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COLONY STIMULATING FACTORS (CSFs) TO PREVENT OPPORTUNISTIC INFECTIONS

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SUMMARY

The colony stimulating factors, i.e., granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF), stimulate the production of phagocytes both *in vitro* and *in vivo*. G-CSF selective stimulates neutrophil formation; GM-CSF stimulates the formation of neutrophils, monocytes and eosinophils. G-CSF levels increase with infections and reduced levels of G-CSF result in severe neutropenia. Administration of the CSFs cause a dose dependent rise in the blood neutrophil count attributed to increased production of these cells by the bone marrow. Randomised clinical trials have established that the CSFs are useful to accelerate marrow recovery in a variety of clinical settings including myelosuppression after chemotherapy and haematopoietic transplantation. G-CSF is also useful for treatment of severe chronic neutropenia due to congenital cyclic or idiopathic neutropenia. It is also used widely for patients with neutropenia due to HIV infection and autoimmune diseases. The potency and relative safety of these agents has prompted the exploration of their use in normal subjects to facilitate the collection of neutrophils and peripheral blood stem cells. In all of these applications, the underlying principle is to increase production of phagocytes because of the vital role of these cells in preventing and containing infections.

INTRODUCTION

The colony stimulating factors or CSFs are a family of glycoproteins which play a regulatory role in the production deployment and function of white blood cells. *Bradley* and *Metcalf* (1966) coined this term after they discovered that mouse bone marrow cells will form clusters and colonies of differentiating haematopoietic cells when grown in a semi-solid medium overlaying other cells capable of producing the CSFs. Concomitantly *Ishikawa* et al. (1966) made similar observations. Subsequent studies showed that the CSFs can be detected in the supernatants from cultures of many types of cells (*Metcalf*, 1991). The CSFs are also found in serum, plasma and urine, particularly with acute infections and after endotoxin administration (*Cebon* et al., 1994; *Selig* and *Nothdurft*, 1995; *Waring* et al., 1995).

The development of molecular biotechnology has greatly expanded our knowledge of the CSFs and related haematopoietic growth factors (HGFs)

and the interleukins (now IL-1 through IL-17). There are three CSFs which have been studied extensively including clinical investigations: granulocyte colony-stimulating factor (G-CSF); granulocyte-macrophage colony-stimulating factor (GM-CSF); and macrophage colony stimulating factor (M-CSF). A fourth factor, once called multi-CSF but now called IL-3, has also been widely studied. Many of the interleukins as well as the HGFs can affect haematopoietic colony formation *in vitro*, but the term CSF is currently not used for these factors. Only G-CSF and GM-CSF are approved for clinical use by governmental authorities in the USA and Europe.

Early studies of the CSFs focused on their effects on cell proliferation. It is now widely recognised that they have many other effects, including acceleration of differentiation and maturation, enhancement of function, modulation of cytokine production and inhibition of apoptosis of both mature and immature cells (*Hill* et al., 1995; *Dale* et al., 1995; *Price* et al., 1996). This diversity of effects has provided many opportunities for clinical applications of the CSFs (*Mertelsman* and *Hermann* 1990; *Morstyn* and *Dexter*, 1994).

COLONY-STIMULATING FACTORS AND THE PRODUCTION AND FUNCTION OF PHAGOCYTES

Clinical development of the CSFs has been based on our understanding of the role of phagocytes, i.e., neutrophils, monocytes, macrophages and eosinophils, in host defences and the physiological effects of these growth factors on the deployment and function of these critical cells. Phagocytes are derived from haematopoietic stem cells. Throughout life, normal production of phagocytes depends on a continual input from these precursor cells (*Quesenberry*, 1995), (*Babior* and *Golde*, 1995); (*Lehrer* and *Ganz*, 1995). Formation of phagocytes from early precursors ordinarily takes ten to fourteen days. For neutrophils there is a marrow storage pool of cells which can be readily released into the circulation in response to endotoxaemia or infection. For monocytes and other leukocytes there are no marrow storage pools, but there are large supplies of these cells and their progeny in many tissues. Blood neutrophils have no proliferative potential; they are all destined to die in the blood or tissues. By contrast, monocytes are released earlier in their development, have considerable proliferative potential and a relatively long tissue life span (*Lehrer* and *Ganz*, 1995). These overlapping and complementary features of leukocytes contribute to the strength of the host defence system.

Infection or tissue injury provokes an acute inflammatory response. Neutrophils are mobilised from the marrow reserve and transit through the blood to the site of invasion and injury. Classical studies of inflammation demonstrate the orderly process of fluid exudation, neutrophil then monocyte accumulation, fibroblast and epithelial cell proliferation and wound healing. In this response, it is the neutrophil supply which is often deficient, leading to enhanced susceptibility to serious infections (*Dale*, 1995).

There are many clinical examples of the critical role of neutrophils. For example, in the leukocyte adhesion deficiency syndromes, neutrophils and other leukocytes are produced abundantly (*Harlan*, 1993). However, the cells cannot adhere to the vascular en-

dothelium normally. Therefore they can not migrate to the tissues and repeated serious infections occur. Glucocorticosteriod therapy predisposes to infection in part by similar mechanisms, i.e., impairment of the cellular component of acute inflammatory response (*Dale*, 1974; *Goldstein*, 1992). Impairment of the neutrophil response is also induced by administration of monoclonal antibodies directed to the critical integrins on the neutrophil surface, e.g., CD11b/18, which are responsible for mediating this response (*Harlan*, 1993). Neutropenia predisposes also to infection because of a deficient acute inflammatory response. Tissue localisation of infection fails and bacteraemia and the sepsis syndrome follow because the invasion by microbes goes unchecked (*Dale*, 1995). In some other rare diseases such as the Chediak-Higashi syndrome and glycogen storage disease 1b, there are deficiencies of both the neutrophil supply and capacity of the neutrophils to kill microbes (*Smolen* and *Boxer*, 1995). Overall, however, it is the supply of cells rather than deficiency in their capacity to kill bacteria or fungi which is the predominant phagocyte disorder predisposing to infections.

With minor infections the body's supply of phagocytes in the marrow, blood and tissues is sufficient to protect the host from serious consequences. With more severe infections, the inflammatory response and the outcome for the patient depends upon the capacity of the host to increase the supply of these cells. Patients recently receiving myelotoxic chemotherapy or radiotherapy are vulnerable to infections because of low circulating neutrophils and a reduced proliferative capacity of their haematopoietic system (*Liles* and *Dale*, 1995). In a variety of haematological disorders causing severe chronic neutropenia, e.g., congenital, cyclic and idiopathic neutropenia, the severity of reduction in the neutrophil supply determines the frequency and severity of bacterial infections (*Dale*, 1979). Agranulocytosis occurring as a idiosyncratic reaction to drugs, nutritional deficiencies such as folate and vitamin B_2 deficiency, alcoholism and ageing are other causes of increased susceptibility to infections due to a reduced neutrophil response.

CLINICAL APPLICATIONS OF THE CSFs

Because phagocytes play such a critical role in preventing opportunistic infections, there have been many efforts to find ways to stimulate their production, including treatment with vitamins, lithium, androgens and the glucocorticosteroids. In general these agents proved to be relatively ineffective (*Dale*, 1995). In the late 1980's, granulocyte colony-stimulating factor (G-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF) became available for clinical investigations. They have proven to be very useful agents for preventing fever and infections by enhancing the phagocyte supply under a variety of circumstances (*Lieschke* and *Burgess*, 1992; *Petersdorf* and *Dale*, 1994).

G-CSF

G-CSF is a 174 amino acid glycoprotein produced *in vitro* and *in vivo* by macrophages, fibroblasts, endothelial cells and other types of cells (*Molineux* and *Dexter*, 1994). Endogenous levels of G-CSF are increased with infections and endotoxaemia (*Cebon* and *Layton*, 1994). This growth factor is essential for maintenance of normal blood neu-

trophil levels. Experimentally induced deficiency states result in reduction of blood neutrophils to about 20% of normal (*Hammond* et al., 1991; *Lieschke* et al., 1994).

Early clinical trials of G-CSF showed that it induces a dose dependent and selective increase in the blood neutrophil count (*Morstyn* et al., 1988; *Gabrilove* et al., 1988). In haematologically normal persons, blood neutrophils can readily be raised to 2 to 3 times the normal level within a few hours after injection of this agent (*Chatta* et al., 1994; *Liles* et al., 1997). With repeated administration, neutrophil levels rise to a dose dependent plateau. This occurs because G-CSF increases proliferation of marrow neutrophil precursors, stimulates the transit of neutrophils through the marrow and releases the maturing cells from the marrow to the blood (*Price* et al., 1996).

Randomised control trials have established that G-CSF can reduce the duration and severity of neutropenia and reduce the occurrence of fever and infection following cancer chemotherapy (*Lieschke* and *Burgess*, 1992). Two randomised control trials established this effect (*Crawford* et al., 1991; *Trillet-Lenoir* et al., 1993). They showed that the duration of severe neutropenia was decreased from approximately 6 days to 3 days when patients with small cell lung cancer treated with a combination of cyclophosphamide, doxorubicin and etoposide received G-CSF at a daily dose of 5 μg/kg/day beginning the day after completion of the six cycles of chemotherapy. The differences in the duration of neutropenia and the occurrence of fever with neutropenia (febrile neutropenia) were statistically highly significant. Although not a primary endpoint in this trial, the occurrence of documented infections was reduced by approximately 50% (*Crawford* et al., 1991). Roughly 1/3 of the febrile patients had a documented infection. There was one important difference between these two trials. In the US study patients were randomised at enrolment, but, if they developed febrile neutropenia, they were allowed to cross over from the placebo to the treatment arm in subsequent cycles. There was a more rapid attrition of patients from the placebo group than from the G-CSF group, complicating analysis of the data. The second randomised study, performed in Europe, did not allow for cross-overs (*Trillet-Lenoir* et al., 1993). It showed similar overall results to the first trial, but also better maintenance of on-schedule administration of chemotherapy. In these studies, antibiotic treatment was not standardised so many potential interrelationships of neutropenia, prophylactic and therapeutic antibiotics and G-CSF administration were not studied. The efficacy of antibiotics, singly or in combination, as an alternative to G-CSF also has not yet been studied carefully. In general, however, prophylactic and excessive use of antibiotics in this setting is to be avoided because of the well recognised frequency of bacterial and fungal superinfection in these highly susceptible hosts (*Goldmann* et al., 1996).

Subsequent to these two randomised trials, numerous studies have confirmed that G-CSF can accelerate marrow recovery after a wide variety of chemotherapeutic agents. The efficacy of G-CSF is largely determined by the capacity of the marrow to respond to treatment. Clinical benefit is reflected by the duration and severity of neutropenia which would occur if the CSFs were or were not given. Most studies suggest that the greatest clinical benefit occurs when this cytokine is administered soon after the completion of chemotherapy, usually the following day (*Ozer*, 1994). The benefit is attributed to stimulating marrow recovery in advance of the nat-

ural response. Because there are few side effects associated with G-CSF treatments and its benefits are so readily apparent, there has been much discussion about the appropriate patients for treatment with this relatively costly therapy (*Boogaerts* and *Demuynck*, 1996). Currently it appears if there is at least a 30% risk of febrile neutropenia in a patient receiving conventional chemotherapy, the cost is justified by the reduction in hospital and other medical expenses (*Smith*, 1996). This estimate does not take into account the patient's loss of income with the occurrence of fever, infection or hospitalisation after chemotherapy or the impact of this medical treatment on the patient's quality of life.

G-CSF is also proven to accelerate bone marrow recovery after intensive chemotherapy and haematopoietic transplantation (*Bierman* et al., 1996). Benefits are in general similar to those seen after chemotherapy but may be of greater magnitude because of the marked susceptibility to infection which follows transplantation. Despite optimal care there is still a period of severe susceptibility to infection after marrow ablation which cannot be overcome by G-CSF or any other growth stimulating factors.

Soon after the introduction of G-CSF it was recognised that the peripheral blood contains large quantities of haematopoietic precursor cells a few days after starting a daily schedule of administration of this cytokine (*Sheridan*, 1996). These cells, now called peripheral blood progenitor cells (PBPCs of PBSCs), can be frozen for later infusion and can be used as an alternative to bone marrow cells for haematopoietic transplantation. Most major transplant centres now use PBPCs for autologous and allogeneic transplantation because infusion of very large numbers of these cells reduces both the duration of neutropenia and thrombocytopenia after transplantation very substantially.

G-CSF is also effective for treatment of patients with severe chronic neutropenia due to congenial, cyclic and idiopathic neutropenia (*Dale* et al., 1993). Neutrophil production in these conditions is impaired because of intrinsic disorders of neutrophil formation. Production can be enhanced by daily subcutaneous injection of G-CSF doses of 1-10 μg/kg/day for most patients. Treatment reduces the occurrence of mouth ulcers and gingivitis as well as the occurrence of fever and requirement for antibiotic treatment and hospitalisation (*Dale* et al., 1993). Continued treatment is required, but it has been well tolerated and well accepted by hundreds of patients without loss of effectiveness due to the development of antibodies to the G-CSF (*Dale*, 1995).

G-CSF is also now used on a long term basis to prevent infections associated with neutropenia in patients with myelodysplasia, human immunodeficiency virus (HIV infection) and the neutropenia associated with autoimmune diseases. In patients with presumed autoimmune neutropenia the presence of antineutrophil antibodies does not appear to reduce the efficacy of G-CSF treatment. As in the chemotherapy setting, the cost of treatment as well as the severity of the susceptibility of infections of the individual patient are critical factors in selecting patients for treatment.

GM-CSF

GM-CSF is a 127 amino acid glycoprotein which also stimulates the proliferation of haematopoietic cells of the granulocytic series *in vitro* and *in vivo* (*Gasson*, 1991). It also stimulates formation of monocytes and eosinophils. In general, the time course for the neutrophil response for GM-CSF is similar

to that for G-CSF but the increase in the blood neutrophils after GM-CSF is less (*Lieschke* and *Burgess*, 1992).

There are now numerous trials of GM-CSF to accelerate marrow recovery after chemotherapy and haematopoietic transplantation utilising both bone marrow and peripheral blood progenitor cells (*Gerhartz* et al., 1993; *Advani* et al., 1992; *Hill* et al., 1995). In both of these circumstances, GM-CSF accelerates recovery of blood neutrophils with similar effects to those observed with G-CSF. GM-CSF has been approved to hasten marrow recovery with delayed engraftment and for treatment of patients whose marrow transplant appears to have failed. It is used for most of these applications somewhat less than G-CSF because of a somewhat more severe side effect profile (*Ozer* 1994; *Hill* et al., 1995).

The use of CSFs in the treatment of acute myelocytic leukaemia (AML) has undergone rapid evolution recently (*Geller*, 1996). Patients with AML are particularly prone to infections because their primary disease impairs neutrophil production and the recovery of haematopoiesis after chemotherapy is slower than for non-haematological malignancies. Several recent randomised control trials have examined the utility of GM-CSF of G-CSF in the treatment of AML. It is very important that neither GM-CSF or G-CSF treatment was found to be associated with an increase in relapse rates for leukaemia, despite the observation that leukaemic blast cells for these patients often will proliferate on exposure to these growth factors. Several studies have demonstrated a significant improvement in neutrophil recovery with associated reduction in severe bacterial infections in most patients (*Geller*, 1996). In one trial with GM-CSF there were fewer severe infections, fewer fatal infections, fewer fatal pneumonias and fewer deaths associated with fungal infections (*Rowe* et al., 1995). As in other clinical trials, most patients in these studies have received extensive antibiotic treatment. Few inferences about the specific relationships of neutropenia, antibiotics and CSFs can be derived from these studies.

OTHER APPLICATIONS OF THE CSFs

Because patients with infections associated with severe neutropenia often have marrow diseases preventing or blunting their responsiveness to the CSFs or because their infection is so severe that they do not have time to respond, there has been recently an awakening interest in neutrophil transfusion therapy for the prevention and treatment of infections in these patients (*Maakestad* et al., 1996). Considerable impetus to these studies came from investigations in normal subjects showing that G-CSF is well tolerated and serves to rapidly increase the blood neutrophil count and expand haematopoiesis without significant side effects (*Chatta* et al., 1994). Initial clinical trials of G-CSF to mobilise and facilitate neutrophil collection from normal subjects have been encouraging and further clinical trials are now underway (*Bensinger* et al., 1993; *Caspar* et al., 1993; *Griggs* et al., 1995). In the past, this type of transfusion support has been limited by the number of cells which can be collected. After G-CSF or G-CSF plus dexamethasone, 100×10^9 neutrophils can often be collected from normal donors. Studies of the efficacy of these cells in protecting the host or accelerating clearance of infections are underway.

OTHER HAEMATOPOIETIC GROWTH FACTORS

There are a number of other HGFs at various stages of clinical development including IL-3, a molecular hybrid of IL-3 and GM-CSF, M-CSF and several interleukins. Many of these factors are regarded as working at an earlier stage of haematopoiesis than G-CSF or GM-CSF. Most appear to have at least some side effects but several of these factors may prove to be useful as adjuncts to G-CSF and GM-CSF.

LITERATURE

Advani, R., Chao, N.J., Horning, S.J., Blume, K.G., Ahn, D.K., Lamborn, K.R., Fleming, N.C., Bonnem, E.M., and Greenberg, P.L.: Granulocyte-macrophage colony-stimulating factor (GM-CSF) as an adjunct to autologous hemopoietic stem cell transplantation for lymphoma. Ann. Int. Med. 116, 183-189 (1992).

Babior, B.M., and Golde, D.W.: Production, distribution, and fate of neutrophils. In: Williams Hematology (Beutler, E,. Lichtman, M.A., Coller, B.S., and Kipps, T.J., Eds.). McGraw-Hill, Inc., New York, 773-779 (1995).

Bensinger, W.I., Price, T.H., Dale, D.C., Clift, R., Lilleby, K., Williams, B., Thomas, E.D., and Buckner, C.D.: The effects of daily recombinant human granulocyte colony-stimulating factor administration on normal granulocyte donors undergoing leukapheresis. Blood 81, 1883-1888 (1993).

Bierman, P.J., Bishop, M.R., and Armitage, J.O.: High-dose chemotherapy with cellular support for hematological malignancies. In: Cell therapy (Morstyn, G., and Sheridan, W., Eds.). Cambridge University Press, Cambridge, England, 337-351 (1996).

Boogaerts, M.A., and Demuynck, H.M.: Consensus on the clinical use of myeloid growth factors. Curr. Opn. Hematol. 3, 241-246 (1996).

Bradley, T.R., and Metcalf, D.: The growth of mouse bone marrow cells *in vitro*. Aust. J. Exp. Biol. Med. Sci. 44, 287-294 (1966).

Caspar, C.B., Seger, R.A., Burger, J., and Gmür, J.: Effective stimulation of donors for granulocyte transfusions with recombinant methionyl granulocyte colony-stimulating factor. Blood 81, 2866-2871 (1993).

Cebon, J., Layton, J.E., Maher, D., and Morstyn, G.: Endogenous haemopoietic growth factors in neutropenia and infection. Br. J. Haematol. 86, 265-274 (1994).

Cebon, J., and Layton, J.: Measurement and clinical significance of circulating hematopoietic growth factor levels. Curr. Opin. Hematol. 1, 228-234 (1994).

Chatta, G.S., Price, T.H., Allen, R.C., and Dale, D.C.: The effects of *in vivo* recombinant methionyl human granulocyte colony stimulating factor (rhG-CSF) on the neutrophil response and peripheral blood colony forming cells in healthy young and elderly volunteers. Blood 84, 2923-2929 (1994).

Crawford, J., Ozer, J., Stoller, R., Johnson, D., Lyman, G., Tabbara, I., Kris, M., Grous, J., Picozzi, V., Rausch, G., Smith, R., Gradishar, W., Yahanda, A., Vincent, M., Stewart, M., and Glaspy, J.: Reduction by granulocyte colony-stimulating factor of fever and neutropenia induced by chemotherapy in patients with small-cell lung cancer. N. Engl. J. Med. 325, 164-170 (1991).

Dale, D.C., Fauci, A.S., and Wolff, S.M.: Alternate-day prednisone: Leukocyte kinetics and susceptibility to infections. N. Engl. J. Med. 29l, 1154-1158 (1974).

Dale, D.C., Guerry, D., Werwerka, J.R., Bull, J.M., and Chusid, M.H.: Chronic neutropenia. Medicine 58, 128-144 (1979).

Dale, D.C., Bonilla, M.A., Davis, M.W., Nakanishi, A., Hammond, W.P., Kurtzberg, J., Wang, W., Jakubowski, A., Winton, E., Lalezari, P., Robinson, W., Glaspy, J.A., Emerson, S., Gabrilove, J., Vincent, M., and Boxer, L.A.: A randomized controlled phase III trial of recombinant human G-CSF for treatment of severe chronic neutropenia. Blood 81, 2496-2502 (1993).

Dale, D.C.: Neutropenia. In: Williams Hematology (Beutler, E., Lichtman, M.A., Coller, B.S., and Kipps, T.J., Eds.). McGraw-Hill, Inc., New York 815-824 (1995).

Dale, D.C., Liles, W.C., Summer, W., and Nelson, S.: Granulocyte-colony-stimulating factor: Role and relationships in infectious diseases. J. Inf. Dis. 172, 1061-1075 (1995).

Dale, D.C.: Hematopoietic growth factors for the treatment of severe chronic neutropenia. Concise Review. Stem Cells 13, 94-100 (1995).

Gabrilove, J.L., Jakubowski, A., Fain, K., Grous, J., Scher, H., Sternberg, C., Yagoda, A., Clarkson, B., Bonilla, M.A., Oettgen, H.F., Alton, K., Boone, T., Altrock, B., Welte, K., and Souza, L.: Phase I study of granulocyte colony-stimulating factor in patients with transitional cell carcinoma of the urothelium. J. Clin. Invest. 82, 1454-1461 (1988).

Ganz, T., and Lehrer, R.I.: Production, distribution, and fate of monocytes and macrophages. In: Williams Hematology (Beutler, E., Lichtman, M.A., Coller, B.S., and Kipps, T.J., Eds.). McGraw-Hill, Inc., New York 875-878 (1995).

Gasson, J.C.: Molecular physiology of granulocyte-macrophage colony-stimulating factor. Blood 6, 1131-1145 (1991).

Geller, R.B.: Use of cytokines in the treatment of acute myelocytic leukemia: A critical review. J. Clin. Oncol. 14, 1371-1382 (1996).

Gerharz, H.H., Engelhard, M., Meusers, P., Brittinger, G., Wilmanns, W., Schlimok, G., Mueller, P., Huhn, D., Musch, R., Siegert, W., Gerhartz, D., Hartlapp, J.H., Thiel, E., Huber, C., Peschl, C., Spann, W., Emmerich, B., Schadek, C., Westerhausen, M., Pees, H.-W., Radtke, H., Engert, A., Terhardt, E., Schick, H., Binder, T., Fuchs, R., Hasfor, J., Brandmair, R., Stern, A.C., Jones, T.C., Erlich, H.J., Stein, H., Parwaresch, M., Tiemann, M., and Lennert, K.: Randomized, double-blind, placebo-controlled, phase III study of recombinant human granulocyte-macrophage colony-stimulating factor as adjunct to induction treatment of high-grade malignant non-Hodgkin's lymphomas. Blood 82, 2329-2339 (1993).

Goldmann, D.A., Weinstein, R.A., Wenzel, R.P., Tablan, O.C., Duma, R.J., Gaynes, R.P., Schlosser, J., and Martone, W.J.: Strategies to prevent and control the emergence and spread of antimicrobial-resistant microorganisms in hospitals: A challenge to hospital leadership. JAMA 275, 234-240 (1996).

Goldstein, R.A., Bowen, D.L., and Fauci, A.S.: Adrenal corticosteroids. In: Inflammation: Basic Principles and Clinical Correlates (2nd edition) (Gallin, J.I., Goldstein, I.M., and Snyderman, R., Eds.). Raven, New York, 1061-1082 (1992).

Grigg, A., Lusk, J., and Szer, J.: G-CSF stimulated donor granulocyte collections for neutropenic sepsis. Leuk. Lymphoma 18, 329-334 (1995).

Hammond, W.P., Csiba, E., Canin, A., Souza, L.M., and Dale, D.C.: Chronic neutropenia: A new canine model induced by human G-CSF. J. Clin. Invest. 87, 704-710 (1991).

Harlan, J.M.: Leukocyte adhesion deficiency syndrome: Insights into the molecular basis of leukocyte emigration. Clin. Immunol. Immunopathol. 67, S16-S224 (1993).

Hill, A.D.K., Naama, H.A., Calvano, S.E., and Daly, J.M.: The effect of granulocyte-macrophage colony-stimulating factor on myeloid cells and its clinical applications. J. Leuko. Biol. 58, 634-642 (1995).

Ishikawa, Y., Pluznick, D.H., and Sachs. L.: *In vitro* control of the development of macrophage and granulocyte colonies. Proc. Natl. Acad. Sci. USA 56, 488-495 (1966).

Lieschke, G.J., and Burgess, A.: Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor, parts I and II. N. Engl. J. Med. 327, 28-35; 99-106, (1992).

Lieschke, G.J., Grail, D., Hodgson, G., Metcalf, D., Stanley, E., Cheers, C., Fowler, K.J., Basu, S., Zhan, Y.F. and Dunn, A.R.: Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell deficiency, and impaired neutrophil mobilization. Blood 84, 1737-1746 (1994).

Liles, W.C., and Dale, D.C.: Current approach to the management of neutropenia. J. Int. Care. Med. 10, 283-293 (1995).

Liles, W.C., Huang, J.E., Llewellyn, C., SenGupta, D., Price, T.H., and Dale, D.C.: A comparative trial of granulocyte colony-

stimulating factor (G-CSF) and dexamethasone alone and in combination for the mobilization of neutrophils in the peripheral blood of normal human volunteers. Transfusion, in press (1997).

Maakestad, K., Mazanet, R., Liles, W.C., and Dale, D.C.: Neutrophil Transfusions for Treatment of Infections. In: Cell Therapy (Morstyn, G., and Sheridan, W.P., Eds.). Cambridge University Press, Cambridge, 510-526 (1996).

Metcalf, D.: Control of granulocytes and macrophages: Molecular, cellular, and clinical aspects. Science 254, 529-533 (1991).

Mertelsmann, R., and Herrmann, F. (Eds.): Hematopoietic growth factors in clinical applications. Marcel Dekker, Inc., New York (1990).

Molineux, G., and Dexter, T.M.: Biology of G-CSF. In: Filgrastim (r-metHuG-CSF) in clinical practice (Morstyn, G., and Dexter, T.M., Eds.). Marcel Dekker, New York 1-21 (1994).

Morstyn, G., Campbell, L., Souza, L.M., Alton, N.K., Keech, J., Green, M., Sheridan, W., Metcalf, D., and Fox, R.: Effect of granulocyte colony-stimulating factor on neutropenia induced by cytotoxic chemotherapy. Lancet i, 667-672 (1988).

Morstyn, G., and Dexter, T.M. (Eds.): Filgrastim (r-metHuG-CSF) in clinical practice. Marcel Dekker, Inc., New York (1994).

Ozer, H. (for: Am. Soc. Clin. Oncol. ad hoc CSSF Guidelines Expert Panel): American Society of Clinical Oncology recommendations for the use of hematopoietic colony-stimulating factors: Evidence-based clinical practice guidelines. J. Clin. Oncol. 12, 2471-2508 (1994).

Petersdorf, S.H., and Dale, D.C.: The biology and clinical applications of erythropoietin and the colony-stimulating factors. In: Advances in internal medicine (Schrier, R.W., Ed.). Mosby-Year Book, Chicago, 40, 395-428, (1994).

Price, T.H., Chatta, G.S., and Dale, D.C.: The effect of recombinant granulocyte colony-stimulating factor on neutrophil kinetics in normal young and elderly humans. Blood 88, 335-340 (1996).

Quesenberry, P.J.: Hemopoietic stem cells, progenitor cells, and cytokines. In: Williams Hematology (Beutler, E., Lichtman, M.A., Coller, B.S., and Kipps, T.J., Eds.). McGraw-Hill, Inc., New York 211-228 (1995).

Rowe, J.M., Andersen, J.W., Mazza, J.J., Bennett, J.M., Paietta, E., Hayes, F.A., Oette, D., Cassleth, P.A., Stadtmauer, E.A., and Wiernik, P.H.: A randomized placebo-controlled phase III study of granulocyte-macrophage colony-stimulating factor in adult patients (≥55 to 70 years of age) with acute myelogenous leukemia: A study of the Eastern Cooperative Oncology Group (E1490). Blood 86, 457-462 (1995).

Selig, C., and Nothdurft, W.: Cytokines and progenitor cells of granulocytopoiesis in peripheral blood of patients with bacterial infections. Infect. Immun. 63, 104-109 (1995).

Sheridan, W.: Cytokine-only approaches to mobilization of progenitor cells. In: Cell therapy (Morstyn, G., and Sheridan, W., Eds.). Cambridge University Press, Cambridge, 146-182 (1996).

Smith, T.J.: Economic analysis of the clinical uses of the colony-stimulating factors. Curr. Opn. Hematol. 3, 175-179 (1996).

Smolen, J.E., and Boxer, L.A.: Functions of neutrophils. In: Williams hematology (Beutler, E., Lichtman, M.A., Coller, B.S., and Kipps, T.J., Eds.). McGraw-Hill, Inc., New York, 779-798 (1995).

Trillet-Lenoir, V., Green, J., Manegold, C., Green, J., Manegold, C., VonPawel, J., Gatzemeier, U., Lebeau, B., Depierre, A., Johnson, P., Decoster, G., Tomita, D., and Ewen, C.: Recombinant granulocyte colony-stimulating factor reduces the infectious complications of cytotoxic chemotherapy. Eur. J. Cancer 29A, 319-324 (1993).

Waring, P.M., Presneill, J., Maher, D.W., Layton, J.E., Cebon, J., Waring, L.-J., and Metcalf, D.: Differential alterations in plasma colony-stimulating factor concentrations in memingococcaemia. Clin. Exp. Immunol. 102, 501-506, (1995).