5. The Influence of Colony-Stimulating Factors on Neutrophil Production, Distribution, and Function

J. Milton Gaviria, W. Conrad Liles, David C. Dale

Introduction

The colony-stimulating factors (ie. interleukin 3, granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor and monocyte/macrophage colony stimulating factor) are a group of glycoproteins that regulate the proliferation and differentiation of hematopoietic precursor cells [1-4]. In addition to their effects on hematopoiesis, colony-stimulating factors (CSFs) modulate the function of fully mature cells and therefore play an important role in regulating inflammatory responses vital to host defense. Here we review recent information that describes the biological activity of CSFs, particularly focusing on their modulation of neutrophil production, distribution, and function. The spectrum of biological activity of granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) is summarized in Table 1.

Differential effect of G-CSF and GM-CSF on neutrophil function.

In vitro marrow culture studies have furthered our understanding of the different effects of CSFs on hematopoietic proliferation and differentiation. For instance, when hematopoietic stem cells were cultured in the presence of 100 U/mL of IL-3, a 100-fold increase in cell number was seen within 7 days. Throughout this time course, more than 95% of the cells exhibited a primitive blast morphology. Very little spontaneous differentiation was observed, but when present, the maturing cells consisted of metamyelocytes [8,9]. When the IL-3 concentration was reduced to 1 U/mL, only limited proliferation was observed, but a greater proportion of mature cells was present following 7 days of culture, including some mature neutrophils. When cells were grown with GM-CSF only, little increase in cell number was seen after 7 days, but the cultures contained more mature neutrophils and macrophages. These data indicate that IL-3 supports the maintenance and proliferation of early cells, whereas GM-CSF acts predominantly as a differentiation factor [8].

Recent studies have shown that a combination of early acting factors affecting the kinetics of cell cycle-dormant primitive stem cells is necessary to stimulate the proliferation and differentiation of primitive hematopoietic stem/progenitor cells. Combinations of stem cell factor (SCF; c-kit ligand) or a ligand for type III receptor tyrosine kinase (flt3 RTK; FL) with IL-3 or GM-CSF showed a distinct synergistic effect on primitive stem cells [10,11]. Furthermore, the combination of three signals through the activation of gp130 (a signal-transducing receptor), c-kit, and IL-3 receptor (IL-3R) exerted a dramatic synergistic action on hematopoietic colony formation [12]. In the presence of these three signals, multipotential progenitors and committed cells could proliferate and differentiate to form colonies in the absence of terminally acting

J. R. Wingard et al. (eds.), *Clinical Applications of Cytokines and Growth Factors*

Table 1. Effects of G-CSF and GM-CSF on Neutrophil Distribution and Function

	IN VITRO		IN VIVO	
	G-CSF	GM-CSF	G-CSF	GM-CSF
Adhesion	↑ [60, 61]	↑ [57,58,62,63]	↑ [64,65,66, 67]	↑ [63]
	n [57, 58]			
Chemotaxis	↑ [68, 69]	↑ [69,74]	↑ [85]	n [71, 83, 84]
	n [70]	↓ [70-72, 75]	n [80-83]	↓ [23, 78, 79]
	↓ [72, 73]		↓ [39, 65-67, 76, 77]	
CD11b/CD18	↑ [57, 58, 60, 70, 72]	↑ [57, 58, 62, 63, 70, 72, 87, 88]	↑ [64, 66, 84, 90, 91]	↑ [23, 57, 92]
L-selectin	↓ [57, 58, 70, 96, 97]	↓ [57, 58, 70]	↓ [57, 96 ,97]	↓ [57]
Phagocytosis	↑ [69, 77, 90, 102, 104]	↑ [69, 87, 90, 102, 103, 105]	↑ [66,77,85,90,107,108]	↑ [78]
			n [82,106]	
FcgRI	↑ [109]	↑ [69]	↑ [65,66,110,112,113]	n [23, 112]
	n [69, 88, 110, 111]	n [88]		
FcgRII	↑ [109]		↑ [66]	n [23]
FcgRIII	↑ [88, 89]	n [88]	n [66]	n [23]
		↓ [115]	↓ [65,73,89,113,115]	↓ [116]
CR 1	n [69]	↑ [69]	↑ [66,67]	
Respiratory Burst	↑ [60,70,72,117,118, 122,143]	↑ [70,72,87,102,117, 119,120, 121,122]	↑ [38,66,67,91,124-126, 127,130-134]	↑ [128-130, 132, 133, 135]
	↓ [136]		n [66]	
			↓ [137]	
Microbicidal Activity	↑ [69,72,105,109, 142,143]	↑ [69,138,139]	↑ [140,144]	↑ [146]
	n [140]	n [103,119]	n [136,145]	
ADCC	↑ [27,145,147,148]	↑ [27,147,148,149]	↑ [93,112]	↑ [150]
Degranulation	↑ [131]	↑ [101,102, 121,139, 151]	↑ [89,154]	↑ [154]
CD14	↑ [92]	↑ [92]	↑ [65,73,92,93,115]	n [92]
Apoptosis	↓ [165,166,168,176]	↓ [-165-170]	↓ [177,178]	↓ [178]

↑- increased; ↓ - decreased; n – no effect.

lineage-specific factors, such as G-CSF, erythropoietin, and thrombopoietin. Moreover, data from single-cell suspension cultures suggested that IL-3 support was important for survival and initial proliferation, whereas SCF was important for enhancement of progenitor cell proliferation [12,13].

Because of the known synergistic activities of hematopoietic growth factor combinations [14,15] and based on the assumption that normal hematopoiesis is regulated

by such combinations, *in vitro* studies have been conducted to stimulate cell proliferation and simultaneously facilitate differentiation and maturation. *In vitro* expansion of progenitors and neutrophil precursors from enriched $CD34^+$ marrow cells has been achieved by the combination of IL-3, GM-CSF and G-CSF, which also increased the proportion of cells expressing the myeloid antigens CD13, CD15, CD11b and CD14 [13]. Addition of SCF to this mixture of factors further increased the production of progenitors and precursors and helped to maintain the cells at a less differentiated stage early in the cultures [13].

In vivo, human studies have shown that IL-3 is a powerful activator of the proliferation of all myelopoietic progenitors. The effect is dose dependent, and the various progenitors have different degrees of sensitivity; megakaryocyte progenitors appear to be the most sensitive, followed by erythroid and granulocyte/monocyte progenitors, orderly [16]. Furthermore, IL-3 acts as a primer for the action of other cytokines. Purified marrow progenitors obtained at the end of 7 days of IL-3 administration are more sensitive *in vitro* to the effect of optimal doses of G-CSF. This priming effect, also observed *in vivo* in the presence of physiological concentrations of G-CSF, could explain the modest increase in the proliferative activity of the morphologically recognizable granulocyte progenitors induced by IL-3 [17]. The same priming effect is seen with GM-CSF, which induces simultaneous stimulation of granulopoiesis, eosinophilopoiesis, and monocytopoiesis [18,19]. Clinical trials with IL-3 alone, however, have resulted in significant side effects with only very modest changes in blood cell counts [20-22]. Likewise, *in vivo* studies in healthy volunteers showed that GM-CSF at a dose of 250 mcg/m^2 causes neutrophilia mainly by accelerating delivery of neutrophils from the bone marrow to the blood and by decreasing migration from the blood to the tissues, with only a modest effect on neutrophil production and blood half-life [23]. Combinations of IL-3 and GM-CSF have been used in an attempt to better stimulate granulopoiesis. A study in cancer patients, showed a 3.2-fold amplification in neutrophil production, which required at least 2 extra divisions in the marrow [19]. The results were comparable with those of a previous trial using GM-CSF alone, but significant ineffective granulopoiesis developed in this earlier study [24]. These findings further suggest that IL-3 serves as a priming factor to increase the proliferative activity of precursors, while GM-CSF functions primarily as a differentiation factor. Similar results were seen in patients after bone marrow transplantation [25,26].

Like other growth factors , G-CSF stimulates cell proliferation and function by interacting with a specific cell surface receptor [27,28]. The G-CSF receptor is a 140-kDa homodimer, similar in structure to the receptors for erythropoietin and some interleukins. In contrast, other hematopoietic growth factor receptors are composed of two or three dissimilar components. Although G-CSF receptors may be found in other tissues, biologic functions are largely limited to hematopoietic cells. Experimental evidence indicates that G-CSF is necessary not only for the maintenance of normal neutrophil levels but also for the development of neutrophilia with infections.

Hammond et al. showed that dogs given repeated injections of human G-CSF produced antibodies that cross-reacted with recombinant canine G-CSF. Dogs with the anti-G-CSF antibodies developed sustained neutropenia that lasted until the antibodies had declined to undetectable levels, suggesting that G-CSF is necessary for the maintenance of a normal neutrophil count [29]. However, the bone marrow of these

animals showed neutrophil development that appeared normal in its early stages, suggesting that other cytokines may support the earlier phases of neutrophil formation. Lieschke et al. reported the presence of chronic neutropenia (with levels 20%-30% of normal) in G-CSF knockout mice, with marrow histology similar to that of dogs with G-CSF deficiency [30]. The mice responded to G-CSF administration by developing normal blood neutrophil levels. It is noteworthy that dogs repeatedly injected with human GM-CSF and mice rendered deficient in GM-CSF by "gene knockout" experiments are not neutropenic [31,32].

Circulating G-CSF levels rise promptly as an acute-phase response to endotoxin injections and in various infectious diseases [33-35]. A dramatic example of this response was reported in a patient who self-injected 1 mg of *Salmonella minnesota* endotoxin and subsequently developed hypotension and sepsis syndrome. Twenty two hours later, the G-CSF level was 277 ng/mL, equivalent to the levels achieved after intravenous injection of approximately 25 mcg/kg recombinant G-CSF [36]. Cebon et al. examined the level of G-CSF, interleukin-6 (IL-6), GM-CSF, and monocyte/macrophage colony-stimulating factor (M-CSF) in normal subjects, febrile neutropenic patients, and bacteremic patients who were not neutropenic [37]. G-CSF, IL-6, and M-CSF levels were elevated with fever, but GM-CSF levels were not. In multiple regression analysis, the investigators showed that G-CSF levels correlated with fever, neutropenia, and pathogen type (higher levels in patients with gram-negative infections compared to those with gram-positive infections). Thus, a growing body of evidence suggests that G-CSF is necessary not only for the maintenance of normal neutrophil levels but also for the development of neutrophilia with infections.

Normally, neutrophil development requires approximately 6 days from the last stage of cell division, the myelocyte stage, before entry into the blood, as measured by *in vivo* labeling with ^{3}H-TdR [38]. Daily injection of G-CSF (30 mcg/day) to healthy young and elderly volunteers reduced this interval to approximately 4.5 days. A higher dose of G-CSF (300 mcg/day) further reduced the transit time to approximately 3 days and expanded the neutrophil mitotic pool, without significantly affecting blood neutrophil circulatory half-life or the distribution of blood neutrophils between the marginal and circulating pools [38,39]. A single injection of G-CSF (300 mcg) increased the blood neutrophil count about 4 fold [40]. Repeated administration of G-CSF over several days in hematologically healthy volunteers causes sustained neutrophilia [39-42]. Thus, daily subcutaneous G-CSF injection induces a progressive elevation of the base line count to a plateau, with superimposed acute increases in the count after each dose is given, which far exceeds the normal variation. On the basis of the observation that the percentage of circulating band neutrophils is increased, the acute neutrophilia after G-CSF administration is generally attributed to the release of maturing cells from bone marrow neutrophil reserves resulting from the dose-dependent shortening of the marrow transit time [38-40].

G-CSF also causes neutrophilia by stimulating proliferation of all stages of neutrophil development through the myelocyte stage [39,41,43]. Cells of the neutrophil lineage, including undifferentiated cells ($CD34^{+}$ $CD33^{-}$), have receptors for G-CSF and respond to this cytokine with increased proliferation [44]. G-CSF is also able to raise circulating $CD34^{+}$ hematopoietic progenitor cells in peripheral blood of healthy volunteers, cancer patients and severely ill patients with the acquired

immunodeficiency syndrome (AIDS) [45]. Histologically, a prompt increase in the percentage of cells engaged in active DNA synthesis is present in marrow aspirate and biopsy samples [46]. Marrow cells exhibit features arising from both stimulated production and accelerated marrow release [38-40]. With aging, there seems to be no apparent difference in the hematopoietic-precursor response to factors that are regarded as early multilineage regulators (i.e., IL-3 and GM-CSF). In addition, bone marrow hematopoietic progenitors from healthy elderly subjects have reduced sensitivity, but similar absolute responsiveness to G-CSF compared to healthy young subjects [39,40,44,47,48].

M-CSF exerts its physiologic activity on cells committed to the monocyte/macrophage lineage, enhancing their replication, differentiation, and protein synthesis. Early clinical studies with M-CSF reported amelioration of neutropenia induced by conventional dose chemotherapy for solid tumors [49], and a more rapid neutrophil recovery in patients undergoing bone marrow transplantation (BMT) [50]. However, more recent clinical studies have been unable to demonstrate any effect of M-CSF on neutrophil recovery after BMT [51-53]. Current available data suggest that this growth factor has no significant activity on neutrophils [54].

Effects of Colony-Stimulating Factors on Neutrophil Distribution

Neutrophils leave the marrow storage compartment and enter the blood, and there is no evidence that they re-enter the marrow or recirculate once they leave the blood and enter the tissues[55]. The total blood neutrophil pool consists of all the neutrophils in the vascular spaces. A substantial percentage of these neutrophils do not circulate freely but adhere to the endothelium of blood vessels. These adherent cells constitute the marginated neutrophil pool, which accounts for approximately half of the total number of neutrophils [56]. The behavior of neutrophils in the blood appears to be controlled predominantly by two classes of membrane-bound adhesion proteins, L-selectin (LAM-1) and the β_2 integrin receptor CD11b/CD18, which regulate neutrophil rolling and transendothelial migration, respectively [57-59].

The mature neutrophil lacks IL-3 receptors and, thus, is not affected by IL-3. However, i*n vitro* stimulation of peripheral blood neutrophils with either G-CSF or GM-CSF leads to rapid (maximal by 30 min) up-regulation of CD11b/CD18 and down-regulation of L-selectin (LAM-1) [57,58]. Early *in vitro* studies reported increased neutrophil adherence after treatment with G-CSF [60,61], but recent investigators have failed to detect an effect of G-CSF on neutrophil adherence [57,58]. In contrast, GM-CSF has consistently been shown to increase neutrophil adhesion to vascular endothelium [57,58,62,63]. In addition, GM-CSF is also able to down-regulate neutrophil L-selectin (LAM-1) expression [57]. When G-CSF was administered to healthy volunteers, neutrophils displayed enhanced expression of CD11b/CD18 and increased adhesion to E-selectin and ICAM-1 (CD54) [64]. G-CSF treatment of patients with cancer and severe neutropenia also resulted in an increased adherence of the induced neutrophils to plastic surfaces [65]. Yong et al. demonstrated that GM-CSF enhances neutrophil adherence to human endothelial monolayers *in vitro,* while G-CSF, despite producing a rise in CD11b expression of similar magnitude to that of GM-CSF, had no effect on neutrophil adherence [57]. G-CSF administered to healthy volunteers and patients with

aplastic anemia caused increased neutrophil adhesion to nylon wool, which peaked on day 4 or 5 of treatment [64,66,67]. *In vivo,* although the time course and magnitude of the margination responses are similar for G-CSF and GM-CSF, the kinetics of margination differ. Following G-CSF, neutrophils marginate earlier with complete recovery of counts by 60 minutes, whereas at 2 hours after GM-CSF, peripheral cell counts were still at 50 percent of preinfusion levels [57].

Effects of Colony-Stimulating Factors on Neutrophil Function

Neutrophil chemotaxis and tissue migration. Chemotaxis refers to the directed migration of neutrophils along a concentration gradient of a particular stimulus, known as a chemotatic factor. G-CSF has been shown to serve as a chemotactic agent for neutrophils *in vitro* [68]. Checkerboard experiments performed using polycarbonate filters showed that maximal induction of migration occurred in the presence of a positive concentration gradient [68]. Bober et al. showed that pre-exposure of neutrophils to G-CSF or GM-CSF for 15 minutes stimulated the motility of the cells toward soluble stimuli such as formyl-methionyl-leucyl-phenylalanine (FMLP) [69]. However, other in vitro experiments have yielded contrasting results, including a dose-related inhibition of neutrophil chemotaxis after priming with GM-CSF [70-72]. Other studies have shown no effect or inhibition of chemotaxis after priming with G-CSF [70,72,73]. In general, the balance of data indicates that both G-CSF and GM-CSF enhance neutrophil chemotaxis *in vitro* at low concentrations and inhibit neutrophil motility at high concentrations [68,70,71,74,75]. This biphasic response presumably serves to attract and immobilize neutrophils at sites of inflammation. Chemokinesis, which refers to the random migration of neutrophils in response to a particular stimulus, is stimulated *in vitro* by both G-CSF [58,68] and GM-CSF [58].

In vivo experiments in healthy volunteers [65,66], patients with cancer [65,66], patients with aplastic anemia [67], and patients following bone marrow transplantation[76] have demonstrated decreased neutrophil chemotaxis after administration of G-CSF. In one study, chemotaxis toward FMLP was decreased after 2-3 d of treatment and returned to normal values 3-5 d after cessation of G-CSF therapy [65]. Furthermore, studies performed in our laboratory in healthy volunteers showed decreased neutrophil migration after administration of 300 mcg/d of G-CSF for 5 days, as demonstrated by the lack of proportional increase in neutrophil count in skin chambers and buccal neutrophils [39]. Similar results were observed after daily administration of GM-CSF (250 mcg/m^2) [23]. Other *in vivo* studies investigating neutrophil function in G-CSF-treated healthy volunteers and patients with cancer have shown decreased neutrophil migration into tissues [65,66,76,77]. During continuous intravenous infusion of GM-CSF, a markedly reduced neutrophil migration was found in a skin chamber assay [78,79]. However, other studies have not shown impairment of neutrophil migration *in vivo* after treatment with G-CSF [80-82] or GM-CSF [83,84]. Lieschke et al. evaluated the presence of neutrophils in the oral cavity in patients after bone marrow transplantation using a mouth rinse assay [80]. The percentage of neutrophils recovered from the oral cavity was not altered by G-CSF administration, suggesting that G-CSF did not impair tissue migration of primed neutrophils. In addition, another study demonstrated that G-CSF was able to improve abnormal neutrophil chemotaxis when given to patients with

myelodysplastic syndrome [85]. Factors that might be responsible for the variable results on neutrophil chemotaxis and tissue migration include the use of different assays, different doses and routes of administration, and different intervals between administration of the growth factor and blood collection.

Integrins and selectins. The β_2 integrin subfamily of adhesion molecules play an important role in the regulation of neutrophil adhesion and migration into tissues [86]. Expression of CD11b, an α-subunit of the β_2 integrin subfamily, is up-regulated on neutrophils by G-CSF [57,58,60,70,72] and GM-CSF [57,58,62,63,70,72,87,88] *in vitro.* Up-regulation of CD11b has been observed following administration of G-CSF to healthy volunteers [64,66,89] and patients with malignancy[90,91] . Likewise, GM-CSF administered *in vivo* stimulated CD11b expression in healthy volunteers [23] and cancer patients[57,92] . Up-regulation of CD11b is a very early event after administration of G-CSF, with maximal expression levels seen approximately 1 hour after administration [90,91,93]. These changes in surface expression of adherence molecules may represent the mechanism underlying the acute but transient neutropenia observed following the administration of either G-CSF [41,94] or GM-CSF [23].

In vitro studies performed in healthy volunteers have shown that both G-CSF and GM-CSF down-regulate expression of L-selectin (LAM-1) on neutrophils [58,70]. Also, both colony stimulating factors have been reported to initially increase the affinity of L-selectin (LAM-1) for its ligand [95], then to decrease L-selectin (LAM-1) surface expression via shedding [57,95,96,97]. Serum concentrations of soluble L-selectin (sL-selectin) and sE-selectin were elevated after administration of G-CSF to healthy volunteers and patients with cancer [98]. sL-selectin retained functional activity and inhibited L-selectin-dependent leukocyte attachment to endothelial cells [99,100]. Conceivably, altered L-selectin expression may play a role in the regulation of the adhesive functions of primed neutrophils.

Phagocytosis. *In vitro*, GM-CSF [69,87,101-104] and G-CSF [69,101,105] stimulate phagocytic activity of normal neutrophils. The phagocytic activity of neutrophils stimulated with GM-CSF [78] and G-CSF [66,77,82,90,106,107] *in vivo* was enhanced or normal in most studies. G-CSF has been found to enhance the abnormal phagocytic activity of neutrophils in patients with myelodysplastic syndrome [85] and chronic graft-versus-host disease [108]. The enhancement of neutrophil phagocytosis by GM-CSF and G-CSF is related to increased expression of Fc receptors for IgG (FcγR) [known as the high-affinity receptor FcγRI (CD64) and the low-affinity receptors FcγRII (CD32) and FcγRIII (CD16)], and receptors for the C3b and C3bi complement fragments known as complement receptor 1 (CR1; CD35) and CR3 (CD11b/CD18) [74,109].

Immunoglobulin and complement receptors. Under natural conditions, expression of FcγRI (CD64) is restricted to monocytes in various stages of development and early precursors of the myelocyte lineage [110]. With maturation into band forms and polymorphonuclear cells, Fcγ RI (CD64) expression diminishes strongly. Studies have shown that GM-CSF and G-CSF have only a marginal effect *in vitro* on CD64 expression by mature neutrophils [69,88,110,111]. However, during G-CSF-induced *in vitro* myeloid differentiation, the CD64 receptor remains expressed during final maturation [110]. Sullivan et al. reported increased expression of FcγRI (CD64) after *in vitro* stimulation of mature neutrophils with G-CSF [109]. A strong induction of FcγRI (CD64)

on mature neutrophils has been observed after administration of G-CSF to healthy volunteers [65,66,110] and patients with cancer [65,112,113], cyclic neutropenia [112] and AIDS [113]. Administration of GM-CSF to healthy volunteers failed to affect on expression of FcγRI (CD64) [23].

Mature neutrophils constitutively express low affinity receptors for multimeric IgG, [FcγRII (CD32) and FcγRIII (CD 16)] which mediate binding and lysis of cells coated with IgG, in a process known as antibody-dependent cellular cytotoxicity (ADCC), as well as binding and phagocytosis of immune complexes [114]. *In vitro*, G-CSF stimulates the expression of FcγRII (CD32) [109] and FcγRIII (CD16) [88,89], while expression of FcγRIII (CD16) is either decreased or unaltered by GM-CSF [88,115]. In studies conducted in healthy volunteers, administration of G-CSF increased neutrophil expression of FcγRII (CD32), whereas FcγRIII (CD16) expression did not differ from base line [66]. Others have found the expression of FcγRIII to be strongly decreased after G-CSF administration to healthy volunteers [65,89], patients following cancer chemotherapy [65,113], and patients with congenital neutropenias [73,115]. GM-CSF *in vivo* produced decreased neutrophil expression of FcγRIII (CD16) in one study [115] and no effect in expression of FcγRII (CD32) and FcγRIII (CD16) in another study [23].

The decreased expression of FcγRIII (CD16) after administration of G-CSF and GM-CSF may be related to either their respective actions on neutrophil precursors, favoring the release of partially immature granulocytes from the marrow, or shedding of the receptor. Kerst et al. found that levels of soluble FcγRIII (sFcγRIII; sCD16) were increased for as long as 10 days in healthy volunteers after a single dose of G-CSF [93]. Similar results have been published by others [113]. More research is needed to clarify the biological and clinical relevance of these changes on neutrophil function.

In vitro studies have shown that expression of CR1 (CD35) and CR3 (CD11b/CD18) is increased in GM-CSF-primed neutrophils. In contrast, G-CSF reportedly does not enhance expression of these cell-surface receptors [69]. Nevertheless, when neutrophil microbicidal activity against *S. aureus* was evaluated, no difference was seen between GM-CSF- or G-CSF-primed neutrophils. In a different set of studies, increased expression of CR1 (CD35) and CR3 (CD11b/CD18) was observed after G-CSF treatment of healthy volunteers and patients with cancer or aplastic anemia [66,67].

Respiratory oxidative burst. *In vitro* stimulation with G-CSF or GM-CSF fails to activate the respiratory burst of neutrophils in suspension, but induces a delayed but substantial respiratory burst in adherent neutrophils [117]. Although the maximal response to both CSFs is roughly equivalent, it is noteworthy that GM-CSF is the more potent stimulus when compared on the basis of weight [117]. After a short incubation time (15 min) with either GM-CSF or G-CSF, neutrophils demonstrate a marked enhancement in their ability to undergo a respiratory burst in response to FMLP or zymosan-activated serum [60,70,72,87,102,117-122]. Although both agents exert similar priming effects on neutrophils, their respective signal transduction pathways differ [123].

We have previously shown that *in vivo* administration of G-CSF [38,124,125,126] and GM-CSF [23] to healthy volunteers primed neutrophils for an enhanced production of reactive oxygen species (ROS). The neutrophil respiratory burst is increased after administration of GM-CSF and G-CSF to patients with cancer [66,91,127-130], myelodysplasia [131-133], aplastic anemia[67], diabetes[134] and after bone marrow trans-

plantation [135]. Other studies, however, have failed to demonstrate this priming effect. Höglund et al. found that G-CSF *in vivo* did not stimulate the neutrophil respiratory burst in healthy volunteers [66]. Others have found a decreased production of ROS by neutrophils of patients with severe congenital neutropenia (SCN) after priming with G-CSF and stimulation with FMLP, but normal values after stimulation with the direct activator, phorbol myristate acetate (PMA) [136,137]. The balance of experimental evidence indicates that GM-CSF and G-CSF enhance the respiratory burst of normal and dysfunctional neutrophils both *in vitro* and *in vivo*.

Microbicidal activity. *In vitro*, G-CSF significantly enhanced the microbicidal activity of normal neutrophils and defective neutrophils from HIV-1-infected patients against *Staphylococcus aureus* [105,109], while GM-CSF stimulated the bactericidal activity of neutrophils obtained from patients following allogeneic bone marrow transplantation against *Staphylococcus aureus* [138]. Other *in vitro* studies showed that GM-CSF and G-CSF were able to correct the decreased neutrophil killing of *S. aureus* in patients with myelodysplasia [72] and in dexamethasone-suppressed neutrophils [69]. However, in other studies GM-CSF failed to augment bactericidal activity of neutrophils *in vitro* despite increasing phagocytic activity and stimulating production of ROS [103,119]. GM-CSF enhanced the fungicidal activity of normal neutrophils *in vitro* against blastospores of *Torulopsis glabrata* [139] and *Candida albicans* [69]. Conflicting results have been reported with G-CSF, however, which failed *in vitro* to enhance the fungicidal activity of neutrophils against *C. albicans* blastospores [69,140], despite its ability to augment the neutrophil respiratory burst in response to blastoconidia and pseudohyphae of the yeast [141]. In contrast, other *in* vitro studies conducted with neutrophils obtained from healthy volunteers showed that neutrophils stimulated with G-CSF had enhanced fungicidal activity against different Candida species [142] and *Aspergillus fumigatus* [143].

Administration of G-CSF to HIV-1-infected patients caused significant enhancement of neutrophil fungicidal activity against *C. albicans* and *C. neoformans* [140]. Furthermore, treatment with G-CSF enhanced neutrophil bactericidal activity in cancer patients [144]. However, in a group of patients with SCN [136] or hairy cell leukemia [145], treatment with G-CSF resulted in normal neutrophil bactericidal activity. Non-human primates treated with GM-CSF showed enhanced neutrophil bactericidal activity against *E. coli* [146].

Antibody-dependent cellular cytotoxicity (ADCC). Both G-CSF and GM-CSF are capable of stimulating neutrophil ADCC *in vitro* as well as *in vivo*. G-CSF has been shown to enhance *in vitro* neutrophil ADCC in healthy volunteers [27,147,148], HIV-infected patients [147] and hairy-cell-leukemia patients [145]. *In vitro*, GM-CSF also enhanced ADCC of neutrophils from healthy volunteers [27,102,147-149] and patients with chronic granulomatous disease (CGD) [147]. Furthermore, G-CSF stimulated *in vivo* ADCC when given to healthy volunteers [93] and cancer patients [112]. A similar effect was observed following administration of GM-CSF to HIV-infected patients [150].

Degranulation. *In vitro* assays have shown that GM-CSF primes neutrophils for an augmented release of constituents from both specific and azurophil granules in response to *C. albicans* [101], *Torulopsis glabrata* [139], Sephadex particles [151], opsonized inflammatory microcrystals [121] and FMLP [102]. *In vitro*, G-CSF is able to induce leukocyte alkaline phosphatase (LAP) mRNA [152] and to increase LAP activity in

neutrophils [153]. Furthermore, G-CSF has been shown to increase LAP activity in neutrophils from patients with myelodysplastic syndrome and chronic myelogenous leukemia (CML) [131]. G-CSF also induces *in vivo* activation of neutrophils, as demonstrated by the mobilization and release of secretory vesicles (LAP and CD11b), specific granules (lactoferrin, CD11b, and CD66b), and azurophil granules (elastase and α-1 antitripsin) [89]. Administration of GM-CSF or G-CSF, but not M-CSF, to patients recovering from autologous bone marrow transplantation primed neutrophils for enhanced neutrophil degranulation [154].

Other receptor expression and function. The receptor for lipopolysacharide (LPS) and LPS-binding protein (LBP) known as CD14 is expressed by neutrophils at low levels under natural conditions [155]. *In vitro*, both G-CSF and GM-CSF stimulated expression of CD14 antigen in mature neutrophils [92]. Increased surface expression of CD14 on neutrophils has been found after administration of G-CSF to healthy volunteers [65,92,93], patients post-chemotherapy [65] and patients with congenital neutropenias [73,115]. In contrast, GM-CSF failed to alter CD14 expression on neutrophils [92]. In addition, the level of soluble CD14 (sCD14) rose in plasma after healthy volunteers received G-CSF[93].

Apoptosis. Programmed cell death or apoptosis refers to the non-pathologic or physiological mode of cell death, which occurs via a characteristic sequence of events with morphologic features distinct from necrosis [156]. Because apoptosis induces the recognition and phagocytosis of senescent neutrophils by monocyte-derived macrophages and phagocytes, death via apoptosis results in the removal of intact neutrophils at tissue sites, presumably preventing the release of their toxic compounds into the tissues [157,158]. Apoptosis is now recognized to play a fundamental role in the regulation of the immune system and the inflammatory response [159,160]. As neutrophils proceed through apoptosis, functional activity declines. Apoptotic neutrophils lose FcγRIII (CD16) expression [161,162] and demonstrate a reduce ability to degranulate, generate respiratory burst, or undergo shape changes in response to external stimuli such as FMLP [163,164].

Recent evidence indicates that the functional life span of mature human neutrophils can be significantly extended *in vitro* by incubation with pro-inflammatory mediators, including G-CSF, GM-CSF, interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), IL-1β, IL-2, endotoxic lipopolysaccharide (LPS) and complement factor 5a (C5a) [165-168], or adenosine triphosphate (ATP) and the diadenosine polyphosphates Ap_3A, Ap_4A, Ap_5A and Ap_6A [169-171], or dexamethasone[166] . *In vitro* studies have shown that the combination of GM-CSF together with ATP and diadenosine polyphosphates results in more pronounced protection from apoptosis than that of any of the components alone [169,170].

Niessen et al. showed that G-CSF delays spontaneous neutrophil apoptosis in part by activation of the vacuolar proton ATPase (v-ATPase) which plays a role in maintaining cellular pH balance. G-CSF may stimulate v-ATPase synthesis or its translocation to the plasma membrane [171]. Other laboratories, including ours, have studied another important mechanism involved in the regulation of spontaneous neutrophil apoptosis, the Fas (APO-1; CD95)/Fas-ligand (FasL) pathway, showing that neutrophils are highly susceptible to death through this system [166,172,173]. Although Fas

is constitutively expressed on human neutrophils, monocytes and eosinophils, constitutive expression of cell surface FasL is relatively restricted to neutrophils [166,172-174]. As a result of constitutive co-expression of both Fas and FasL, neutrophils may be destined to undergo rapid cell death via the Fas apoptotic pathway. *In vitro* experiments have shown that G-CSF, GM-CSF, IFN-γ, TNF-α, or dexamethasone can suppress Fas-mediated neutrophil apoptosis and partly maintain neutrophil function [166,168,175].

Neutrophil apoptosis has been found to be accelerated in patients with advanced HIV infection (AIDS) [176]. The defect appears to be intrinsic and not related to serum factors. *In vitro* incubation of neutrophils from AIDS patients with G-CSF significantly delayed apoptosis [176]. *In vivo* administration of G-CSF to healthy volunteers and cancer patients increased subsequent neutrophil survival *ex vivo* by approximately 24 hours longer than that observed in controls which did not receive G-CSF [177]. Other studies examining neutrophil apoptosis in the acute respiratory distress syndrome (ARDS) have shown that the proportion of apoptotic neutrophils recovered from the lungs of patients with ARDS is low throughout the course of ARDS. In addition, the alveolar microenviroment of patients with established ARDS contains factors that prolong the survival of normal human neutrophils *in vitro*. G-CSF and GM-CSF appear to account for the majority of anti-apoptotic activity detected in the bronchoalveolar fluid from patients with ARDS [178].

Summary

Scientific data support the concept that IL-3 in combination with other early acting factors (SCF; c-kit ligand and flt3 RTK; FL) are required to stimulate the proliferation and differentiation of primitive hematopoietic stem/progenitor cells. GM-CSF, on the other hand, acts predominantly as a differentiation factor for granulocyte/monocyte progenitors. G-CSF has a more restricted activity, limited mainly to the granulocyte lineage, where it is necessary not only for the maintenance of normal neutrophil level but also for the development of neutrophilia with infections. In contrast, M-CSF exerts its activity on the comminted monocyte/macrophage cell lineage, with very little or no activity on the granulocyte cell lineage. Mature neutrophils lack IL-3 receptors and are, therefore, not affected by IL-3. In contrast, GM-CSF and G-CSF act on mature neutrophils modulating not only their distribution in blood and tissues but also their function.

References

1. Metcalf D. The granulocyte-macrophage colony-stimulating factors. Science 229: 16-22 , 1985.
2. Clark SC, Kamen R. The human hematopoietic colony-stimulating factors. Science 236: 1229-1237, 1987.
3. Demetri GD, Griffin JD. Granulocyte colony-stimulating factor and its receptor. Blood 78: 2791-2808, 1991.
4. Lieschke GJ, Burgess AW. Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. N Engl J Med 327: 28-35, 1992.
5. Metcalf D. The molecular control of normal and leukemic granulocytes and macrophages. Proc R Soc London 230: 389-423, 1987.

6. Spivak J, Smith RR, Ihle JN. Interleukin 3 promotes the *in vitro* proliferation of murine pluripotent hematopoietic stem cells. J Clin Invest 76: 1613-1621, 1985.
7. Lord BI, Molineux G, Pojda Z, et al. Myeloid cell kinetics in mice treated with recombinant interleukin-3, granulocyte colony-stimulating factor (CSF), or granulocyte-macrophage CSF in vivo. Blood 77: 2154-2159, 1991.
8. Heyworth CM, Dexter TM, Kan O, Whetton AD. The role of hemopoietic growth factors in self-renewal and differentiation of IL-3 dependent multipotential stem cells. Growth Factors 2: 197-211, 1990.
9. Spooncer E, Heyworth CM, Dunn A, Dexter TM. Self renewal and differentiation of interleukin 3 dependent multipotent stem cells are modulated by stromal cells and serum factors. Differentiation 31: 111-118, 1986.
10. Sonoda Y, Sakabe H, Ohmisono Y, et al. Synergistic actions of stem cell factor and other burst-promoting activities on proliferation of CD34+ highly purified blood progenitors expressing HLA-DR or different levels of c-kit protein. Blood 84: 4099-4106, 1994.
11. Sonoda Y, Kimura T, Sakabe H, et al. Human flt3 ligand acts on myeloid as well as multipotential progenitors derived from purified CD34+ blood progenitors expressing different levels of c-kit protein. Eur J Haematol 58: 257-262, 1997.
12. Kimura T, Sakabe H, Tanimukai S, et al. Simultaneous activation of signals through gp130, c-kit, and interleukin-3 receptor promotes a trilineage blood cell production in the absence of terminally acting lineage-specific factors. Blood 90: 4767-4778, 1997.
13. Smith SL, Bender JG, Maples PB, et al. Expansion of neutrophil precursors and progenitors in suspension cultures of $CD34^+$ cells enriched from human bone marrow. Exp Hematol 21: 870-877, 1993.
14. Broxmeyer HE, Williams DE, Hangoc G, et al. Synergistic myelopoietic actions *in vivo* after administration to mice of combinations of purified natural murine colony stimulating factor 1, recombinant murine interleukin 3, and recombinant murine granulocyte/macrophage colony stimulating factor. Proc Natl Acad Sci USA 84: 3871-3875, 1987.
15. Heyworth CM, Ponting ILO, Dexter TM. The response of hemopoietic cells to growth factors: developmental implications of synergistic interactions. J Cell Sci 91: 239-247, 1988.
16. Aglietta M, Pasquino P, Sanavio F, et al. Granulocyte-macrophage colony-stimulating factor and interleukin 3: target cells and kinetics of response in vivo. Stem Cells 11(suppl 2): 83-87, 1993.
17. Lemoli RM, Fortuna A, Fogli M, et al. Proliferative response of human marrow myeloid progenitor cells to in vivo treatment with granulocyte colony-stimulating factor alone and in combination with interleukin-3 after autologous bone marrow transplantation. Exp Hematol 23: 1520-1526, 1995.
18. Aglietta M, Sanavio F, Stacchini A, et al. Interleukin 3 in vivo: kinetics of response of target cells. Blood 82: 2054-2061, 1993.
19. Lord BI, Testa NG, Bretti S, et al. Haemopoietic progenitor and myeloid cell kinetics in humans treated with interleukin-3 and granulocyte/macrophage colony-stimulating factor in combination. Int J Cancer 59: 483-490, 1994.
20. Ganser A, Ottmann OG, Seipelt G, et al. Effect of long term treatment with recombinant human interleukin-3 in patients with myelodisplastic syndromes. Leukemia 7: 696-701, 1993.
21. Tepler I, Elias A, Kalish L, et al. Effect of recombinant human interleukin-3 on haematological recovery from chemotherapy-induced myelosuppression. Br J Haematol 87: 678-686, 1994.
22. Scadden DT, Levine JD, Bresnahan J, et al. *In vivo* effects of interleukin 3 in HIV type 1-infected patients with cytopenia. AIDS Res Hum Retrov 11: 731-739, 1995.
23. Dale DC, Liles WC, Llewellyn C, Price TH. Effects of granulocyte-macrophage colony-stimulating factor (GM-CSF) on neutrophil kinetics and function in normal human volunteers. Am J Hematol 57: 7-15, 1998.
24. Lord BI, Gurney H, Chang J, et al. Haematopoietic cell kinetics in humans treated with rGM-CSF. Int J Cancer 50: 26-31, 1992.
25. Crump M, Couture F, Kovacs M, et al. Interleukin-3 followed by GM-CSF for delayed engraftment after autologous bone marrow transplantation. Exp Hematol 21: 405-410, 1993.
26. Albin N, Douay L, Fouillard L, et al. *In vivo* effects of GM-CSF and IL-3 on hematopoietic cell recovery in bone marrow and blood after autologous transplantation with mafosfamide-purged marrow in lymphoid malignancies. Bone Marrow Transplant 14: 253-259, 1994.
27. Avalos BR, Gasson JC, Hedvat C, et al. Human granulocyte colony-stimulating factor: biologic activities and receptor characterization of hematopoietic cells and small cell lung cancer cell lines. Blood 75: 851-857, 1990.

28. D'Andrea AD. Cytokine receptors in congenital hematopoietic disease. N Engl J Med 330: 839-846, 1994.
29. Hammond WP, Csiba E, Canin A, et al. Chronic neutropenia: a new canine model induced by human G-CSF. J Clin Invest 87: 704-710, 1991.
30. Lieschke GJ, Grail D, Hodgson G. Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell deficiency, and impaired neutrophil mobilization. Blood 84: 1737-1746, 1994.
31. Stanley E, Lieschke GJ, Grail D, et al. Granulocyte-macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoiesis but developed a characteristic pulmonary pathology. Proc Natl Acad Sci USA 91: 5592-5596, 1994.
32. Dranoff G, Crawford AD, Sadelain M, et al. Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis. Science 294: 713-716, 1994.
33. Cheers C, Haigh AM, Kelso A, et al. Production of colony-stimulating factors (CSFs) during infection: separate determinations of macrophage-, granulocyte-, and multi-CSFs. Infect Immun 56: 247-251, 1988.
34. Dale DC, Lau S, Nash R, et al. Effect of endotoxin on serum granulocyte and granulocyte-macrophage colony-stimulating factor levels in dogs. J Infect Dis 165: 689-694, 1992.
35. Kawakami M, Tsutsumi H, Kumakawa T, et al. Levels of serum granulocyte colony-stimulating factor in patients with infections. Blood 76: 1962-1974, 1990.
36. Taveira Da Silva AMT, Kaulbach HC, Chuidian FS, et al. Brief report: shock and multiple-organ dysfunction after self-administration of salmonella endotoxin. N Engl J Med 328: 1993 1457-1460.
37. Cebon J, Layton J, Maher D, Morstyn G. Endogenous haemopoietic growth factors in neutropenia and infection. Br J Haematol 86: 265-274, 1994.
38. Price TH, Chatta GS, Dale DC. The effect of recombinant granulocyte colony-stimulating factor (G-CSF) on neutrophil kinetics in normal human subjects. (abstract) Blood 80: 350a, 1992.
39. Price TH, Chatta GS, Dale DC. Effect of recombinant granulocyte colony-stimulating factor on neutrophil kinetics in normal young and elderly humans. Blood 88: 335-340, 1996.
40. Chatta GS, Price TH, Allen RC, Dale DC. Effects of in vivo recombinant methionyl human granulocyte colony-stimulating factor on the neutrophil response and peripheral blood colony-forming cells in healthy young and elderly adult volunteers. Blood 84: 2923-2929, 1994.
41. Morstyn G, Campbell L, Souza LM, et al. Effect of granulocyte colony-stimulating factor on neutropenia induced by cytotoxic chemotherapy. Lancet 1: 667-672, 1988.
42. Lieschke GJ, Burgess AW. Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. N Engl J Med 327: 28-35, 99-106, 1992.
43. Linderman A, Herrmann F, Oster W, et al. Hematologic effects of recombinant human granulocyte colony-stimulating factor in patients with malignancy. Blood 74: 2633-2651, 1989.
44. Chatta GS, Andrews RG, Roger E, et al. Hematopoietic progenitors and aging: alterations in granulocyte precursors and responsiveness to recombinant human G-CSF, GM-CSF, and IL-3. J Gerontol 48: M207-212, 1993.
44. Nielsen SD, Dam-Larsen S, Nielsen C, et al. Recombinant human granulocyte colony-stimulating factor increases circulating CD34-positive cells in patients with AIDS. Ann Hematol 74: 215-220, 1997.
46. Lord BI, Bronchud MH, Owens S, et al. The kinetics of human granulopoiesis following treatment with granulocyte colony-stimulating factor in vivo. Proc Natl Acad Sci USA 86: 9499-9503, 1989.
47 Lipschitz D, Udupa K, Milton K, Thompson C. Effect of age on hematopoiesis in man. Blood 63: 502-509, 1984.
48. Timmafy M. A comparative study of bone marrow function in young and old individuals. Geront Clin 4: 13-18, 1962.
49. Motoyoshi K, Takaku F, Maekawa T, et al. Protective effect of partially purified human urinary colony-stimulating factor on granulocytopenia after antitumor chemotherapy. Exp Hematol 14: 1069-1075, 1986.
50. Masaoka T, Motoyoshi K, Takaku F, et al. Administration of human urinary colony-stimulating factor after bone marrow transplantation. Bone Marrow Transplant 3: 121-127, 1988.
51. Khwaja A, yong K, Jones HM, et al. The effect of macrophage colony-stimulating factor on haematopoietic recovery after autologous bone marrow transplantation. Br J Haematol 81: 288-295, 1992.
52 Laughlin MJ, Kirkpatrick G, Sabiston N, et al. Hematopoietic recovery following high-dose combined alkylating-agent chemotherapy and autologous bone marrow support in patients in phase-

I clinical trials of colony-stimulating factors: G-CSF, GM-CSF, IL-1, IL-2, M-CSF. Ann Hematol 67: 267-276, 1993.
53. Schuening FG, Neumunaitis J, Appelbaum FR, Storb R. Hematopoietic growth factors after allogeneic marrow transplantation in animal studies and clinical trials. Bone Marrow Transplant 14: S74-S77, 1994.
54. Nemunaitis J. A comparative review of colony-stimulating factors. Drugs 54: 709-729, 1997.
55. Zimmerman GA, Prescott SM, McIntyre TM. Endothelial cell interactions with granulocytes: Tethering and signaling molecules. Immunol Today 13: 93-98, 1992.
56. Athens JW, Raab SO, Haab OP, et al. Leukokinetic studies. III. The distribution of granulocytes in the blood of normal subjects. J Clin Invest 40: 159-167, 1961.
57. Yong KL, Linch DC. Differential effects of granulocyte and granulocyte-macrophage colony-stimulating factors (G- and GM-CSF) on neutrophil adhesion *in vitro* and *in vivo*. Eur J Haematol 49: 251-259, 1992.
58. Yong KL. Granulocyte colony-stimulating factor (G-CSF) increases neutrophil migration across vascular endothelium independent of an effect on adhesion: comparison with granulocyte-macrophage colony-stimulating factor (GM-CSF). Br J Haematol 94: 40-47, 1996.
59. Yong KL, Linch DC. GM-CSF differentially regulates neutrophil migration across IL-1 activated, and nonactivated, human endothelium. J Immunol 150: 2449-2456, 1993.
60. Yuo A, Kitagawa S, Ohsaka A, et al. Recombinant human granulocyte colony-stimulating factor as an activator of human granulocytes: potentiation of responses triggered by receptor-mediated agonists and stimulation of C3bi receptor expression and adherence. Blood 74: 2144-2149, 1989.
61. Okada Y, Kawagishi M, Kusaka M. Effect of recombinant human granulocyte colony-stimulating factor on human neutrophil adherence *in vitro*. Experientia 46: 1050-1053, 1990.
62. Devereux S, Bull HA, Campos-Costa D, et al. Granulocyte-macrophage colony-stimulating factor induced changes in cellular adhesion molecule expression and adhesion to endothelium: *in vitro* and *in vivo* studies in man. Br J Haematol 71: 323-330, 1989.
63. Yong KL, Rowles PM, Patterson KG, Linch DC. Granulocyte-macrophage colony-stimulating factor induces neutrophil adhesion to pulmonary vascular endothelium *in vivo*: role of β_2 integrins. Blood 80: 1565-1575, 1992.
64. Håkansson L, Höglund M, Jonsson U-B, et al. Effects of *in vivo* administration of G-CSF on neutrophil and eosinophil adhesion. Br J Haematol 98: 603-611, 1997.
65. Spiekermann K, Emmendoerffer A, Elsner J, et al. Altered surface marker expression and function of G-CSF induced neutrophils from test subjects and patients under chemotherapy. Br J Haematol 87: 31-38, 1994.
66. Höglund M, Håkansson L, Venge P. Effects of *in vivo* administration of G-CSF on neutrophil functions in healthy volunteers. Eur J Haematol 58: 195-202, 1997.
67. Yasui K, Tsuno T, Miyabayashi M, et al. Effects of high-dose granulocyte colony-stimulating factoron neutrophil functions. Br J Haematol 92: 571-573, 1996.
68. Wang JM, Chen ZG, Colella S, et al. Chemotactic activity of recombinant human granulocyte colony-stimulating factor. Blood 72: 1456-1460, 1988.
69 Bober LA, Grace MJ, Pugliese-Sivo C, et al. The effect of GM-CSF and G-CSF on human neutrophil function. Immunopharmacology 29: 111-119, 1995.
70. Wheeler JG, Huffine ME, Childress S, Sikes J. Comparison of colony stimulating factors on in vitro rat and human neutrophil function. Biol Neonate 66: 214-220, 1994.
71. Buescher ES, McIlheran SM, Vadhan-Raj S. Effects of in vivo administration of recombinant human granulocyte-macrophage colony-stimulating factor on human neutrophil chemotaxis and oxygen metabolism (letter). J Infect Dis 158: 1140-1142, 1988.
72. Boogaerts M, Meeus P, Verhoef G, et al. Differential effects of IL-3, GM-CSF and G-CSF on preleukemic granulocytes. (abstract) Blood 72: 110a, 1988.
73. Idenbach T, Roesler J, Elsner J, et al. *In vitro* functions of granulocyte colony-stimulating factor induced neutrophils. In: Freund M, Link H, Schmidt RE, Welte K (eds). Cytokines in Hemopoiesis, Oncology, and AIDS II. Springer. Berlin pp. 521-527, 1992.
74. Wang JM, Colella S, Allavena P, Mantovani A. Chemotactic activity of human recombinant granulocyte-macrophage colony-stimulating factor. Immunology 60: 439-444, 1987.
75. Weisbart RH, Golde DW. Physiology of granulocyte and macrophage colony-stimulating factors in host disease. Hematol Oncol Clin North Am 3: 401-409, 1989.
76. Turilli I, Bowers D, Aronson I, et al. G-CSF and neutrophil chemotaxis (letter). Br J Haematol 90: 232-233, 1995.

77. Bronchud MH, Potter MR, Morgenstern G, et al. In vitro and in vivo analysis of the effects of recombinant human granulocyte colony-stimulating factor in patients. Br J Cancer 58: 64-69, 1988.
78. Peters WP, Stuart A, Affronti ML, et al. Neutrophil migration is defective during recombinant human granulocyte-macrophage colony-stimulating factor infusion after autologous bone marrow transplantation in humans. Blood 72: 1310-1315, 1988.
79. Addison IE, Johnson B, Devereux S, et al. Granulocyte-macrophage colony-stimulating factor may inhibit neutrophil migration in vivo. Clin Exp Immunol 76: 149-153, 1989.
80. Lieschke GJ, Ramenghi U, O'Connor MP, et al. Studies of oral neutrophil levels in patients receiving G-CSF after autologous marrow transplantation. Br J Haematol 82: 589-595, 1992.
81. Hammond WP, Price TH, Souza LM, Dale DC. Treatment of cyclic neutropenia with granulocyte colony-stimulating factor. N Engl J Med 320: 1306-1311, 1989.
82. Bowski AA, Souza L, Kelly F, et al. Effects of human granulocyte colony-stimulating factor in a patient with idiopathic neutropenia. N Engl J Med 320: 38-42, 1989.
83. Toner GC, Jakubowski AA, Crown JP, et al. Colony-stimulating factors and neutrophil migration (letter). Ann Intern Med 110: 846-847, 1989.
84. Montgomery B, Bianco JA, Jacobsen A, Singer JW. Localization of transfused neutrophils to site of infection during treatment with recombinant human granulocyte-macrophage colony-stimulating factor and pentoxifylline (letter). Blood 78: 533-534, 1991.
85. Negrin RS, Haeuber DH, Nagler A, et al. Treatment of myelodysplastic syndromes with recombinant human granulocyte colony-stimulating factor. A phase I-II trial. Ann Intern Med 110: 976-984, 1989.
86. Carlos TM, Harlan JM. Leukocyte-endothelial adhesion molecules. Blood 84: 2068-2101, 1994.
87. Lopez AF, Williamson DJ, Gamble JR, et al. Recombinant human granulocyte-macrophage colony-stimulating factor stimulates in vitro mature human neutrophil and eosinophil function, surface receptor expression, and survival. J Clin Invest 78: 1220-1228, 1986.
88. Buckle AM, Hogg N. The effect of IFN-γ and colony-stimulating factors on the expression of neutrophil cell membrane receptors. J Immunol 143: 2295-2301, 1989.
89. Haas M, Kerst JM, Van der Schoot CE, et al. Granulocyte colony-stimulating factor administration to healthy volunteers: analysis of the immediate activating effects on circulating neutrophils. Blood 84: 3885-3894, 1994.
90. Katoh M, Shirai T, Shikoshi K, et al. Neutrophil kinetics shortly after initial administration of recombinant human granulocyte colony-stimulating factor: neutrophil alkaline phosphatase activity as an endogenous marker. Eur J Haematol 49: 19-24, 1992.
91. Ohsaka A, Kitagawa S, Sakamoto S, et al. *In vivo* activation of human neutrophil functions by administration of recombinant human granulocyte colony-stimulating factor in patients with malignant lymphoma. Blood, 74: 2743-2748, 1989.
92. Hansen PB, Kjaersgaard E, Johnsen HE, et al. Different membrane expression of CD11b and CD14 on blood neutrophils following *in vivo* administration of myeloid growth factors (published erratum appears in Br Haematol 1994 86: 240). Br J Haematol 85: 50-56, 1993.
93. Kerst JM, de Haas M, van der Schoot E, et al. Recombinant granulocyte colony-stimulating factor administration to healthy volunteers: induction of immunophenotypically and functionally altered neutrophils via an effect on myeloid progenitor cells. Blood 82: 3265-3272, 1993.
94. Demetri GD, Griffin JD. Granulocyte colony-stimulating factor and its receptor. Blood 78: 2791-2808, 1991.
95. Spertini O, Kansas GS, Munro JM, et al. Regulation of leukocyte migration by activation of the leukocyte adhesion molecule-1 (LAM-1) selectin. Nature 349: 691-694, 1991.
96. Liles WC, Rodger ER, Dale DC. Differential regulation of human neutrophil surface expression of CD14, CD11b, CD18, and L-selectin following the administration of G-CSF in vivo and in vitro (abstract). Clin Res 42:304A, 1994.
97. Ohsaka A, Saionji K, Sato N, et al. Granulocyte colony-stimulating factor down-regulates the surface expression of the human leukocyte adhesion molecule-1 on human neutrophils in vitro and in vivo. Br J Haematol 84: 574-580, 1993.
98. Ohsaka A, Saionji K, Igari J. Granulocyte colony-stimulating factor administration increases serum concentrations of soluble selectins. Br J Haematol 100: 66-69, 1998.
99. Schleiffenbaum B, Spertini O, Tedder TF. Soluble L-selectin is present in human plasma at high levels and retains functional activity. J Cell Biol 119: 229-238, 1992.
100. Spertini O, Callegari P, Cordey A-S, et al. High levels of the shed form of L-selectin are present

in patients with acute leukemia and inhibit blast cell adhesion to activated endothelium. Blood 84: 1249-1256, 1994.
101. Fabian I, Kletter Y, Mor S, et al. Activation of human eosinophil and neutrophil functions by haemopoietic growth factors: comparisons of IL-1, IL-3, IL-5 and GM-CSF. Br J Haematol 80: 137-143, 1992.
102. Burgess AW, Begley G, Johnson GR, et al. Purification and properties of bacterially synthesized human granulocyte-macrophage colony-stimulating factor. Blood 69: 43-51, 1987
103. Fleischman J, Golde DW, Weisbart RH, Gasson J. Granulocyte-macrophage colony-stimulating factor enhances phagocytosis of bacteria by human neutrophils. Blood 68: 708-711, 1986.
104. Richardson MD, Chung I. GM-CSF-modulated phagocytosis of *Trichosporon beigelii* by human neutrophils. J Med Microbiol 46: 321-325, 1997.
105. Roilides E, Walsh TJ, Pizzo P, Rubin M. Granulocyte colony-stimulating factor enhances the phagocytic and bactericidal activity of normal and defective human neutrophils. J Infect Dis 163: 579-583, 1991.
106. Katoh M, Takada M, Nakatani N, et al. Chemotaxis and phagocytosis of the neutrophils mobilized by granulocyte colony-stimulating factor in healthy donors for granulocyte transfusions. Am J Hematol 49: 96-97, 1995.
107. Lawry J, Smith MO, Lorigan PC. Monitoring neutrophil phagocytosis in lung cancer patients or normal stem cell donors, treated with G-CSF. Biochem Soc Trans 25: 197S, 1997.
108 Stoppa AM, Fossat C, Sainty D, et al. *In vivo* administration of recombinant granulocyte colony-stimulating factor corrects acquired neutrophil function deficiency associated with chronic graft-versus-host disease. Br J Haematol 83: 169-170, 1993.
109. Sullivan GW, Gelrud AK, Carper HT, et al. Interaction of tumor necrosis factor-α and granulocyte colony-stimulating factor on neutrophil apoptosis, receptor expression, and bactericidal function. Proc Assoc Amer Phys 108: 455-466, 1996.
110. Kerst JM, Van de Winkel GJ, Evans AH, et al. Granulocyte colony-stimulating factor induces hFcgRI (CD64 antigen)- positive neutrophils via an effect on myeloid precursor cells. Blood 81: 1457-1464, 1993.
111. Buckle AM, Jayaram Y, Hogg N. Colony-stimulating factors and interferon-γ differentially affect cell surface molecules shared by monocytes and neutrophils. Clin Exp Immunol 81: 339-342, 1990.
112. Alerius T, Repp R, de Wit TPM, et al. Involvement of the high-affinity receptor for IgG (FcγRI CD64) in enhanced tumor cell cytotoxicity of neutrophils during granulocyte colony-stimulating factor therapy. Blood 82: 931-939, 1993.
113. Ersten MJ, de Jong S, Evers LM, et al. Addition of granulocyte colony-stimulating factor to chemotherapy in patients with AIDS-related lymphoma: effects on neutrophil Fcg receptor expression and soluble FcgRIII plasma levels. Br J Haematol 99: 537-541, 1997.
114. Ravetch JV, Kinet JP. Fc-receptors. Ann Rev Immunol 9: 457-492, 1991.
115. Elsner J, Roesler J, Emmendoerffer A, et al. Altered function and surface marker expression of neutrophils induced by rhG-CSF treatment in severe congenital neutropenia. Eur J Haematol 48: 10-19, 1992.
116. Maurer D, Fischer GF, Felzmann T, et al. Ratio of complement receptor over Fc-receptor III expression: a sensitive parameter to monitor granulocyte-macrophage colony-stimulating factor effects on neutrophils. Ann Hematol 62: 135-140, 1991.
117. Nathan CF. Respiratory burst in adherent human neutrophils: triggering by colony-stimulating factors CSF-GM and CSF-G. Blood 73: 301-306, 1989.
118. Kitagawa S, You A, Souza LM, et al. Recombinant human granulocyte colony-stimulating factor enhances superoxide release in human granulocytes stimulated by chemotactic peptide. Biochem Biophys Res Commun 144: 1143-1146, 1987.
119. Weisbart RH, Golde DW, Clark SC, et al. Human granulocyte-macrophage colony-stimulating factor is a neutrophil activator. Nature 314: 361-363, 1985.
120. Eisbart RH, Kwan L, Golde DW, Gasson JC. Human GM-CSF primes neutrophils for enhanced oxidative metabolism in response to the major physiological chemoattractants. Blood 69: 18-21, 1987.
121. Burt HM, Jackson JK. The priming action of tumor necrosis factor-alpha (TNF-α) and granulocyte-macrophage colony-stimulating factor (GM-CSF) on neutrophils activated by inflammatory microcrystals. Clin Exp Immunol 108: 432-437, 1997.
122. Meyer CN, Nielsen H. Priming of neutrophil and monocyte activation in human immunodeficiency

virus infection. APMIS 104: 640-646, 1996.
123. Balazovich KJ, Almeida HI, Boxer LA. Recombinant human G-CSF and GM-CSF prime human neutrophils for superoxide production through different signal transduction mechanisms. J Lab Clin Med 118: 576-584, 1991.
124. Liles WC, Rodger ER, Dale DC. *In vivo* administration of granulocyte colony-stimulating factor (G-CSF) to normal human subjects: priming of the respiratory burst and inhibition of apoptosis in neutrophils. (abstract) Blood 84: 24a, 1994.
125. Liles WC, Huang JE, van Burik J-A H, et al. Granulocyte colony-stimulating factor administered in vivo augments neutrophil-mediated activity against opportunistic fungal pathogens. J Infect Dis 175: 1012-1015, 1997.
126. Allen RC, Stevens PR, Price TH, et al. In vivo effects of recombinant human granulocyte colony-stimulating factor on neutrophil oxidative functions in normal human volunteers. J Infect Dis 175: 1184-1192, 1997.
127. Lindermann A, Herrmann F, Oster W, et al. Hematologic effects of recombinant human granulocyte colony-stimulating factor in patients with malignancy. Blood 74: 2644-2651, 1989.
128. Harazmi A, Nielsen H, Hovgaard D, et al. Modulation of neutrophil and monocyte function by recombinant human granulocyte-macrophage colony-stimulating factor in patients with lymphoma. Eur J Clin Invest 21: 219-224, 1991.
129. Kaplan SS, Basford RE, Wing EJ, Shadduck RK. The effect of recombinant human granulocyte-macrophage colony-stimulating factor on neutrophil activation in patients with refractory carcinoma. Blood 73: 636-638, 1989.
130. Wiltschke C, Krainer M, Nanut M, et al. *In vivo* administration of granulocyte-macrophage colony-stimulating factor and granulocyte colony-stimulating factor increases neutrophil oxidative burst activity. J Interf Cytok Res 15: 249-253, 1995.
131. Uo A, Kitagawa S, Okabe T, et al. Recombinant human granulocyte colony-stimulating factor repairs the abnormalities of neutrophils in patients with myelodysplastic syndromes and chronic myelogenous leukemia. Blood 70: 404-411, 1987.
132. Iki S, Yuo A, Yagisawa M, et al. Increased neutrophil respiratory burst in myeloproliferative disorders: selective enhancement of superoxide release triggered by receptor-mediated agonists and low responsiveness to in vitro cytokine stimulation. Exp Hematol 25: 26-33, 1997.
133. Zabernigg A, Hilbe W, Eisterer W, et al. Cytokine priming of the granulocyte respiratory burst in myelodysplastic syndromes. Leukemia Lymphoma 27: 137-143, 1997.
134. Sato N, Kashima K, Tanaka Y, et al. Effect of granulocyte colony-stimulating factor on generation of oxygen-derived free radicals and myeloperoxidase activity in neutrophils from poorly controlled NIDDM patients. Diabetes 46: 133-137, 1997.
135. Zimmerli W, Zarth A, Gratwohl A, et al. Granulocyte-macrophage colony-stimulating factor for granulocyte defects of bone marrow transplant patients. Lancet I: 494-497, 1989.
136. Roesler J, Emmendorffer A, Elsner J, et al. *In vitro* functions of neutrophils induced by treatment with rhG-CSF in severe congenital neutropenia. Eur J Haematol 46: 112-118, 1991.
137. Elsner J, Roesler J, Emmendoerffer A, et al. Abnormal regulation in the signal transduction in neutrophils from patients with severe congenital neutropenia: relation of impaired mobilization of cytosolic free calcium to altered chemotaxis, superoxide anion generation and F-actin content. Exp Hematol 21: 38-46, 1993.
138. Fabian I, Kletter Y, Bleiberg I, et al. Effect of exogenous recombinant human granulocyte and granulocyte-macrophage colony-stimulating factor on neutrophil function following allogeneic bone marrow transplantation. Exp Hematol 19: 868-873, 1991.
139. Kowanko IC, Ferrante A, Harvey DP, et al. Granulocyte-macrophage colony-stimulating factor augments neutrophil killing of *Torulopsis glabrata* and stimulates neutrophil respiratory burst and degranulation. Clin Exp Immunol 83: 225-230, 1991.
140. Vecchiarelli A, Monari C, Baldelli F, et al. Beneficial effect of recombinant human granulocyte colony-stimulating factor on fungicidal activity of polymorphonuclear leukocytes from patients with AIDS. J Infect Dis 171: 1448-1454, 1995.
141. Roilides E, Uhlig K, Venzon D, et al. Neutrophil respiratory burst in response to blastoconidia and pseudohyphae of *Candida albicans*. J Infect Dis 166: 668-673, 1992.
142. Roilides E, Holmes A, Blake C, et al. Effects of granulocyte colony-stimulating factor and interferon-γ on antifungal activity of human polymorphonuclear neutrophils against pseudohyphae of different medically important *Candida* species. J Leukoc Biol 57: 651-656, 1995.
143. Roilides E, Uhlig K, Venzon D, et al. Enhancement of oxidative response and damage caused by

human neutrophils to *Aspergillus fumigatus* hyphae by granulocyte colony-stimulating factor and gamma interferon. Infect Immun 61: 1185-1193, 1993.
144. Fossat C. Stoppa AM, Sainty D, et al. G-CSF *in vivo* improves neutrophil bactericidal activity against *Pseudomonas aeruginosa* in cancer patients. (abstract) Blood 76: 137a, 1990.
145. Glaspy JA, Baldwin JC, Robertson PA, et al. Therapy for neutropenia in hairy cell leukemia with recombinant human granulocyte colony-stimulating factor. Ann Intern Med 109: 789-795, 1988.
146. Mayer P, Lam C, Obenaus H, et al. Recombinant human GM-CSF induces leukocytosis and activates peripheral blood polymorphonuclear neutrophils in non-human primates. Blood 70: 206-213, 1987.
147. Baldwin GC, Fuller ND, Roberts RL, et al. Graanulocyte- and granulocyte-macrophage colony-stimulating factors enhance neutrophil cytotoxicity toward HIV-infected cells. Blood 74: 1673-1677, 1989.
148. Baldwin GC, Chung GY, Kaslander C, et al. Colony-stimulating factor enhancement of myeloid effector cell cytotoxicity towards neuroectodermal tumor cells. Br J Haematol 83: 545-553, 1993.
149. Metcalf D, Begley CG, Johnson GR, et al. Biologic properties *in vitro* of a recombinant human granulocyte-macrophage colony-stimulatimg factor. Blood 67: 37-45, 1986.
150. Baldwin GC, Gasson JC, Quan SG, et al. Granulocyte-macrophage colony-stimulating factor enhances the neutrophil function in acquired immunodeficiency syndrome patients. Proc Natl Acad Sci USA 1988 85: 2763-2766.
151. Carlson M, Peterson C, Venge P. The influence of IL-3, IL-5, and GM-CSF on normal human eosinophil and neutrophil C3b-induced degranulation. Allergy 48: 437-442, 1993.
152. Sato N, Mizukami H, Tani K, Asano S. Regulation of mRNA levels of alkaline phosphatase gene in neutrophilic granulocytes by granulocyte colony-stimulating factor and retinoic acid. Eur J Haematol 46: 107-111, 1991.
153. Sato N, Takatani O, Koeffler HP, et al. Modulation by retinoids and interferons of alkaline phosphatase activity in granulocytes induced by granulocyte colony-stimulating factor. Exp Hematol 17: 258-262, 1989.
154. Tsakona CP, Goldstone AH. Patterns of primary degranulation as indicated by the mean myeloperoxidase index (MPXI) during bacteremia in lymphoma transplants treated with growth factors. Clin Lab Haematol 14: 273-280, 1992.
155. Ziegler-Heitbrock HWL, Ulevitch RJ. CD14: cell surface receptor and differentiation marker. Immunol Today 14: 121-125, 1993.
156. Ellis RE, Yuan J, Horvitz HR. Mechanisms and functions of cell death. Annu Rev Cell Biol 7: 663-698, 1991.
157. Newman SL, Henson JE, Henson PM. Phagocytosis of senescent neutrophils by human monocyte-derived macrophages and rabbit inflammatory macrophages. J Exp Med 156: 430-442, 1982.
158. Savill JS, Wyllie AH, Henson JE, et al. Macrophage phagocytosis of aging neutrophils in inflammation: programmed cell death in the neutrophil leads to its recognition by macrophages. J Clin Invest 83: 865-875, 1989.
159. Cohen JJ. Programmed cell death in the immune system. Adv Immunol 50: 55-85, 1991.
160. Cohen JJ, Duke RC, Fadok VA, Sellins KS. Apoptosis and programmed cell death in immunity. Annu Rev Immunol 10: 267-293, 1992.
161. Dransfield I, Buckle A, Savill A, et al. Neutrophil apoptosis is associated with reduction in CD16 (FcγRIII) expression. J Immunol 153: 1254-1263, 1994.
162. Homburg CHE, de Haas M, Von dem Borne AEG, et al. Human neutrophils lose their surface FcgRIII and acquire annexin V binding sites during apoptosis in vitro. Blood 85: 532-540, 1995.
163. Dransfield I, Stocks SC, Haslett C. Regulation of cell adhesion molecule expression and function associated with neutrophil apoptosis. Blood 85: 3264-3273, 1995.
164. Whyte MKB, Meagher LC, MacDermot J, Haslett C. Impairment of function in aging neutrophils is associated with apoptosis. J Immunol 150: 5124-5134, 1993.
165. Colotta F, Re F, Polentarutti N, et al. Modulation of granulocyte survival and programmed cell death by cytokines and bacterial products. Blood 80: 2012-2020, 1992.
166. Liles WC, Kiener PA, Ledbetter JA, et al. Differential expression of Fas (CD95) and Fas ligand on normal human phagocytes: implications for the regulation of apoptosis in neutrophils. J Exp Med 184: 429-440, 1996.
167. Lee A, Whyte MKB, Haslett C. Inhibition of apoptosis and prolongation of neutrophil functional longevity by inflammatory mediators. J Leukoc Biol 54: 283-288, 1993.
168. Hu B, Yasui K. Effects of colony-stimulating factors (CSFs) on neutrophil apoptosis: possible roles

at inflammatory site. Int J Hematol 66: 179-188, 1997.
169. Gasmi L, McLennan AG, Edwards S. The diadenosine polyphosphates Ap_3A and Ap_4A and adenosine triphosphate interact with granulocyte-macrophage colony-stimulating factor to delay neutrophil apoptosis: implications for neutrophil:platelet interactions during inflammation. Blood 87: 3442-3449, 1996.
170. Gasmi L, McLennan AG, Edwards S. Neutrophil apoptosis is delayed by the diadenosine polyphosphates, Ap_5A and Ap_6A: synergism with granulocyte-macrophage colony-stimulating factor. Br J Haematol 95: 637-639, 1996.
171. Niessen H, Meisenholder GW, Li HL, et al. Granulocyte colony-stimulating factor upregulates the vacuolar proton ATPase in human neutrophils. Blood 90: 4598-4601, 1997.
172. Liles WC, Dale DC, Klebanoff SJ. Fas (APO-1 CD95) activation induces apoptosis in human phagocytes. (abstract) Blood 84: 371a, 1994.
173. Iwai K, Miyawaki T, Taakizawa T, et al. Differential expression of bcl-2 and susceptibility to anti-Fas-mediated cell death in peripheral blood lymphocytes, monocytes, and neutrophils. Blood 84: 1201-1208, 1994.
174. Matsumoto K, Schleimer RP, Saito H, et al. Induction of apoptosis in human eosinophils by anti-Fas antibody treatment in vitro. Blood 86: 1437-1443, 1995.
175. Liles WC, Dale DC, Klebanoff SJ. Glucocorticoids inhibit apoptosis of human neutrophils. Blood 86: 3181-3188, 1995.
176. Pitrak DL, Tsai HC, Mullane KM, et al. Accelerated neutrophil apoptosis in the acquired immunodeficiency syndrome. J Clin Invest 98: 2714-2719, 1996.
177. Adachi S, Kubota M, Lin YW, et al. *In vivo* administration of granulocyte colony-stimulating factor promotes neutrophil survival *in vitro.* Eur J Haematol 53: 129-134, 1994.
178. Matute-Bello G, Liles WC, Radella II F, et al. Neutrophil apoptosis in the acute respiratory distress syndrome. Am J Respir Crit Care Med 156: 1969-1977, 1997.