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Haematopoietic growth factors and lung cancer treatment

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Normal haematopoiesis calls for a complicated but integrated process of proliferation and differentiation, with about 7×10^9 granulocytes and 1×10^{10} erythrocytes replaced hourly. The haematopoietic colony stimulating factors (CSFs) are glycoproteins that were found to stimulate and promote the proliferation of granulocyte-monocyte progenitor cells on semi-solid media in clonogenic assays (table 1, fig 1). The diverse but ordered interactions of colony stimulating factors, target cells, and stroma are responsible for the complex process of haematopoiesis. The colony stimulating factors have effects other than proliferation and differentiation, including maintenance of cell viability, membrane integrity, and functional stimulation of mature cells—for example, granulocyte phagocytosis and superoxide production (table 2).[1-3]

The growth factors have very different structures and specific receptors exist on target cells, though a target cell may possess many more than one type of colony stimulating factor receptor. Growth factors are also unusual in having very high biological activity and in being able to increase rapidly given appropriate stimuli. The production of growth factors from endothelial and stromal cells, fibroblasts, lymphocytes, and macrophages is controlled by a network of interactions between the various cell types and external stimuli, such as foreign antigen and endotoxin. In addition, we are becoming increasingly aware of important synergistic intereactions between growth factors, which again emphasise the complexity of the system and the potential difficulties of full clinical exploitation.[1-3]

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*Table 1 Human haematopoietic growth factors of current clinical interest**

Name	*Abbreviation*	*Molecular weight (daltons)*	*Location of gene*
Erythropoietin	Epo	39 000	7q 11–22
Granulocyte colony stimulating factor	G-CSF	20 000	17q 11·2–21
Granulocyte-macrophage colony stimulating factor	GM-CSF	18–30 000	5q 23–31
Macrophage colony stimulating factor	M-CSF (CSF-1)	70–90 000, 45–50 000	5q 33·1
Multipotential colony stimulating factor	Multi-CSF (IL-3)	15–30 000	5q 23–31
Interleukin-4	IL-4	16–20 000	5q 31
Interleukin-6	IL-6	19–21 000	7p 15

*Modifed from Metcalf[2].

Various factors other than the colony stimulating factors, such as erythropoietin and the interleukins, cause proliferation and differentiation of cells, the latter on B and T lymphocytes. Some, such as interleukin-3 (IL-3) and IL-6, also have an effect on myeloid cells (table 1, fig 1). Growth factors such as IL-3 that act on multipotent progenitor cells of early lineage give rise to a range of mature cell types, including erythrocytes platelets, monocytes, and the various granulocytes. Granulocyte-macrophage colony stimulating factor (GM-CSF) stimulates production of granulocytes and monocytes and increases the levels of eosinophils and lymphocytes, and, in some cases, platelets and erythrocytes. G-CSF is much more lineage restricted and acts specifically on neutrophil granulocytes (table 1, fig 1).

The genes coding for IL-3, GM-CSF, and monocyte (M) CSF are localised to a small area of chromosome 5, whereas the gene for G-CSF resides on chromosome 17 (table 1). Recombinant DNA technology has enabled these factors to be further examined both in vitro and in early clinical studies. Data are now available on erythropoietin, G-CSF, and GM-CSF and preliminary results will soon be available for IL-3, IL-4, and IL-6.

Clinical applications of growth factors in lung cancer

Haematopoietic growth factors are now the centre of feverish clinical activity in a wide range of malignant and non-malignant diseases, including solid tumours, haematopoietic malignancies, myelodysplastic syndromes, aplastic anaemia, AIDs, and the idiopathic neutropenias (table 3).[1-3]

ERYTHROPOIETIN

Erythropoietin has produced dramatic improvement in the anaemia and quality of life of patients with end stage renal failure, provided that sufficient iron stores are present. Patients with malignant tumours, including lung cancer, often have normochromic-normocytic anaemia with a block in iron transfer from stores to the erythroid precursor cell. They may also have anaemia due to blood loss or marrow infiltration and, interestingly, the erythropoietin level tends to be lower in patients with malignant disease (including lung

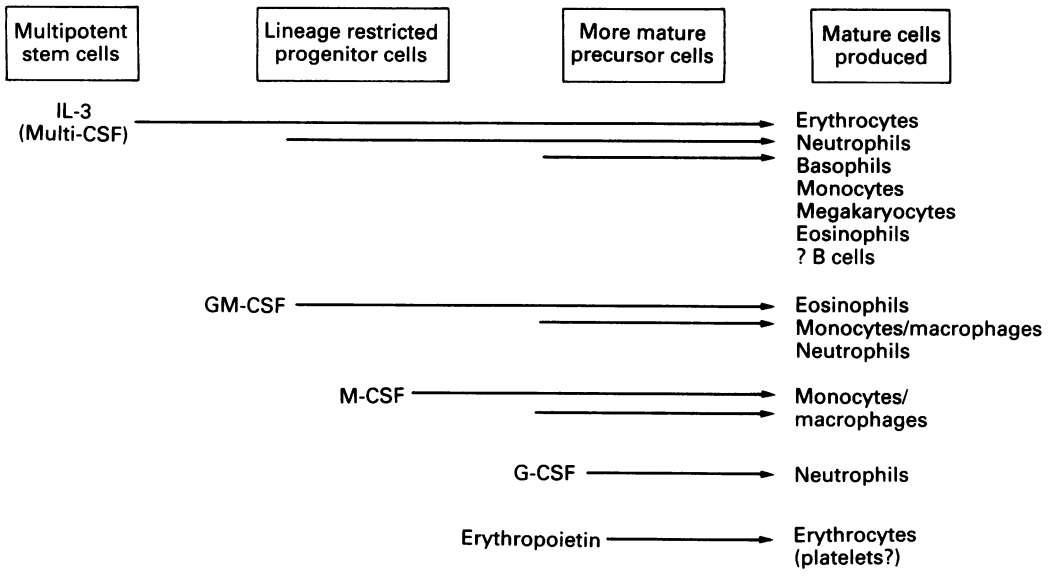

Figure 1 Targets of haematopoietic growth factors. IL-3—interleukin-3; CSF—colony stimulating factor; M—macrophage; G—granulocyte.

cancer) than in patients with anaemia due to other causes but of similar severity.[4,5] The erythropoietin response was also found to be decreased in patients receiving chemotherapy, though this was not due particularly to the use of nephrotoxic drugs such as cisplatin.[5]

A randomised study, reported so far only in preliminary form, examined the effect of erythropoietin at two doses and compared this with outcome in a control group.[6] All patients had small cell lung cancer and were treated with an intensive carboplatin-ifosamide-etoposide regimen. Suppression of bone marrow made frequent blood and platelet transfusions mandatory. Erythropoietin (150 or 300 IU/kg three times a week subcutaneously) resulted in transfusion of significantly less blood in both groups receiving erythropoietin than in the control group and in a trend towards fewer platelet transfusions. The latter trend strengthens the controversial experimental observation of megakaryocyte precursor stimulation by erythropoietin. There may therefore be a case for the use of erythropoietin to reduce blood transfusions in selected patients with cancer.

*Table 2 In vitro actions of myeloid colony stimulating factors**

1	Maintain survival at all stages of development of granulocytes and monocytes
2	Presence required to induce cell division; concentration determines length of cell cycle
3	Commit bipotential granulocyte-macrophage precursor cells to enter granulocytic or monocytic lineage
4	Stimulate functional activity of mature polymorphs and monocyte-macrophages; effects on chemotaxis, expression of membrane antigens, phagocytosis, superoxide production, killing of microorganisms and tumour cells, and production of biologically active agents (for example, interferons, tumour necrosis factor, prostaglandins)

*Modified from Steward *et al.*[1]

*Table 3 Potential clinical applications of myeloid colony stimulating factors**

1	Treatment of bone marrow failure: (i) idiopathic (ii) neoplastic (iii) iatrogenic
2	Augment rate of recovery after bone marrow transplantation
3	Reduce duration or degree (or both) of leucopenia following chemotherapy
4	Increase granulocyte number and function (for example, in patients with AIDS)
5	Treatment of leukaemia—altering the rates of cell reproducton and differentiation
6	Treatment of myelodysplasia—increase normal differentiation and reduce blast population
7	Treatment of established bacterial and fungal infections
8	Improve host defence against potential infection after major trauma—for example, burns

*Modified from Steward *et al.*[1]

GRANULOCYTE COLONY STIMULATING FACTOR AND CONVENTIONAL CHEMOTHERAPY

The first clinical study of a colony stimulating factor, G-GSF, was conducted in Manchester in patients with small cell lung cancer receiving chemotherapy.[7] This and subsequent studies examined the effect of G-CSF after conventional dose chemotherapy in preventing drug induced neutropenia. In the Manchester study patients received up to six cycles of treatment with doxorubicin, ifosfamide, and etoposide and were randomised to receive G-CSF in odd (Nos 1, 3, 5) or even (2, 4, 6) cycles. The G-CSF was given by continuous 14 day intravenous infusion through an ambulatory pump, starting the day after chemotherapy. In addition, the dose-response relationship (from 1 to 40 μg/kg/day) was examined over five days before the first course of chemotherapy. In this study the maximum response to G-CSF occurred with the 10 μg/kg/day dose and G-CSF was extremely effective in reducing severe neutropenia (defined as less than 1×10^9/l neutrophils—a level considered to be critical as below this patients' vulnerability to life threatening infections and death from septicaemia is greatly increased). The duration of neutropenia was substantially reduced (median 80%) with G-CSF (fig 2) and normal or above normal neutrophil counts were obtained within two weeks of chemotherapy. Of particular importance was the observation that all six life threatening infections occurred after cycles of chemotherapy without G-CSF and no severe infection occurred after cycles in which patients were protected with G-CSF. The severe infections resulted in 30 extra days in hospital for intravenous antibiotic treatment and other supportive measures.[7]

Other studies have confirmed these observations, particularly the specific effect on neutrophils and the fact that no toxic effects, or only

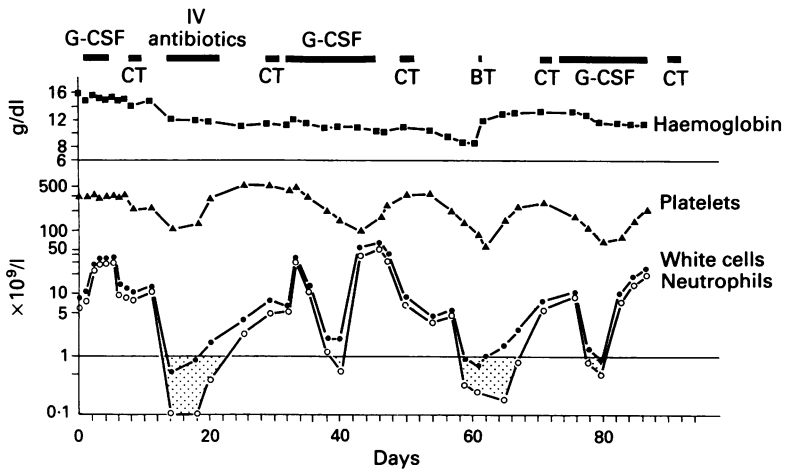

Figure 2 Haematological response to granulocyte colony stimulating factor (G-CSF) showing changes before chemotherapy (CT) and after four cycles. IV—intravenous; BT—blood transfusion. The shaded area indicates the total area of absolute neutropenia.

very minor side effects, occurred with G-CSF. Gabrilove investigated patients with advanced transitional cell bladder cancer receiving doxorubicin, cisplatin, vinblastine, and methotrexate chemotherapy who had a short intravenous infusion of G-CSF over 30 minutes.[8] They found a reduction in the number of days of neutropenia (less than 1×10^9 neutrophils/l), and all patients were able to receive the planned chemotherapy on day 14, compared with only 29% in courses with no protection from G-CSF. Interestingly, there was also a reduction in mucositis. These investigations suggested the possibility of accelerated chemotherapy, with courses given at two week rather than the conventional three or four week intervals.

In a larger study in 126 patients with small cell lung cancer reported by Crawford (in abstract form) patients were given conventional doses of cyclophosphamide, doxorubin, and etoposide and randomised to receive G-CSF or no G-CSF on days 4–17 of a three week treatment cycle.[9] G-CSF was given as a subcutaneous bolus of 230 $\mu g/m^2$/day. There was a significant reduction in the incidence and duration of neutropenia and in the incidence of severe infection as manifest by febrile neutropenia. Benefit occurred both in the first cycle of chemotherapy, when deaths from infection in patients with small cell lung cancer receiving chemotherapy appear to be predominant and in subsequent cycles. The number of days spent in hospital and receiving intensive antibiotic treatment was 40–50% less in patients treated with G-CSF. A similar study is now being performed in some European centres with the same combination of drugs. Preliminary results indicate a reduction in febrile neutropenia with a protective effect extending through all six cycles of chemotherapy, and a substantial reduction in the time spent in hospital with infections. In addition, about 90% of patients having G-CSF were able to receive full dose chemotherapy on time compared with only 65% of patients receiving placebo.[10]

Another group of investigators, in Australia, compared the response to G-CSF given by short bolus intravenous injections, subcutaneous bolus, and subcutaneous infusion at various doses.[11,12] G-CSF again reduced the duration of neutropenia after melphalan chemotherapy, though patients previously given chemotherapy or radiotherapy did not appear to respond as well to G-CSF as did untreated patients. When G-CSF was given subcutaneously to 31 patients, including nine with lung cancer, it was again very well tolerated and substantially reduced the neutropenia, even when G-CSF was started several days after the melphalan. As in other studies using G-CSF, there were no changes in the counts of cells other than neutrophils. A dose of 3 $\mu g/kg$/day produced similar increases in neutrophil counts when given by bolus and by continuous subcutaneous infusion, the neutrophils increasing within 24 hours with both routes.[11] In a Japanese phase I/II study of 33 patients with primary lung cancer of all histological types various chemotherapy regimens were used,[13] The optimal dose of G-CSF in these patients was 100–200 $\mu g/m^2$ given as a 30 minute intravenous infusion over 14 days; 400 $\mu g/m^2$ was recommended for patients who had had previous chemotherapy, because the bone marrow response would be impaired.[13]

GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR AND CONVENTIONAL CHEMOTHERAPY

In the first published clinical trial with intravenous GM-CSF patients with AIDS and bone marrow failure showed a dose dependent increase in circulating leucocytes, most being mature granulocytes. The subject has been reviewed recently.[15] In this and other studies peripheral eosionophlia was a feature. The stimulation of all types of granulocytes is a feature of GM-CSF, whereas G-CSF specifically stimulates neutrophil granulocytes. Combinations of GM-CSF with zidovudine are now under investigation in patients with AIDS to improve tolerance to zidovudine and reduce requirements for antibiotics. In some patients with myelodysplastic syndromes an increase in platelet and reticulocyte counts and a reduction in tranfusion requirements have been observed.[15]

In 1989 some phase I studies investigated routes of administration and the GM-CSF dose-response relationship in patients with refractory, advanced solid tumours, many of whom had been treated previously. In another study from Manchester a dose-response relationship was observed with GM-CSF, with significant increases in total leucocyte, neutrophil, and eosinophil counts.[16] Of particular interest was one patient who had received a large amount of treatment for liposarcoma, in whom a 50% reduction of the tumour occurred after GM-CSF; the response lasted for six months. Seven other patients, with tumours that were progressing, were stabilised.[16] Further investigation of the potential anticancer activity of GM-CSF (possibly mediated through the macrophage system) are warran-

ted. Leucocytosis, largely due to an increase in granulocytes was also reported with subcutaneous GM-CSF given once daily (3–15 μg/kg/day).[17] Thus GM-CSF is an effective stimulator of haemotopoiesis with a role in reducing cytopenia after chemotherapy and bone marrow transplantation.[1 15–18]

GM-CSF was examined by Antman *et al* in 16 adults with sarcoma treated with doxorubicin, ifosfamide, and dacarbazine.[19] GM-CSF was given in the first cycle and the outcome compared with that of chemotherapy alone in the second cycle. Chemotherapy induced neutropenia was reduced with GM-CSF, which was well tolerated in doses up to 32 μg/kg a day given by continuous intravenous infusion. The number of febrile episodes, however, was similar for the two courses of chemotherapy, and it is not clear whether these were due to infection or to pyrexia arising from the GM-CSF. A study of GM-CSF in small cell lung cancer from Manchester had the same design as that of Bronchud *et al*—that is, randomisation betwen odd and even cycles of chemotherapy.[7 20] With subcutaneous GM-CSF intravenous antibiotics were required in seven of 26 cycles, a proportion similar to the 10 out of 30 cycles without GM-CSF. Despite the reduction in neutropenia after chemotherapy the incidence of infection and requirement for intravenous antibiotics were very similar, indicating the need to examine all these clinical measures in studies of the efficacy of haemopoietic growth factors.[20] In a German randomised investigation in patients with small cell lung cancer subcutaneous GM-CSF shortened the duration of neutropenia induced by chemotherapy that include doxorubicin and ifosfamide, and it reduced the incidence of infections requiring antibiotics.[21]

PHARMACOLOGY AND TOXICITY

The qualitative effects of G-CSF and GM-CSF on peripheral blood neutrophils are similar. Within 30 minutes of administration of the colony stimulating factor there is a transient fall in peripheral blood neutrophils followed by a rapid and substantial increase to above baseline levels about five or six hours after administration. The transient depression in the count may be caused by margination to endothelial cells. The increase in neutrophil count with G-CSF and the leucocytosis with GM-CSF reflect demargination, accelerated release of cells from the bone marrow, and an increase in cell proliferation. The dose dependent increases in cell count may be many times greater than the baseline count, tending to plateau from two days onwards.[1 11 12 14 16–18] For GM-CSF the increase in white cell count may be phasic, affecting both neutrophils and (to a lesser extent) eosinophils.[16] Retreatment with GM-CSF produced similar changes in the leucocyte counts but with a more rapid increase and somewhat higher peak counts.[16 17] After G-CSF or GM-CSF is withdrawn the cell counts decline rapidly and by 24–48 hours they are back to baseline levels. When intravenous and subcutaneous administration of G-CSF and GM-CSF were examined the increase in peripheral blood counts appeared somewhat greater with the subcutaneous route, an important practical observation.[1 11 16–18] The neutrophils produced in response to G-CSF have an in vitro functional capacity similar to that of baseline neutrophils.[14] Phagocytic function as reflected by chemoluminescence is usually increased by G-CSF and less consistently with GM-CSF.[14 20]

G-CSF has been extremely well tolerated, with only occasional bone pain and some musculoskeletal discomfort occurring with higher doses. The discomfort is mainly in the medullary areas (sternum, jaw, pelvis, back, and limbs), usually lasts for only a few hours, and does not necessarily recur with subsequent doses. G-CSF has been associated with reversible increases in lactate dehydrogenase, alkaline phosphatase, liver transaminases, and uric acid and in some patients a reduction of serum cholesterol.[1–3 15 18] More severe side effects have been reported with GM-CSF but these occurred with the higher doses in phase I studies. Side effects of GM-CSF also include bone pain and fever in some patients. A capillary leak syndrome has resulted in fluid retention with pericardial and pleural effusions, fever, arthralgia, hypotension, and renal dysfunction, but only with higher doses. Activation of inflammatory cells with overexpression of adhesion molecules resulting in aggregation of these cells, particularly in the microvasculture, may explain in part the capillary leak.

Nevertheless, the increase in leucocytes occur with doses of GM-CSF that are tolerable plus an improvement in platelet and reticulocyte counts in some studies.[1–3 15 18] In an occasional patient considerable leucocytosis ($>30 \times 10^9$/l) has occurred, though this is very uncommon with the dosages now recommended—for example, 5 μg/kg/day G-CSF subcutaneously for 14 days or until the counts have recovered. Such severe leucocytosis resolves rapidly once the colony stimulating factor has been discontinued.

There are theoretical drawbacks to the use of growth factors, including diversion of haematopoiesis to a specific cell type with reductions in other cells lines, toxin production, and marrow hypoplasia after "badly timed" chemotherapy; in addition, colony stimulating factors are not recommended in the 24 hours before or after conventional chemotherapy. There is also the potential for transformation with increased growth rate of malignant cells. This is potentially important as receptors to colony stimulating factors are capable, in some cases, of modulating cell proliferation in lung cancer cell lines.[22–24] Similar receptors in fresh biopsy material from small cell lung cancers (J Hampson, unpublished data) have not, however, been identified by our group.

With the growth factors currently examined there has been no evidence of late marrow failure due to "marrow exhaustion" and no evidence of the type of wasting illness seen in rodents undergoing long term administration of GM-CSF, thought to be due to excessive macrophage activation. Neutralising anti-

bodies to the growth factors have not been detected and there have been no reports of unexpected exacerbation of malignant disease.[1-3 9 15 18]

G-CSF AND GM-CSF FOR INTENSIFICATION OF CHEMOTHERAPY

The initial studies of G-CSF and GM-CSF reporting a reduction in neutropenic infections induced by chemotherapy and in antibiotic requirements and a low incidence of side effects led to the examination of growth factors in the context of dose intensification in chemotherapy. The issue is important as there is increasing evidence that dose intensification may be associated with improved response and survival in patients with various solid tumours, including lung cancer.[25]

Accelerated chemotherapy

G-CSF has been used in patients with advanced breast and ovarian cancer to facilitate escalation of the dose and rate of administration of doxorubicin. Doxorubicin could be given safely only at doses of 75 mg/m^2 every three weeks without G-CSF but 125 and 150 mg/m^2 could be administered every two weeks with G-CSF, an increase in dose intensity of up to sixfold.[26] The higher dose rates were associated with a much improved rate of complete and partial response. All patients treated with the two higher doses of 125 and 150 mg/m^2 responded, though non-myelosuppressive toxicity, particularly epithelial damage, then became dose limiting.[26] The study emphasised that the choice of the cytotoxic drugs is critical and must take into account the potential for severe non-myelosuppressive toxicity at doses higher than the conventional ones.

In a small study by Ardizzoni, in which accelerated chemotherapy with cyclophosphamide, doxorubicin, and vincristine alternating with cisplatin and etoposide was given without dose modification, a twofold increase in dose intensity was achieved in patients with small cell lung cancer by giving GM-CSF.[29] In the German study in which GM-CSF is being used preliminary results indicate that this may allow chemotherapy to be given at two weekly intervals with a schedule of doxorubicin, ifosfamide, and vincristine alternating with cisplatin and etoposide.[21] A further investigation is now being conducted in Manchester with intensive chemotherapy—with carboplatin, ifosfamide, and etoposide—in which dosages are not modified because of previous toxicity. This regimen or a variation (alternating carboplatin with cisplatin) has resulted in two year survival rates of 30% or more in patients with limited disease who had not had computed tomography and in patients with extensive disease with no other adverse factors.[27 28] In the new investigation patients are randomised to receive or not receive G-CSF and whenever possible the chemotherapy is given at much shorter intervals than the 4–6 weeks usually required for this intensive regimen. The study should identify any survival benefits that arise from dose intensification with G-CSF and show whether reduction in neutropenia is accompanied by fewer infections and sequelae.

Dose intensive chemotherapy with and without bone marrow transplantation

Repeated courses of cisplatin, etoposide, and cyclophosphamide have been given in doses for which transplantation is usually necessary to 24 patients with refractory malignancies (including some with lung cancer). G-CSF again shortened the duration of severe neutropenia in a dose related fashion and enabled these patients to have fewer days of antibiotic treatment than a control group, but their stay in hospital was not reduced. The protective effect of G-CSF (given as a 30 minute intravenous infusion) was sustained with repeated cycles of chemotherapy.[30] In another study, from Australia, subcutaneous G-CSF was given to patients receiving high dose busulphan and cyclophosphamide with autologous bone marrow transplantation. Fifteen patients with non-myeloid but chemosensitive malignancies, all of whom had been previously treated with chemotherapy and some with radiotherapy, were compared with a historical control group of 18 patients who had received the same high dose treatment alone. The 15 patients had a faster neutrophil recovery (over $0{\cdot}5 \times 10^9$/l) than the control group (mean day 11 *v* day 20). When compared with the controls there was a fewer days of parenteral antibiotic treatment (11 *v* 18 days), and a fewer days of parenteral nutrition for severe oral mucositis (10 *v* 16 days). The number of days spent in hospital (23 *v* 30) did not differ significantly. Doses of G-CSF were decreased stepwise once the neutrophil count exceeded 1×10^9/l for three consecutive days and needed to be reintroduced to maintain the count in only three of the original 15 patients.[31]

The use of GM-CSF after autologous bone marrow transplantation in patients with lymphoid malignancies has been reviewed by Appelbaum.[18] Again there was accelerated recovery in 15 patients given treatment, not only of granulocytes but also of platelets by one to two weeks, with fewer infections and earlier discharge from hospital, whereas less than 5% of 100 patients recovered by this time in the control group without GM-CSF. GM-CSF has been used to treat graft failure in patients who after a period of successful engraftment lose their grafts. Sustained increases in granulocyte counts occurred within two weeks of the start of GM-CSF treatment and this was associated with improved survival.[18] In patients with refractory breast cancer and metastatic melanoma given high dose treatment with cyclophosphamide, carmustine, and cisplatin followed by autologous bone marrow transplantation GM-CSF doses unassociated with substantial toxicity resulted in accelerated recovery of the total white cell count. The patients receiving GM-CSF tolerated chemotherapy with considerably less subjective and objective evidence of toxicity than the controls, with fewer episodes of septicaemia and less hepatotoxicity and nephrotoxicity, possibly as a result of fewer infections.[32] These and other studies suggest that accelerated and high dose chemotherapy, with repeated courses, are possible, and this will reopen the issue of repeated high dose treatment for chemosensitive tumours such as small cell lung cancer.

Peripheral blood stem cell harvesting and intensive chemotherapy

Haematopoietic colony stimulating factors also have the ability to increase levels of progenitor cells in peripheral blood. The progenitors can be harvested from the peripheral blood by leucapheresis and used as an adjunct to marrow cells, or possibly in place of them, for reconstitution after myeloablative treatment.[1 3 33–36] As an example, after high dose cyclophosphamide chemotherapy there is a 30 fold increase in peripheral blood stem cells during the rapid recovery phase of the drug induced pancytopenia. The increase can be augmented by GM-CSF up to a 1000 fold in some circumstances.[34 35] Collection of peripheral blood stem cells by leucapheresis by means of a cell separator, cryopreservation, and later reinfusion has substantially accelerated haematopoietic reconstitution after high dose chemotherapy and radiotherapy in patients with advanced malignancy, including small cell lung cancer.[35 36] Even in patients who have not received high dose chemotherapy G-CSF and GM-CSF are capable of increasing progenitor cells of both the myeloid and the erythroid series.[33] The most dramatic result is enhanced granulocyte recovery but the number of platelet transfusions may also be reduced.[35 37] The number of peripheral blood stem cells may be so much augmented that the cells can be collected with very few leucaphereses—indeed, the number of cells thought to be needed for reconstitution might be obtained from only 500 ml of whole blood after chemotherapy and treatment with colony stimulating factor.

Dose intensification is possible by shortening the intervals between courses. The results of a preliminary study with peripheral blood stem cells obtained by sequential harvesting and reinfusion after repeated courses of high dose carboplatin and GM-CSF in ovarian cancer have been described. Myelosuppression and thrombocytopenia were much less severe, with less need for transfusions, intravenous antibiotics, and stay in hospital than in patients receiving GM-CSF only. The use of the peripheral blood stem cells also allowed a substantial increase (38%) in dose intensity.[38] These techniques may well be applied in pilot studies of selected patients with small cell lung cancer.

The future

The clinical use of recombinant human haematopoietic growth factors has already led to important advances in the management of patients with cancer. Bone marrow impairment is the dose limiting complication for many chemotherapeutic agents and amelioration of the profound neutropenia and associated life threatening infections is now possible. The optimal schedules for colony stimulating factors are still unknown and not all studies with G-CSF and GM-CSF have shown a reduction in infection. Our current administration of these growth factors is unphysiological and a periodic pulse delivery of colony stimulating factors, given the physiological example of hormone release, would be perhaps more appropriate. Nevertheless, colony stimulating factors have produced clinical effects during profound bone marrow suppression when release of growth factors is likely to be at a physiological maximum. The possibility of giving a fixed dose (the contents of a single phial of G-CSF) is an attractive proposition and should be considered, given the data from the clinical dose ranging experiments. This would allow outpatient treatment with administration by the patient or a family member much more feasible. The recovery of haematopoetic stem cells from peripheral blood is likely to be of great importance in enhancing haematological recovery after myelosuppressive treatment and may even replace marrow transplantation and its associated difficulties. Much work still needs to be performed to determine the correct timing for the harvest and to find out how to obtain the optimal number of peripheral blood stem cells with colony stimulating factors used alone or in conjunction with chemotherapy.

Combinations of colony stimulating factors, including G-CSF and GM-CSF, with other growth factors, such as IL-3, cause synergistic effects in animal models. The combinations are potentially capable of restoring all aspects of haematopoiesis after marrow suppression more rapidly than a single colony stimulating factor. IL-6 is of particular interest as it has a considerable effect on megakaryocytes and may well diminish thrombocytopenia. The introduction of megakaryocytic CSF and thrombopoietin also is eagerly awaited for overcoming thrombocytopenia. Treatment with IL-3 followed by GM-CSF produces a manyfold increase in the number of progenitors within a few days of administration in primates. Such combinations should improve the yield of peripheral blood stem cells for marrow rescue after repeated courses of intensive treatment in the future. There is also the possibility of obtaining sufficient numbers of peripheral blood stem cells by venesection, and thus avoiding the logistic difficulties of leucapheresis.

Further clinical exploitation of combinations of growth factors is likely to follow in the very near future. It would seem reasonable, for example, to use IL-6 to stimulate primitive stem cells followed by IL-3 to augment multipotent progenitors and GM-CSF to improve myelopoiesis. These would be followed by other factors, such as G-CSF or erythropoietin to act on more mature precursor cells. This type of schedule might lead to maximum stimulation and more rapid recovery of all cell types after marrow suppression.

Synthetic materials also help to protect against myelosuppression and other forms of toxicity induced by chemotherapy. Ethyol WR2721 was developed as a radioprotective drug and is taken up differentially by normal rather than malignant cells. Some clinical studies indicate that ethyol can protect patients against myelosuppression, neurotoxicity, and nephrotoxicity associated with cyclophosphamide and cisplatin, an approach also worthy of further investigation.[39 40]

Some growth factors, such as GM-CSF, IL-4 and IL-6, modify the cell cycle. GM-CSF shortens the total cycle and the duration of the S phase of bone marrow cells; once it is stopped the rate of marrow proliferation falls dramatically to below the pretreatment rate. Scheduling cytotoxic drugs, with GM-CSF to reduce myelotoxicity is another interesting prospect particularly for drugs that are cell cycle specific. If haematopoietic growth factors and interleukins are required for growth of malignant cells (for example, IL-6 for the myeloma cell) via autocrine or paracrine loops, inhibitors of these factors may be of value. Antibodies to IL-6 may help patients with myeloma, for example. GM-CSF and other factors may have antitumour effects in their own right by enhancing the activity of myeloid cells and inducing release of cytokines (such as interferons) that are capable of tumour cell killing. Intraperitoneal GM-CSF in patients with ovarian cancer is currently being assessed and similar consideration could be given to malignant pleural effusions in patients with mesothelioma. Another new approach would be to use inhibitors of haematopoiesis with cancer chemotherapy. Several short (3–5) chain amino acid peptides inhibit cell proliferation and some inhibit normal haematopoiesis. Such inhibitors when clinically available could protect the bone marrow by reducing normal bone marrow cell cycling and thereby the sensitivity of the marrow to the effects of chemotherapy.

The use of recombinant DNA technology has produced a range of colony stimulating factors, which are now available for clinical study. The early results indicate that these factors substantially ameliorate some of the toxic effects of chemotherapy with conventional and even accelerated or high dose treatment. Fuller exploration of the factors currently available and those likely to be available in the near future will require some carefully designed clinical studies. We must hope not only that the colony stimulating factors will improve the safety of cancer chemotherapy but also, because they allow greater flexibility in chemotherapy, that the problems of dose intensity can be addressed and survival and other benefits examined. It is fitting that the first clinical study with a colony stimulating factor took place in patients with the most common malignancy in Britain. Further studies in these and other patients are already providing very valuable information and should lead to improvements in the management of patients with other cancers. The development of recombinant haematopoietic factors is a very potent illustration of the benefit to be obtained when basic science is linked with cancer medicine.

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