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Review paper

Recombinant human granulocyte macrophage colony-stimulating factor: current status of clinical trials and potential future applications

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Recombinant human granulocyte macrophage colony-stimulating factor (rhGM-CSF) was one of the first of the myeloid growth factors to become available for clinical trials. Phase I studies have demonstrated that the optimal administration is by continuous intravenous infusion or subcutaneous injections at doses of 4–5 μg/kg/day. Phase II trials in patients with a variety of malignancies who receive rhGM-CSF after standard doses of chemotherapy have demonstrated significant reductions of the duration of leucocytopenia. Use of rhGM-CSF after high-dose chemotherapy (with or without bone marrow rescue) suggest that this agent decreases the time to recovery of a normal blood count and reduces infective complications. Results in myelodysplasia and aplastic anemia have been less encouraging. The potential value of rhGM-CSF in the treatment of a variety of other conditions including AIDS and the leukemias is being tested and the early results are discussed.

Key words: Cancer immunotherapy, GM-CSF, granulocyte macrophage colony-stimulating factor, growth factors, hemopoiesis, interleukin.

Introduction

The hematopoietic colony-stimulating factors (CSFs) play a vital role in the regulated production of the eight different types of mature cells found in peripheral blood.[1–6] These cells are multifunctional and intrinsic to a complex cellular signaling system that determines the controlled growth and differentiation of immunohematopoietic cells and modulates the function of their progeny. In almost every instance the CSFs were characterized by their ability to influence clonal proliferation and colony formation *in vitro*[3] and their nomenclature is based on that ability. Advances in recombinant DNA technology have allowed the genes of several of the main CSFs to be cloned and we now have relatively large amounts of the individual synthesized polypeptides, allowing use in preclinical and clinical trials in situations where increases in the numbers and functions of myeloid cells in the peripheral blood would be useful.

The various CSFs may be considered as three overlapping groups of macromolecules. Erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF) and macrophage colony-stimulating factor (M-CSF) are relatively lineage-restricted and act fairly distally in the developmental hematopoietic pathway to produce the terminal differentiation of erythroid cells, granulocytes and macrophages, respectively.[1,7,8] G-CSF and M-CSF also modulate the mature function of neutrophils and macrophages, respectively.[9,10] The second group are less lineage-restricted, acting at a more proximal level in hematopoiesis: interleukin 3 (IL-3) and granulocyte macrophage colony-stimulating factor (GM-CSF) act on myelomonocytic progenitor cells, early erythroid colony formation,[11,12] and megakaryocyte precursors.[13] Lastly, there are CSFs which act on a primitive population of cells probably responsible for marrow repopulation: interleukin 1 (IL-1) and interleukin 6 (IL-6). The role of these factors is less well understood, but seems to include stimulation of proliferation of stem cells and priming them for the effects of factors such as IL-3 and GM-CSF which act at a later stage of differentiation.[14,15]

All of these CSFs are active at picomolar concentrations on cells containing relatively low numbers (200–1000/cell) of specific high-affinity receptors. These macromolecules are all glycopro-

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teins containing disulfide bonds which are critical for biological activity. In the native state they are all glycosylated. For GM-CSF, however, glycosylation is less important, and may not even be necessary for biological efficacy.[16,23] It has even been reported that non-glycosylated GM-CSF is over 10-fold more active in stimulating myeloid proliferation than is the fully glycosylated form. All the molecules are monomers except M-CSF which is a dimer. The genes encoding all of these proteins, except G-CSF and erythropoietin, are on chromosome 5.

The potential clinical use of these CSFs has been inferred from *in vitro* and *in vivo* animal activities which have included stimulation of differentiation and proliferation of the different lineages of the hematopoietic system. Thus the potential for these growth factors would be to enhance peripheral blood and marrow responses in situations in which the hematopoietic system is compromised. The potential clinical uses of myeloid CSFs are as follows:

(a) treatment of aplastic anemia;
(b) treatment of myelodysplasia;
(c) treatment of bone marrow failure following toxin or radiation exposure;
(d) augmentation of hematopoietic recovery following bone marrow transplantation;
(e) abrogation of cytotoxic drug-induced myelosuppression;
(f) treatment of leukemia, to alter rate of differentiation or induce leukemic cells to cycle before chemotherapy;
(g) enhancement of immunohematopoietic and host defence function following major trauma or burns;
(h) enhancement of peripheral blood stem cell harvest for autologous reconstitution;
(i) radioprotection.

The purpose of this paper is to provide an overview of the biology and actions of recombinant human GM-CSF and to review the current clinical trials using this cytokine.

Biology and characteristics

Molecular biology

The cloning of murine and human GM-CSF has been essential for the study of their biological properties. Recombinant cDNA for human GM-CSF was originally generated from T-lymphocyte messenger RNA[18,19] and murine material was subsequently cloned from the EL-4 thymoma cell line.[20] Recombinant GM-CSF has been synthesized in prokaryotic and eukaryotic cells[21,22] and when generated from these systems has identical biological properties to native GM-CSF.[23]

The human and mouse genes for GM-CSF are almost identical in their structure. They are both 2.5 kb in length and contain three introns and four exons.[21] The GM-CSF gene exists as a single gene copy and is localized to chromosome region 5q21 to 5q32,[24] in common with the genes of other cytokines including M-CSF and IL-3.[25] The mouse and human cDNAs have 60–90% sequence homology.[19] The initiation codon for transcription is located 35 base pairs, 5′ to a single translation initiation codon, and 28 base pairs, 3′ to a TATA box promoter sequence.[26] The mechanisms of regulation of transcription of GM-CSF are poorly understood. Human GM-CSF is translated as a peptide containing 144 amino acids and undergoes subsequent proteolytic cleavage of a 17 amino acid peptide to leave a 127 amino acid protein. The polypeptide contains sulfhydryl bonds that are essential for biological activity. The mature protein has a molecular weight of approximately 22 000 Daltons.[17] The multibiological actions of rhGM-CSF seem to be contained in relatively small amino acid sequences.[27,28]

Cell sources and *in vitro* actions

GM-CSF is produced by many cell types in response to various stimuli. Cells which produce GM-CSF include fibroblasts, endothelial cells, and monocytes. It is either produced constitutively or in response to stimulation with lectin, antigen, tumor necrosis factor[29,30] or IL-1.[31,32] Once secreted by cells, it is unclear what the actual local microenvironmental concentration is. It has been suggested that GM-CSF may be locally sequestered by glycosoaminoglycans of the stromal matrix.[33]

There are no reports of serum concentrations of GM-CSF which are detectable in health, and whether GM-CSF has any role to play in constitutive hematopoiesis, i.e. in the absence of hematopoietic stress, remains uncertain. GM-CSF acts in conjunction with other cytokines and indeed its production is mediated by other cytokines in a complex intracellular network. The mechanisms for reducing GM-CSF secretion remain largely uncharacterized.

The *in vitro* actions of GM-CSF include influences on the growth, differentiation and function of the granulocyte, macrophage and eosinophil lineages at a progenitor level, as well as cloned T-lymphocytes. Its presence is essential for cell division to occur in the myeloid progenitor compartment. It directs bipotential granulocyte macrophage precursors along granulocyte or macrophage differentiation pathways.[34] It acts functionally to prime and activate mature neutrophils to combat pathogens, by promoting chemotaxis, phagocytosis, microbial killing and enhancing oxidative metabolism.[35–37] These effects have also been demonstrated for eosinophils and macrophages.[38,39] The action of GM-CSF may be enhanced *in vitro* by a number of different cytokines including G-CSF,[40] M-CSF,[41] erythropoietin, IL-3[42] and IL-6.[43]

GM-CSF receptor structure and function

The GM-CSF receptor is found in fairly low numbers (20–200/cell), but is characterized by having high affinity.[44] At least two different functional classes of receptor have been identified. The neutrophil GM-CSF receptor exclusively binds GM-CSF, but a second receptor, detectable in some leukemic cell lines such as KG1 and MO-7E, also binds IL-3 in a competitive manner.[45]

Chemical crosslinking of [^{125}I] GM-CSF to its receptor on neutrophils reveals the presence of a single crosslinked complex of 98 000 Daltons, implying a molecular weight for the GM-CSF receptor of 84 000. The receptor is immediately internalized and processed through lysosomes after binding to GM-CSF. A significant proportion of the receptors are then recycled to the cell surface.[45] Signal transduction is mediated through a protein kinase and several proteins are phosphorylated. The relationship of protein phosphorylation to effects on cell mechanisms is unclear. Interestingly, GM-CSF receptors have also been identified on a number of non-hematopoietic cell lines including small cell carcinoma and *cos* 7 African green monkey kidney cells.[46] GM-CSF receptor expression is down-regulated by IL-3.[47]

In vivo actions of GM-CSF

The biological properties of GM-CSF *in vitro* prompted subsequent experiments in animals. Studies in mice[48,49] and non-human primates[50–54] indicated that function[48,49,57] and circulating numbers[48,50,51] of neutrophils and macrophages were augmented by the administration of rhGM-CSF. Administration of rhGM-CSF also stimulated recovery of neutrophils one week earlier than controls in monkeys receiving ablative irradiation and autologous marrow grafting.[53,54] On the basis of these experiments, studies in humans were undertaken to achieve two main objectives: (1) to increase circulating numbers of neutrophils in the peripheral blood which would be useful in a number of situations, particularly cytotoxic induced myelosuppression; and (2) to improve the function of circulating neutrophils and macrophages in situations which could be advantageous (e.g. the immunocompromised host).

Clinical studies of recombinant human GM-CSF

Phase I trials

rhGM-CSF was brought to phase I clinical trials based on the observed *in vivo* effects of the growth factor. A number of trials have now been completed in patients with advanced cancer to determine hematopoietic responses and toxicity.

The immediate effect of rhGM-CSF administration in humans is an acute transient fall in circulating neutrophils, monocytes and eosinophils seen at all dose levels above 1 μg/kg.[55] Circulating white blood cell counts return to normal after 4–6 h[56–58] and primitive myeloid cells then appear in the circulation. Generally there is a biphasic response with an initial rise during the first five days after starting rhGM-CSF followed by a plateau and then a second rise continuing until rhGM-CSF administration is discontinued.[57,58] This is presumably related to an initial release of mature leucocytes from the marrow followed by recruitment and proliferation of myeloid progenitor cells. Initial phase I studies documented only small increments in total white blood cell counts after intravenous bolus injection of rhGM-CSF even at doses as high as 1000 μg/m^2/day.[56,59] Continuous intravenous infusion seemed to be significantly more effective in producing leucocyte increments, doses of 1000 μg/m^2/day producing a 17-fold increase in total mean white blood cell count.[56] The superiority of continuous intravenous infusion has been confirmed in other patients with advanced solid tumors[60,61] and primary or secondary bone marrow failure.[59] So far eight phase I studies have been undertaken in which rhGM-CSF in doses up

to 1000 $\mu g/m^2$/day and/or 50 μg/kg/day have been administered. Most have demonstrated a dose-dependent rise in neutrophils, eosinophils and monocytes. Subcutaneous injection of rhGM-CSF has been investigated and has the advantage of ease of administration, although skin rashes may occur in up to 40% of patients.

In general, the results of phase I studies have suggested that continuous exposure to rhGM-CSF produces optimal leucocyte increments with least toxicity. In all of these studies the toxicities were similar and included fever, myalgia, bone discomfort, dyspnoea, pruritus and headache. More serious side-effects include capillary leak syndrome, pericarditis and pulmonary infiltrates. In one patient (with heavily pretreated liposarcoma) an antitumor effect has been seen.[58] Two further patients with chronic lymphatic leukemia have had reductions in lymphocyte counts during rhGM-CSF administration.[62] There are no reported instances of rhGM-CSF accelerating tumor growth, although this is a theoretical possibility as some tumor cell lines have receptors for GM-CSF.[46,63]

Aplastic anemia

Four main studies have been carried out to investigate the biological activities of rhGM-CSF in aplastic anemia,[64–67] all in relatively small numbers of patients (4–15) at varying dose levels. In three studies improvements in neutrophil counts were observed, but were usually temporary and tended to return to pretreatment levels after discontinuation of rhGM-CSF. Increased numbers of eosinophils and monocytes were also seen[65–67] and an increase in circulating myeloid precursor or blast cells occurred in two studies.[65,66] Improvements in marrow cellularity were seen in two studies.[66,67] In one study of only four patients, however, rhGM-CSF failed to produce worthwhile increments of circulating myeloid cells even at high dosage (32 μg/kg/day) in all but one patient.[67] In all of these studies, toxicity was generally mild and included fever, arthralgia and back pain.

Myelodysplasia

To date, five studies have been carried out in patients with myelodysplastic syndromes.[65,68–71] Administration schedules included continuous infusions given for one to two weeks and repeated up to five times at doses up to 750 $\mu g/m^2$/day. In total, 45 patients have been treated with increments in neutrophil counts obtained in 38 (84%), reticulocyte counts in 14 (31%) and platelets in 8 (18%). Increases in the number of circulating myeloid blast cells have been seen in 12 patients (26.6%). Seven patients to date have progressed to acute myeloid leukemia. Only three patients had decreased red cell transfusion requirements. In a study in which rhGM-CSF was given for 2–9 weeks continuously, persistently elevated neutrophil counts were only seen in one of five patients.[71]

In a recent study[72] rhGM-CSF has been used in combination with low-dose cytosine arabinoside to treat myelodysplastic syndromes. Seven of 24 patients had worthwhile and persistent increments in neutrophil counts and improvements in marrow cellularity, although thrombocytopenia remained a problem. Combination with other differentiation agents such as retinoic acid or 1,25-hydroxy vitamin D3 may be the next option to be explored in the treatment of myelodysplasia.

Chemotherapy-induced myelosuppression

The potential impact of GM-CSF on the treatment of malignancy is mainly related to modification of the neutropenia induced by chemotherapy. Improvements in outcome could result from reductions in incidence of neutropenic sepsis and also from enhancement of the antitumor effects of anticancer drugs whose efficacy is often limited by dose reductions caused by myelosuppression. There is increasing evidence that dose intensity is of importance in obtaining lasting remissions in patients with cancer.[73,74]

The first study to combine GM-CSF with chemotherapy was conducted by Antman and coworkers in 16 patients with soft tissue sarcomas.[75] These patients received 3 to 7 days of rhGM-CSF by continuous intravenous infusion. They were then given chemotherapy with subsequent reintroduction of rhGM-CSF. The second cycle of therapy was given without growth factor. There were significantly higher total leucocyte and platelet counts after the first cycle compared to the second. Obviously there are limitations to interpretation of this study since sequential courses of chemotherapy tend to produce cumulative myelosuppression, but even so the results were interpreted as encouraging. The rate of infection in this study was not affected by rhGM-CSF.

In a second study, patients with myeloma, lymphoma or solid tumors again received conventional chemotherapy with rhGM-CSF after the first cycle, but not after the second.[76] The neutrophil nadir was significantly shorter and shallower in the cycles accompanied by rhGM-CSF and episodes of infection and incidence of dose reduction were both reduced. Using relatively intensive chemotherapy, Morstyn and colleagues administered rhGM-CSF to patients with small-cell lung cancer and used previously treated patients who had received the same chemotherapy as historical controls.[77] Only one of 11 evaluable patients had grade 4 (WBC $< 0.5 \times 10^9$/l) neutropenia compared to 31% of patients in the historical control group. These studies suggested that dose intensification of chemotherapy may be possible in the presence of rhGM-CSF.

Increasing the amount of chemotherapy delivered to a patient can be achieved in two ways: either by shortening the period between doses (dose intensification), or by giving high-dose chemotherapy (either alone or in combination with bone marrow transplantation). Studies in primates revealed that rhGM-CSF reduced the period of neutropenia after total body irradiation and autologous bone marrow transplantation.[78] High-dose chemotherapy with autologous or allogeneic bone marrow transplantation is being increasingly used for selected groups of patients with acute leukemia, chronic granulocytic leukemia, non-Hodgkin's lymphomas and Hodgkin's disease. A potential role for rhGM-CSF would therefore be to accelerate hematopoietic reconstitution in these patients who commonly suffer prolonged neutropenia after receiving their chemotherapy and bone marrow graft. Several studies in this area have now been completed.

Brandt *et al.* reported a study of 19 patients with breast cancer and melanoma given high-dose chemotherapy followed by autologous bone marrow transplantation (ABMT).[79] Following chemotherapy and reinfusion of bone marrow, patients were given a 14-day continuous infusion of rhGM-CSF at escalating doses to sequential patient groups. Historical controls were used. The period of neutropenia was reduced at doses of rhGM-CSF above 8 μg/kg/day. There were fewer episodes of septicemia, hepatotoxicity and nephrotoxicity in patients receiving rhGM-CSF.

In a study by Nemunitis *et al.*, patients undergoing ABMT for lymphoid malignancies received rhGM-CSF by daily 2-h infusions at doses between 15 and 240 μg/m^2/day for 14 days after grafting.[80] Patients receiving more than 60 μg/m^2/day showed accelerated neutrophil recovery when compared to historical controls (14 vs 25 days). They also became independent of platelet transfusions more quickly, had fewer febrile episodes and were discharged from hospital earlier than patients in the control group. There was no significant toxicity associated with rhGM-CSF administration.

In a series of patients with Hodgkin's disease receiving high-dose chemotherapy and ABMT, rhGM-CSF was subsequently administered in doses ranging from 100 to 400 μg/m^2/day.[81] The median duration of neutropenia ($<0.5 \times 10^9$/l) was 16.3 days compared with 25 days in 19 concurrent controls. However, in this study there was no significant effect of rhGM-CSF administration on the rate of infection or days of hospitalization. A more limited study in patients with acute lymphoblastic leukemia has shown similar results. Patients receiving purged autologous bone marrow transplants were given rhGM-CSF at doses of 16–250 μg/m^2/day, but only those receiving more than 60 μg/m^2/day showed an acceleration of hematopoietic recovery.[82]

More recently Herrmann *et al.* have administered subcutaneous rhGM-CSF to patients with solid tumors with and without autologous bone marrow support after their second cycle of chemotherapy.[46,84] The structure of this study thus eliminated the difficulty of interpretation associated with giving rhGM-CSF with the first cycle, but not the second.[75] These patients received a single daily subcutaneous dose of rhGM-CSF (250 μg/m^2/day) beginning 48 h after high-dose chemotherapy and continued treatment with rhGM-CSF for 10 days. Within-patient comparison with the first course (when no rhGM-CSF was given) revealed a significant reduction in the duration of neutropenia and hospitalization and decreased antibiotic requirement. Again, no significant toxicity was encountered.

A recent study has investigated the role of rhGM-CSF in a dose of 250 μg/m^2/day after high-dose melphalan[83] without bone marrow rescue. At this dose rhGM-CSF hastened bone marrow recovery with a median time of 21 days to reach a granulocyte count of $>0.5 \times 10^9$/l and 26 days for platelets to reach $>50\,000 \times 10^9$/l. These results were obtained in a relatively selected group of patients with minimal prior therapy and whose ages were generally less than 50. Their results were nevertheless encouraging when compared with the period to hematopoietic reconstitution in historical

controls who received the same dose of melphalan without cytokine.

An interesting approach to improve further the rate of bone marrow recovery after administration of high doses of chemotherapy has been reported by Gianni and coworkers.[85] In previous studies it was shown that GM-CSF strikingly increased circulating granulocyte macrophage progenitor numbers[86] and more recently Gianni and coworkers demonstrated that the effect was even greater when rhGM-CSF was administered following high-dose cyclophosphamide. These authors now report that the GM-CFF cells in peripheral blood can be harvested and reinfused following further chemotherapy and ABMT with the result that, compared with historical controls, neutrophil and platelet recovery is significantly more rapid.

Acquired immune deficiency syndrome

The rationale for investigating the effects of rhGM-CSF in acquired immune deficiency syndrome arises from the fact that patients with HIV infection frequently manifest a marked leukopenia in addition to lymphopenia and T-cell mediated immune functional deficiencies. Treatment involves use of agents which include Zidovudine (azidothymidine, AZT), pentamidine isothionate and gancyclovir. Unfortunately these drugs all cause neutropenia as a side-effect and this may necessitate withdrawal of treatment. rhGM-CSF has therefore been investigated in patients with HIV infection. Interestingly rhGM-CSF *in vitro* has demonstrated additional potentially useful reductions in HIV replication.[87] There have also been recent reports of enhanced inhibition of monocytropic HIV strains in human monocytes when GM-CSF was combined with AZT *in vitro*.[88,89] However, two publications have suggested that GM-CSF may have potentially deleterious effects when combined with AZT in that it may increase the replication of HIV *in vitro*,[90] and may reduce the phosphorylation of AZT in bone marrow cells.[91] The first clinical study to be reported used glycosylated rhGM-CSF in male patients with AIDS who had leucocyte counts $<3 \times 10^9$/l purely attributable to HIV infection (i.e. not on drugs which could produce leucopenia as a toxic effect).[92] Patients received rhGM-CSF by continuous intravenous infusion in doses between 0.5 and 8 μg/kg/day. This produced a dose-related increase in granulocytes, monocytes and eosinophils, but failed to induce any worthwhile increments in lymphocytes or platelets. Improvements in bone marrow cellularity were seen in 11 out of 14 patients. Defects of neutrophil function were detected at the outset in two patients and were corrected by administration of rhGM-CSF.[93] In a subsequent study of long-term subcutaneous administration of rhGM-CSF (up to 3 months) in doses ranging from 0.25 to 4 μg/kg/day, persistent improvements in leucocyte numbers were observed.[94] Assessment of expression of HIV p24 antigen during administration of rhGM-CSF showed that the rate of expression did not alter during the period of treatment.

A phase I study of rhGM-CSF in patients with AIDS or AIDS-related complex who had myelosuppression from AZT has been completed.[95] Seventeen patients receiving at least 1.2 g/day of AZT with leucocyte counts of $<1 \times 10^9$/l were treated with 1–5 μg/kg/day of subcutaneous rhGM-CSF. All patients experienced increments in leucocyte numbers above 1×10^9/l, the majority at doses >1 μg/kg/day. HIV p24 antigen expression again was not altered by administration of rhGM-CSF. The most recent study using rhGM-CSF in conjunction with AZT included 10 patients with AIDS or ARC who could not tolerate conventional doses of AZT.[96] These patients received rhGM-CSF subcutaneously at a dose of 2 μg/kg/day for 12 days. After 2 days without treatment they began taking AZT 1200 mg daily for 7 days. After 2 days off AZT treatment, rhGM-CSF was restarted at the same dose. The cycle was repeated every 4 weeks for up to 6 months. During the first 12 days of rhGM-CSF administration there was an increase in the mean total leucocyte count. In addition, rhGM-CSF stimulated the function of circulating monocytes. Unfortunately serum p24 antigen expression rose in six evaluable patients. However, in the subsequent period of AZT followed by rhGM-CSF, serum HIV p24 antigen fell below the day 14 value in the majority of patients. Hematopoietic toxicity was reduced compared with the effects of AZT alone (as experienced prior to entry to the study). Two patients with previously severe AZT-related myelotoxicity were able to tolerate the regimen for 25 weeks.

The co-administration of rhGM-CSF and gancyclovir for patients with cytomegalovirus infection (who had experienced severe myelosuppression with this antiviral agent alone) resulted in improvements in leukocyte counts allowing continued administration of gancyclovir.[97] rhGM-CSF has also been shown to facilitate administration of myelosuppressive chemotherapy for AIDS-

related non-Hodgkin's lymphomas[98] and has allowed the administration of the combination of α-interferon and AZT in patients with Kaposi's sarcoma.[99]

Leukemia

An area of potential value for rhGM-CSF is in patients with acute and chronic myelogenous leukemias as hemopoietic growth factors may induce differentiation commitment in responding neoplastic populations.[100] A concern is that growth factors may also stimulate proliferation of leukemic myeloid cells. However, this has not been seen in one study in which rhGM-CSF was administered to patients with acute leukemia who were profoundly leucopenic after chemotherapy. Neutrophil recovery appeared to be stimulated.[101] There is also *in vitro* evidence to suggest that rhGM-CSF enhances the sensitivity of clonogenic cells to long-term low-dose cytosine arabinoside with sparing of normal clonogenic cells.[102] The administration of rhGM-CSF to stimulate proliferation of leukemic cells followed by the use of cytotoxic drugs is a theoretically interesting approach to increasing neoplastic cell kill.

Radioprotection

There is evidence *in vivo* to suggest that rhGM-CSF may protect mice from death caused by neutropenia following lethal irradiation.[103] This information was used when an abandoned cesium-137 source in Brazil exposed 14 people to 2–5 Gy of radiation.[104] Eight patients with granulocyte counts $<0.5 \times 10^9$/l at 35 days post exposure were treated with rhGM-CSF as a continuous infusion at a dose of 0.5 mg/m^2/day until granulocytes reached $>2 \times 10^9$/l for 3 consecutive days. rhGM-CSF appeared to accelerate hematopoietic recovery causing rapid rises in granulocyte counts approximately 12 h after commencement of the infusion. However, whether the rhGM-CSF administration increased the survival of some of these patients (four of the eight recovered) is unanswerable. The authors suggest that rhGM-CSF should, in future, be given to patients exposed to potentially lethal doses of radiation.

Prospects for future development

A great deal of information is now available about the biology, *in vitro* and *in vivo* actions and benefits of rhGM-CSF and other hematopoietic growth factors. Future studies should be designed rationally on the basis of laboratory and *in vivo* research. The clinical studies so far show that rhGM-CSF, when administered by continuous intravenous infusion or subcutaneously, produces a rise in circulating neutrophils and eosinophils and, less profoundly, monocytes and lymphocytes. In general, information about the effects of rhGM-CSF on thrombopoiesis is less clear and inconsistent. However, a recent study by Edmondson *et al.* has shown that twice-daily subcutaneous administration of rhGM-CSF is accompanied by improved platelet counts in patients being treated with carboplatin and cyclophosphamide.[105] The improvements in cell counts and in the function of phagocytic cells has not yet clearly been shown to be translated into a consistent reduction in the incidence of infections in patients treated with conventional chemotherapy. It is possible that rhGM-CSF will have an important role when combined with aggressive chemotherapy in patients with tumours that are potentially curable if dose intensification can be achieved (i.e. lymphomas, small-cell lung cancer and testicular tumors). This growth factor may also, by limiting cytotoxic-induced myelosuppression, lead to improved tumor responses and survival in patients with an otherwise poor prognosis and there is some evidence to suggest that rhGM-CSF has antitumor effects itself.[58] Possibly the best method of using rhGM-CSF, however, is suggested by work from Milan where rhGM-CSF was used to stimulate progenitor proliferation following chemotherapy. These cells were collected by leucophoresis of peripheral blood and then reinfused resulting in improved myeloid recovery after the cytotoxic therapy.[85] A further development could be to use cytokines such as IL-1 and IL-3 to prime and then stimulate multipotential progenitor cells, followed by rhGM-CSF to enhance myelopoesis, and finally factors such as G-CSF, M-CSF, erythropoietin and megakaryocytic CSF which enhance terminal differentiation of specific cell lineages. Using other cytokines in sequences shown to be optimal in the laboratory may improve the overall therapeutic index of rhGM-CSF.

A concern with early studies using rhGM-CSF was the toxicity observed. The side-effects appear to be related to high peak serum concentration,[106] but such serum concentrations do not in fact correlate with proliferative responses. Several toxicities, such as fever and pericarditis, are dose-related. Finding the dose which produces optimal proliferative responses and minimal toxicity

must be the aim of future clinical studies. Of importance, also, will be the determination of any stimulatory action that rhGM-CSF may have on malignant cells in solid tumors or malignant clones of cells in myeloproliferative disorders. Since rhGM-CSF can stimulate the growth of several neoplastic hematopoietic and non-hematopoietic cell lines, the question is raised as to whether patients (particularly with preleukemic states) can undergo leukemic transformation after receiving rhGM-CSF.[68–70] If neoplastic cells do have receptors for rhGM-CSF and the ligand is a paracrine or autocrine stimulator then there is the therapeutic possibility of using rhGM-CSF to stimulate proliferation in some malignancies in conjunction with cytotoxic agents to improve tumor cell kill. Conversely, the possibility of using antibodies against the rhGM-CSF receptor, to break any autocrine/paracrine proliferative loops, needs to be studied in the laboratory. Another concern about the use of rhGM-CSF and other stimulating CSFs is that continued use may have long-term adverse effects on bone marrow primitive stem cells, i.e. to specifically divert these cells to differentiate along the myeloid pathway and so reduce stem cell regeneration, and possibly leading to marrow failure when this compartment becomes exhausted. There is, however, no clinical evidence that marrow progenitor cells are depleted, even after prolonged exposure to hematopoietic growth factor.

The availability of rhGM-CSF and other cytokines has been one of the most exciting developments in oncology in the past five years. The rapid accumulation of information which has occurred has only been made possible by the simultaneous development of recombinant DNA technology and the co-operation between molecular biologists, cell biologists, protein chemists, pharmacologists and clinical oncologists in an effort to design research programs and clinical trials aimed at understanding the biology of rhGM-CSF *in vitro* and *in vivo*. Only by continuing this co-operation can further developments be made with the aims of improving cure rates of patients with cancer.

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