The duration of neutropenia was reduced from a mean of 7.4 (cycle 2) to 3.5 days (cycle 1). There were seven hospital admissions for febrile neutropenia during each cycle, with one culture-documented septic episode in cycle 2.

More recently, Gianni et al (79) treated 15 patients with breast cancer and lymphoma with cyclophosphamide 7 g/m² and recombinant human GM-CSF 5.5 µg/kg by daily continuous intravenous infusion for seven to 14 days beginning at one or five days after chemotherapy. A nonrandomized control group received the same chemotherapy without recombinant human GM-CSF. The median recovery of the absolute neutrophil count to more than $1.0 \times 10^9/L$ for patients receiving recombinant human GM-CSF was reduced from 20 to 14 days, and patients on recombinant human GM-CSF experienced fewer infectious complications (7 versus 24%). (Platelet transfusions were also reduced due to more rapid platelet recovery.) The authors emphasized an important clinical problem: distinguishing fever due to recombinant human GM-CSF from that due to infection. They emphasized the absence of systemic symptoms and the rapid disappearance of fever after stopping recombinant human GM-CSF as distinguishing features. However, the possibility of masking of an infection-related fever while using acetaminophen or an NSAID to control other symptoms remains. Indeed, 20% of Gianni's patients had myalgias and bone pain; 40% had skin rash; and 40% had fever over 38.2°C.

Recombinant human GM-CSF has improved the morbidity associated with therapy intensification using autologous bone marrow transplantation support in ways similar to that seen with recombinant human G-CSF. Brandt et al (80) demonstrated earlier dose-dependent increases in neutrophils and total white blood cell counts (by day 14 post transplant) and reduction in number of neutropenia days from 17 to 12, all compared to historical controls. Nadler and Rabinowe are conducting a randomized trial of patients with relapsed non-Hodgkin's lymphoma receiving high dose chemotherapy. In a preliminary report of the first 23 patients, they reported significantly fewer days to an absolute neutrophil count above $0.5 \times 10^9/L$ (19 versus 23), a shorter duration of

intravenous antibiotic support (10 versus 19 days), fewer hospitalized days (22 versus 26), and fewer platelet transfusions (81). An update of this study was recently published (82). Another patient group that may benefit from recombinant human GM-CSF is that with autologous or allogeneic bone marrow graft failure. Primary infection due to persistent neutropenia is a major reason for morbidity and mortality. Nemunaitis et al (83,84) treated 37 patients in graft failure with recombinant human GM-CSF as a daily intravenous infusion for 14 to 21 days. Twenty-one patients responded, with the absolute neutrophil count exceeding $0.5 \times 10^9/L$; only the neutrophil lineage responded. The survival of all patients was significantly better than a matched historical cohort with graft failure (at one year, 44 versus 23%, $P=0.003$).

One further application of CSFs is in the area of peripheral blood stem cell transplantation. Siena et al (85) have shown that the peripheral blood of cancer patients recovering from high dose cyclophosphamide therapy is enriched for CD34⁺ hematopoietic stem cells. This enrichment is markedly improved when intravenous recombinant human GM-CSF is given daily for 14 days after chemotherapy. The yield of stem cells was 10- to 20-fold higher than that achieved through conventional autologous bone marrow transplantation. This method provides much simpler stem cell reconstitution by eliminating general anesthesia and pain from multiple biopsies, and circumventing bone marrow which may have been previously irradiated. (Du et al [86] have reported that recombinant human G-CSF may also increase circulating early progenitor cells in the peripheral blood of cancer patients.)

More clinical trials are needed before the full clinical benefits of recombinant human G-CSF and recombinant human GM-CSF can be conclusively determined. To date, the most convincing studies are those which compare the clinical infection outcomes of patients who receive one cycle of treatment with a CSF and one without (inpatient controls). However, this design is flawed by the fact that results of the second cycle may be affected by exposure of the bone marrow to chemotherapy during cycle 1. Assigning patients to receive the CSF in cycle 1 or 2 by a randomization scheme reduces this problem but does not eliminate it. The best design would involve the random allocation of patients with the same disease receiving the same treatment to a CSF or 'no CSF' arm (interpatient controls). Several such trials are in progress, such as that reported by Nadler et al (82), and a recently completed but not yet published study comparing the clinical infection out-

TABLE 3
Potential therapeutic role of granulocyte colony-stimulating factor (G-CSF), granulocyte/macrophage colony-stimulating factor (GM-CSF) and interleukin-1 (IL-1) in humans

Factors	Diseases for which there is a potential therapeutic role	
G-CSF	Congenital neutropenia ? Myelodysplastic syndromes Hairy cell leukemia Low grade lymphomas Adjunct to autologous bone marrow transplantation	Idiopathic neutropenia Acquired immune deficiency syndrome Chronic lymphocytic leukemia Myelodysplasia High dose chemotherapy
GM-CSF	Some congenital and idiopathic neutropenias High dose chemotherapy Adjunct to stem cell reconstitution	Adjunct to autologous bone marrow transplantation Parasitic infections ? Myelodysplastic syndromes
IL-1	Adjunct to treatment of various disease states using other colony-stimulating factors Adjunct to antibiotic therapy in established infections	

comes of patients receiving recombinant human GM-CSF during therapy for small cell lung cancer. Patients were randomly assigned to receive either of two doses of recombinant human GM-CSF or no recombinant human GM-CSF during each cycle of chemotherapy. However, such trials require large numbers of patients to detect significant differences in clinical outcomes, and may be difficult to conduct in diseases such as chronic neutropenia.

CANADIAN STUDIES OF RECOMBINANT HUMAN GM-CSF AND RECOMBINANT HUMAN G-CSF

At present, clinical trials are being planned or have begun in the areas of bone marrow transplant support and chemotherapy dose intensification. Investigators in Vancouver are conducting trials of recombinant human GM-CSF and recombinant human interleukin-3 in patients undergoing autologous bone marrow transplantation. The National Cancer Institute of Canada's Clinical Trials Group has organized a Biologics Working Group through which several trials of recombinant human GM-CSF are being conducted. These are presently phase I trials assessing the safety of recombinant human GM-CSF and its effectiveness in protecting patients from myelosuppression while they receive higher than standard doses of chemotherapy. Priority has been set on trials in patients with ovarian cancer, small cell lung cancer, and lymphoma. Clinical outcomes being monitored include febrile neutropenia, intravenous antibiotic requirements and hospitalization. A trial of recombinant human interleukin-3 in patients with ovarian cancer is being planned. To date, no trials of recombinant human G-CSF have begun, but discussions with a major commercial producer of this agent have been indicated.

FUTURE THERAPEUTIC APPLICATIONS

The intensification of cancer therapy is expanding as a concept and as a basis for the design of a

growing number of clinical trials. Along with this comes increased risk to the patient in an attempt to find more curative therapies. Reduction in infection risk will continue to be a major priority associated with these trials, and it is likely that recombinant human G-CSF, recombinant human GM-CSF and recombinant human interleukin-3 alone or in combination will become standard adjunctive therapies for an as yet unknown variety of intensive treatment regimens. Already these agents are the main therapeutic hope of patients suffering from congenital neutropenia, in whom morbidity and mortality from infection are a life-long problem. An untouched but potentially productive area of application is the treatment of parasitic diseases. Recombinant human GM-CSF, in particular, has the potential not only to reduce treatment-induced neutropenia, but also to act as an immunostimulant to enhance eosinophilic responses and perhaps improve the response and survival of patients receiving antibiotic therapy. Finally, recombinant human interleukin-1 appears to have a broad spectrum of therapeutic value in conjunction with other CSFs, and perhaps on its own, as an adjunct to antibiotics in the treatment of serious Gram-negative infections. As a new modality of treatment, the CSFs and other cytokines are exciting additions to the established therapeutic armamentarium against infections and cancer (Table 3).

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