G-CSF: Boosting endogenous production - a new strategy?

Sonja von Aulock, Isabel Diterich, Lars Hareng & Thomas Hartung*

Address
University of Konstanz
Biochemical Pharmacology
POB M655
78457 Konstanz
Germany
Email: Thomas.Hartung@uni-konstanz.de

*To whom correspondence should be addressed

Current Opinion in Investigational Drugs 2004 **5**(11):**1148-1152**
 ISSN 1472-4472

Granulocyte colony-stimulating factor (G-CSF) has been in clinical use for over a decade. Its main applications are in adjunctive medication to chemotherapy and in mobilizing stem cells for bone marrow transplantation. However, it has additional effects in that it primes neutrophilic granulocytes for improved host defense, and reduces the release of pro-inflammatory cytokines. These effects have prompted trials for numerous other indications. New research into the production and regulation of G-CSF in health and disease may now enable tailored strategies to induce or boost G-CSF formation. Similarly, new forms of application may increase its effectiveness.

Keywords Clinical applications, endogenous production, granulocyte colony-stimulating factor, Lyme borreliosis, sepsis

Introduction

Granulocyte colony-stimulating factor (G-CSF) was first described as a glycoprotein, isolated from a bladder carcinoma cell line, which stimulated the growth of granulocyte colonies in bone marrow preparations on soft agar. It was cloned in 1986, and developed with the intention of using its activity to protect cancer patients undergoing chemotherapy from the infectious risk posed by declining neutrophil counts. G-CSF injection stimulated the production and release of neutrophils from the bone marrow. Furthermore, less mature cells, ie, CD34+ stem cells, were also released following administration of G-CSF, which led to the less invasive strategy of harvesting these cells from peripheral blood and using them for bone marrow transplantation. More than three million patients have been treated with recombinant G-CSF since regulatory approval for these indications was granted. This review will consider additional potential applications of G-CSF based on its immunomodulatory properties, especially with regard to new investigations of the production and regulation of endogenous G-CSF, which might allow the modulation of endogenous G-CSF formation.

Improved neutrophil function by G-CSF suggests additional applications

As well as reversing neutropenia to limit the risk of infection in cancer patients, G-CSF treatment also primed mature neutrophils for more effective responses with regard to phagocytic activity, oxidative burst and degranulation, as well as increasing their lifespan in the blood (Figure 1) [1]. These findings implied that G-CSF would also be effective in other forms of disease in which the functions of neutrophils are adversely affected. This was confirmed in HIV patients with granulocyte dysfunction [2] and, more recently, in patients with melioidosis, a form of community-acquired sepsis caused by *Burkholderia pseudomallei*, which often occurs in patients with co-morbidities associated with impaired neutrophil function [3•]. Diabetic foot infection also appeared to be a prime candidate for G-CSF therapy on account of reported defects in neutrophil activity, but the results of more recent clinical trials have lowered expectations [4-8].

Effects of G-CSF on monocytes and lymphocytes suggests further applications

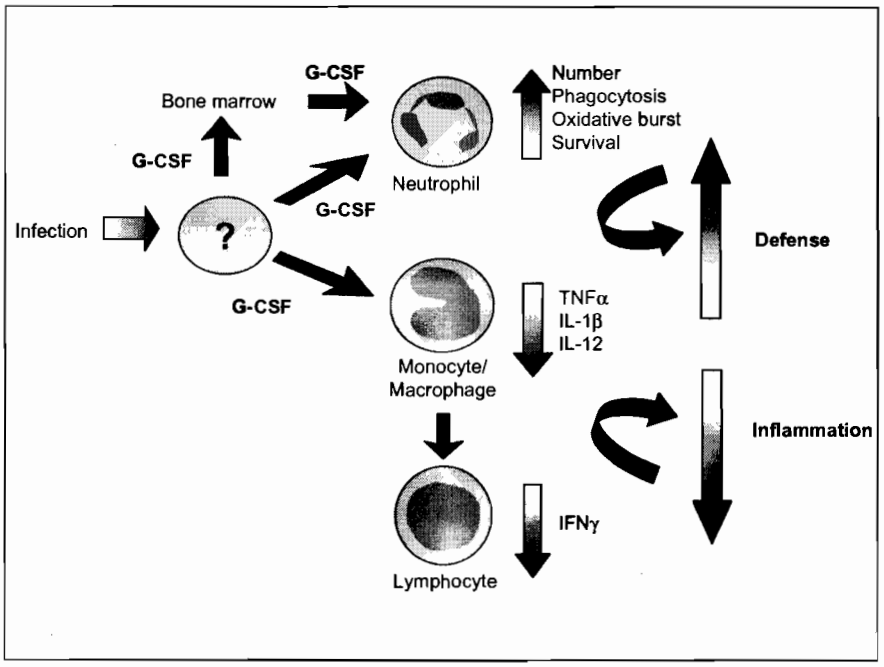
Despite its name, the biological actions of G-CSF do not rest solely with granulocytes, but also encompass effects on monocyte and lymphocyte numbers and functions. Monocytes are primed by G-CSF to release more anti-inflammatory and less pro-inflammatory cytokines upon stimulation. Furthermore, while the *ex vivo* lipopolysaccharide (LPS)-stimulated release of tumor necrosis factor (TNF)α, interleukin (IL)-1β and IL-12 is decreased per monocyte, the release of antagonists of these cytokines, such as soluble TNF receptors and IL-1 receptor antagonists are increased significantly [9•]. Since *ex vivo* LPS-stimulated TNFα and IL-12 production by monocytes was decreased under G-CSF therapy, lymphocytic pro-inflammatory interferon (IFN)γ release, which depends on these two monokines, was also reduced [11•,12•].

Repeated administration of G-CSF (over a 4-day period) not only augmented granulocyte counts, but also led to an increase in monocyte counts and all populations of lymphocytes, without inducing the expression of activation or proliferation markers, indicating that this increase resulted from increased lymphopoiesis, rather than from peripheral lymphocyte proliferation [10]. However, daily treatment for more than 8 days led to a decline in lymphocyte populations, reverting to numbers found in healthy volunteers [11•]. Therefore, exploitation of this activity would depend on the development of a suitable application strategy to maximize this effect.

These 'side effects' of G-CSF raised interest in its use as an immunosupportive adjuvant, eg, promoting immuno-reconstitution in HIV patients by stimulating the production of new lymphocytes [13,14], via perioperative administration after elective surgery, in order to prevent the sepsis and immune dysfunction that may result from this procedure [15•,16,17], or to treat septic preterm infants whose immune systems are not fully developed [18,19]. Some of these strategies appear to be beneficial, however, they have not yet attained widespread acceptance or regulatory approval.

As an example, the potential of G-CSF to reactivate the immune system and aid in the eradication of persistent *Borrelia* infection is discussed in more detail below.

Figure 1. Biological activity of G-CSF.



Infection leads to the production of G-CSF by as yet unidentified cells. G-CSF stimulates the production of neutrophils by the bone marrow and primes them for improved host defense. G-CSF also acts on monocytes, by decreasing their production of pro-inflammatory mediators and increasing the production of their inhibitors. As a result, the production of pro-inflammatory lymphokines is decreased indirectly. Together, G-CSF combines anti-infective and anti-inflammatory activities. **IFN** interferon, **IL** interleukin, **TNF** tumor necrosis factor.

Could G-CSF substitution improve eradication of persistent *Borrelia* infection?

Borrelia infection, transmitted by infected ticks, may become persistent. Increasingly, bacteria-induced immune suppression is recognized as a possible persistence mechanism.

In the blood of patients with Lyme borreliosis challenged with LPS from *Salmonella abortus equi*, or with a lysate from *Borrelia burgdorferi*, less release of TNFα, IFNγ and G-CSF, compared with healthy individuals, was reported in response to both stimuli [20•]. This led to the hypothesis that *Borrelia* generally downregulate the immune response, thus enabling them to persist within the tissue [21,22]. An experimental treatment of a patient with persistent *Borrelia* infection, who had failed with antibiotic therapy, was attempted by combining G-CSF with ceftriaxone administration (1 week ceftriazone, 1 week G-CSF, 1 week both). This treatment regimen was well tolerated. The subjective arthritic symptoms disappeared during the 6 weeks following treatment, and the patient regained fine mechanical skills. After 3 months, the *Borrelia* immunoglobulin G titer was negative, and within two years the immunoblot also became negative [I Diterich, unpublished data].

In order to address a possible effect of G-CSF on *Borrelia* eradication more systematically, mice were infected with *Borrelia* and their clearance under G-CSF treatment was monitored. Fewer *Borrelia* were found in the bladder and ankles of G-CSF-treated mice compared to the placebo-treated group, but infection resulted in severe arthritis in both groups. In severe combined immunodeficiency mice, the number of *Borrelia* was lower in all organs tested in the G-CSF-treated group, compared with the placebo-treated group [I Diterich, unpublished data]. This showed that G-CSF treatment alone had some effect in improving host defense, but combination with antibiotics was necessary. A clinical study investigating G-CSF as an immunosupportive therapy of lyme borreliosis is currently underway.

Investigations of the endogenous production of G-CSF

Surprisingly, little is known about the *in vivo* production and regulation of G-CSF in states of both health and disease. Despite evidence that immune cells, different tumor cells and structural cells, ie, endothelial cells and fibroblasts, are able to produce G-CSF *in vitro*, few published data are available describing the endogenous production of G-CSF *in vivo* [23]. We found that diverse murine organs and tissues could produce G-CSF mRNA and protein in models of LPS-shock or in *Salmonella typhimurium* infection. In contrast, intraperitoneal injection of heat-killed *Salmonella* only induced G-CSF production in the peritoneum, suggesting that G-CSF serum levels may not directly reflect local tissue levels. The structural cells producing G-CSF *in vivo* upon infection with *Salmonella typhimurium* were endothelial cells; in the case of LPS administration, Kupffer cells were additional producers of G-CSF, as identified by *in situ* hybridization [24]. This implies that every tissue can be involved in G-CSF production, and that the site of infection determines the locus of endogenous G-CSF formation.

G-CSF production should now be addressed in animal models of different local or systemic diseases, eg, infection in diabetes, to further our understanding of cells and tissues contributing to the natural G-CSF response, and of situations of inadequate production. What local concentrations are necessary to be physiologically relevant? Does the production of G-CSF follow diurnal rhythms or a pulsatile profile providing additional information? The answers to these questions could allow the definition of clearer therapeutic windows and improve trial designs, as well as indicating methods of counteracting inadequate endogenous production of G-CSF.

Can new strategies to boost G-CSF levels be exploited?

Both transcriptional and translational regulation were found to impact upon G-CSF formation [23]. Regulatory promoter elements have been identified in the human G-CSF promoter, nuclear factor kappa B (NFκB) and nuclear factor for IL (NF-IL)6 binding sites, and more recently a cAMP-responsive element, mediating G-CSF transcription (Figure 2) [25•,26,27]. The combination of NFκB-activating and cAMP-elevating mediators, ie, TNFα and prostaglandin E_2, is sufficient to induce monocytic G-CSF release [25•].

Our knowledge of gene regulatory pathways could be exploited to modulate endogenous G-CSF release. This might be achieved by adenosine or synthetic adenosine receptor agonists, which induce G-CSF production by activating NFκB [28,29]. In combination with these, or in the case of inflammatory states leading to NFκB or NF-IL6 activation, cAMP-elevating drugs, such as phosphodiesterase (PDE) inhibitors, would have the capacity to increase G-CSF formation. The selective inhibition of PDE4 with piclamilast, but not the inhibition of PDE3 with motapizone, significantly increased LPS-induced G-CSF release *in vitro* in whole blood studies [30]. Multiple family members, gene copies and splice variants provide a count of more than 50 different PDE isoenzymes, which differ in their tissue distribution, subcellular localization and substrate specificity [31]. Selective inhibitors of the relevant subtypes might therefore provide a valuable tool to augment G-CSF formation in distinct cell types and tissues, as required.

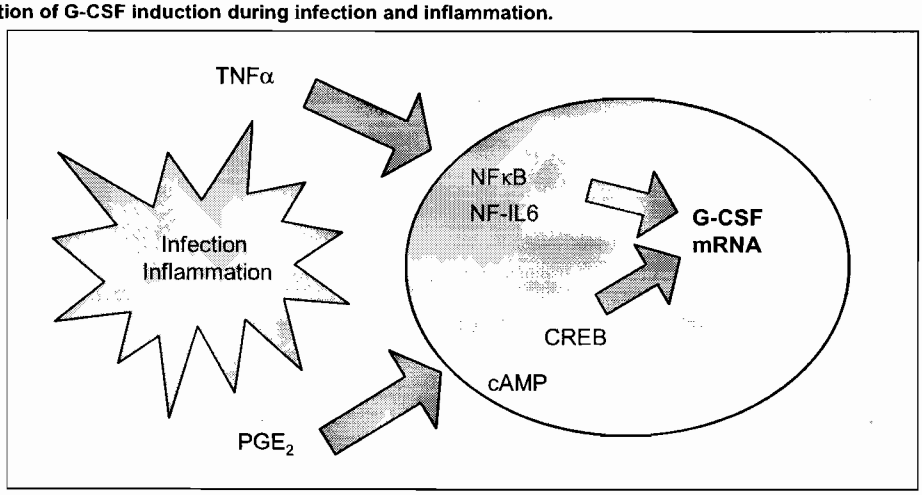
An alternative method of boosting endogenous production of G-CSF on a local scale, may be by local viral transfection of wounded skin [32]. The incorporation of G-CSF and other cytokines into wound dressings is a novel application, which had the beneficial local effect of improving host defense against infection in mice [33]. Leridistim is an engineered chimeric dual agonist of IL-3 and G-CSF receptors, which has stronger effects on colony formation *in vitro* than either IL-3 or G-CSF alone [34].

Longer-lasting systemic application of G-CSF is now also possible using pegfilgrastim, but may also become possible by injection of non-replicating Epstein-Barr virus (EBV)-based expression plasmids. One injection of the G-CSF gene in an EBV-based plasmid increased white blood cell counts for 2 months, by repeatedly re-transfecting immunocompetent hosts [32,35]. Another strategy is the fusion of G-CSF to albumin, which has resulted in a longer half-life of G-CSF in monkeys [36]. These applications may replace daily injection of G-CSF and lead to better compliance over a longer treatment period, as well as reducing costs.

What effects does exogenous G-CSF application have on endogenous G-CSF levels and disease outcome?

It is unclear whether or not the application of G-CSF may benefit patients with burn wounds. Patients with septic burn wounds have measurable serum levels of G-CSF [37]. When challenged with *Pseudomonas aeruginosa*, mice with burns also responded with G-CSF release [38]. In patients with burns and ensuing systemic inflammatory response syndrome, serum levels of G-CSF and TNF diverged in patients who succumbed to the disease, ie, TNF levels increased while G-CSF levels

Figure 2. Modulation of G-CSF induction during infection and inflammation.



The production of G-CSF is regulated on a transcriptional level by nuclear factors and cAMP. These are modulated in turn by mediators such as TNFα and PGE_2. **CREB** cAMP-responsive element-binding protein.

decreased [39]. Administration of G-CSF had beneficial effects in rodents; bacterial translocation was reduced and macrophage suppression was reversed [40,41•]. In a study in patients with burns, in which G-CSF was administered in addition to standard regimens, the survival rate was 42/51 (82%) in the group receiving G-CSF, compared with 9/27 (33%) in the control group [42•].

Two independent studies demonstrated that G-CSF serum levels were high in sepsis patients, but decreased in patients who survived, while remaining elevated in patients who died [43,44•]. The phagocytic and bactericidal activities of the neutrophils were normal during the course of disease in septic and trauma patients [44•]. These two observations, sufficient endogenous production and normal neutrophil function, provide an argument against administering G-CSF to patients who already have sepsis. However, G-CSF administration did show beneficial effects in patients with granulocytopenia and sepsis. Out of 24 patients, 19 responded to G-CSF administration, with an increase in granulocyte counts and a concomitant decrease in G-CSF levels. These patients all survived, while the five non-responders died, retaining high levels of endogenous G-CSF [45]. This indicates that desensitization towards endogenous G-CSF may be overridden by exogenous application in some cases. From a study in which patients were treated with G-CSF after major abdominal surgery, a decrease in the incidence of infectious complications (5/40 in the G-CSF-treated group against 6/20 in the placebo group) was observed [17].

Conclusions

The studies described above illustrate that there are a variety of settings in which application of exogenous G-CSF might be of benefit to patients, including use against many forms of granulocytopenia or granulocyte dysfunction. Although these disorders are often associated with high endogenous levels of G-CSF, additional exogenous application still appears to be beneficial without concomitant detrimental side effects. It is not yet known by which mechanism this desensitization to endogenous G-CSF is caused.

Immunomodulation is a likely explanation for the persistence of some forms of pathogens in host tissue, and this has been shown for viral, bacterial and parasitic infections. The observation that patients diagnosed with a *Borrelia* infection released less G-CSF and inflammatory cytokines in response to endotoxin or a *Borrelia*-specific challenge than healthy individuals, implied that the bacteria influenced the patients' host defenses. Pilot applications of G-CSF in mice, as well as a patient, suggest a possible therapeutic impact of exogenous or boosted endogenous G-CSF. This hypothesis may be expanded to other persistent infections if concomitant G-CSF and antibiotic treatment proves more effective in the elimination of the pathogens in further studies.

The prophylactic use of G-CSF to prime host defenses and pre-empt infections may find applications in elective surgery, or in generally boosting immune defense, eg, in patients with HIV and immune dysfunction.

Naturally, the indiscriminate use of G-CSF as a 'wonder drug' is not a rational scientific strategy. On the other hand, it is not surprising that the potential fields of application are so varied, as many diseases include an element of inflammation and can only be overcome by a functioning immune system, or be ameliorated by strengthening host defense. Inclusion of information on the endogenous production and regulation of G-CSF could optimize rational treatment and improve outcome. Boosting endogenous formation represents a promising novel treatment regimen.

References

1. Hartung T: **Immunomodulation by colony-stimulating factors.** *Rev Physiol Biochem Pharmacol* (1999) **136**:1-164.

2. Frumkin LR: **Role of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor in the treatment of patients with HIV infection.** *Curr Opin Hematol* (1997) **4**(3):200-206.

3. Stephens DP, Fisher DA, Currie BJ: **An audit of the use of granulocyte colony-stimulating factor in septic shock.** *Intern Med J* (2002) **32**(4):143-148.

• *This paper reports on how adjunctive G-CSF administration is highly beneficial with regard to survival in individuals with a form of community-acquired sepsis (melioidosis).*

4. Yonem A, Cakir B, Guler S, Azal OO, Corakci A: **Effects of granulocyte-colony stimulating factor in the treatment of diabetic foot infection.** *Diabetes Obes Metab* (2001) **3**(5):332-337.

5. Bennett SP, Griffiths GD, Schor AM, Leese GP, Schor SL: **Growth factors in the treatment of diabetic foot ulcers.** *Br J Surg* (2003) **90**(2):133-146.

6. de Lalla F, Pellizzer G, **Strazzabosco M**, Martini Z, **Du Jardin G, Lora** L, Fabris P, Benedetti P, Erle G: **Randomized prospective controlled trial of recombinant granulocyte colony-stimulating factor as adjunctive therapy for limb-threatening diabetic foot infection.** *Antimicrob Agents Chemother* (2001) **45**(4):1094-1098.

7. Gough A, Clapperton M, Rolando N, Foster AV, Philpott-Howard J, Edmonds ME: **Randomised placebo-controlled trial of granulocyte-colony stimulating factor in diabetic foot infection.** *Lancet* (1997) **350**(9081):855-859.

8. Kastenbauer T, Hornlein B, Sokol G, Irsigler K: **Evaluation of granulocyte-colony stimulating factor (Filgrastim) in infected diabetic foot ulcers.** *Diabetologia* (2003) **46**(1):27-30.

9. Hartung T, Docke WD, Gantner F, Krieger G, Sauer A, Stevens P, **Volk** HD, Wendel A: **Effect of granulocyte colony-stimulating factor treatment on *ex vivo* blood cytokine response in human volunteers.** *Blood* (1995) **85**(9):2482-2489.

• *This paper describes the anti-inflammatory effect of G-CSF treatment in volunteers, ie, priming for increased soluble TNF receptors and IL-1 receptor antagonists, and decreased TNFα and IFNγ release, upon stimulation.*

10. von Aulock S, Boneberg EM, Hartung T: **Intermittent G-CSF (filgrastim) treatment cannot induce lymphocytosis in volunteers.** *Clin Pharmacol Ther* (2000) **68**(1):104.

11. Hartung T, Doecke WD, Bundschuh D, Foote MA, Gantner F, Hermann C, Lenz A, Milwee S, Rich B, Simon B, Volk HD *et al*: **Effect of filgrastim treatment on inflammatory cytokines and lymphocyte functions.** *Clin Pharmacol Ther* (1999) **66**(4):415-424.

• *This paper describes the effects of daily G-CSF treatment, especially on lymphocyte counts and functions, over 12 days.*

12. Boneberg EM, Hareng L, Gantner F, Wendel A, Hartung T: **Human monocytes express functional receptors for granulocyte colony-stimulating factor that mediate suppression of monokines and interferon-γ.** *Blood* (2000) **95**(1):270-276.

• *The mechanism by which G-CSF exerts its anti-inflammatory activity is elucidated.*

13. Hartung T, Pitrak DL, Foote M, Shatzen EM, Verral SC, Wendel A: **Filgrastim restores interleukin-2 production in blood from patients with advanced human immunodeficiency virus infection.** *J Infect Dis* (1998) **178**(3):686-692.

14. von Aulock S, Hartung T: **Potential for immune reconstitution through G-CSF treatment of HIV patients.** *Arch Immunol Ther Exp* (2002) **50**(2):111-120.

15. Hubel K, Mansmann G, Schafer H, Oberhauser F, Diehl V, Engert A: **Increase of anti-inflammatory cytokines in patients with esophageal cancer after perioperative treatment with G-CSF.** *Cytokine* (2000) **12**(12):1797-1800.
• *In this study, patients were given G-CSF daily from 2 days before, up until 7 days after, surgery. Patients had increased serum levels of anti-inflammatory cytokines and a drastically lower rate of infection than in an historical control.*

16. Lorenz W, Stinner B, Bauhofer A, Rothmund M, Celik I, Fingerhut A, Koller M, Lorijn RH, Nystrom PO, Sitter H, Schein M *et al*: **Granulocyte-colony stimulating factor in the prevention of postoperative infectious complications and sub-optimal recovery from operation in patients with colorectal cancer and increased preoperative risk (ASA 3 and 4). Protocol of a controlled clinical trial developed by consensus of an international study group. Part one: Rationale and hypothesis.** *Inflamm Res* (2001) **50**(3):115-122.

17. Schneider C, von Aulock S, Zedler S, Schinkel C, Hartung T, Faist E: **Perioperative recombinant human granulocyte colony-stimulating factor (Filgrastim) treatment prevents immunoinflammatory dysfunction associated with major surgery.** *Ann Surg* (2004) **239**(1):75-81.

18. Ahmad A, Laborada G, Bussel J, Nesin M: **Comparison of recombinant granulocyte colony-stimulating factor, recombinant human granulocyte-macrophage colony-stimulating factor and placebo for treatment of septic preterm infants.** *Pediatr Infect Dis J* (2002) **21**(11):1061-1065.

19. Bernstein HM, Calhoun DA, Christensen RD: **Use of myeloid colony-stimulating factors in neonates with septicemia.** *Curr Opin Pediatr* (2002) **14**(1):91-94.

20. Diterich I, Härter L, Hassler D, Wendel A, Hartung T: **Modulation of cytokine release in *ex vivo*-stimulated blood from borreliosis patients.** *Infect Immun* (2001) **69**(2):687-694.
• *Blood from borreliosis patients responds to both LPS and borrelia lysate stimulation, with reduced cytokine release in comparison to blood from control individuals.*

21. Diterich I, Hartung T: ***Borrelia burgdorferi* s.l., the infectious agent of Lyme borreliosis.** *Contrib Microbiol* (2001) **8**:72-89.

22. Diterich I, Rauter C, Kirschning CJ, Hartung T: ***Borrelia burgdorferi*-induced tolerance as a model of persistence via immunosuppression.** *Infect Immun* (2003) **71**(7):3979-3987.

23. Hareng L, Hartung T: **Induction and regulation of endogenous granulocyte colony-stimulating factor formation.** *Biol Chem* (2002) **383**(10):1501-1517.

24. Hareng L, Hasiwa N, Niedobitek G, Lehner MD, van Rooijen N, Hartung T: **Characterization and localization of endogenous G-CSF production in murine endotoxemia and peritonitis with *Salmonella typhimurium*.** *Inf Immun* (2004): Manuscript submitted.

25. Hareng L, Meergans T, von Aulock S, Volk HD, Hartung T: **Cyclic AMP increases endogenous granulocyte colony-stimulating factor formation in monocytes and THP-1 macrophages despite attenuated TNF-α formation.** *Eur J Immunol* (2003) **33**(8):2287-2296.
• *The role of cAMP in the stimulation of G-CSF production is elucidated by mutation of a novel CRE sequence in the G-CSF promoter and modulation of cAMP levels.*

26. Shannon MF, Pell LM, Lenardo MJ, Kuczek ES, Occhiodoro FS, Dunn SM, Vadas MA: **A novel tumor necrosis factor-responsive transcription factor which recognizes a regulatory element in hemopoletic growth factor genes.** *Mol Cell Biol* (1990) **10**(6):2950-2959.

27. Dunn SM, Coles LS, Lang RK, Gerondakis S, Vadas MA, Shannon MF: **Requirement for nuclear factor (NF)-κB p65 and NF-interleukin-6 binding elements in the tumor necrosis factor response region of the granulocyte colony-stimulating factor promoter.** *Blood* (1994) **83**(9):2469-2479.

28. Fishman P, Bar-Yehuda S, Farbstein T, Barer F, Ohana G: **Adenosine acts as a chemoprotective agent by stimulating G-CSF production: A role for A_1 and A_3 adenosine receptors.** *J Cell Physiol* (2000) **183**(3):393-398.

29. Bar-Yehuda S, Madi L, Barak D, Mittelman M, Ardon E, Ochaion A, Cohn S, Fishman P: **Agonists to the A_3 adenosine receptor induce G-CSF production via NF-κB activation: A new class of myeloprotective agents.** *Exp Hematol* (2002) **30**(12):1390-1398.

30. Hareng L, Hartung T: **Regulation of G-CSF formation by cAMP: Role of prostanoids, epinephrine and histamine.** *Eur J Immunol* (2004): Manuscript submitted.

31. Hatzelmann A, Schudt C: **Anti-inflammatory and immunomodulatory potential of the novel PDE4 inhibitor roflumilast *in vitro*.** *J Pharmacol Exp Ther* (2001) **297**(1):267-279.

32. Meuli M, Liu Y, Liggitt D, Kashani-Sabet M, Knauer S, Meuli-Simmen C, Harrison MR, Adzick NS, Heath TD, Debs RJ: **Efficient gene expression in skin wound sites following local plasmid injection.** *J Invest Dermatol* (2001) **116**(1):131-135.

33. Grzybowski J, Janiak MK, Oldak E, Lasocki K, Wrembel-Wargocka J, Cheda A, Antos-Bielska M, Pojda Z: **New cytokine dressings. II. Stimulation of oxidative burst in leucocytes *in vitro* and reduction of viable bacteria within an infected wound.** *Int J Pharm* (1999) **184**(2):179-187.

34. Abegg AL, Vickery LE, Bremer ME, Donnelly AM, Doshi PD, Evans ML, Thurman TL, Braford SR, Caparon MH, Bauer SC, Gir JG *et al*: **The enhanced *in vitro* hematopoietic activity of leridistim, a chimeric dual G-CSF and IL-3 receptor agonist.** *Leukemia* (2002) **16**(3):316-326.

35. Tu G, Kirchmaier AL, Liggitt D, Liu Y, Liu S, Yu WH, Heath TD, Thor A, Debs RJ: **Non-replicating Epstein-Barr virus-based plasmids extend gene expression and can improve gene therapy *in vivo*.** *J Biol Chem* (2000) **275**(39):30408-30416.

36. Halpern W, Riccobene TA, Agostini H, Baker K, Stolow D, Gu ML, Hirsch J, Mahoney A, Carrell J, Boyd E, Grzegorzewski KJ: **Albugranin, a recombinant human granulocyte colony stimulating factor (G-CSF) genetically fused to recombinant human albumin induces prolonged myelopoietic effects in mice and monkeys.** *Pharm Res* (2002) **19**(11):1720-1729.

37. Struzyna J, Pojda Z, Braun B, Chomicka M, Sobiczewska E, Wrembel J: **Serum cytokine levels (IL-4, IL-6, IL-8, G-CSF, GM-CSF) in burned patients.** *Burns* (1995) **21**(6):437-440.

38. Gamelli R, He LK, Hahn E: **Granulocyte colony-stimulating factor: Release is not impaired after burn wound infection.** *J Trauma* (2002) **53**(2):284-289.

39. Sun Y, Yan R, Yu D: **The relationship between severe burn injury and systemic inflammatory response syndrome.** *Zhonghua Wai Ke Za Zhi* (1998) **36**(2):110-112.

40. Yalcin O, Soybir G, Koksoy F, Kose H, Ozturk R, Cokneseli B: **Effects of granulocyte colony-stimulating factor on bacterial translocation due to burn wound sepsis.** *Surg Today* (1997) **27**(2):154-158.

41. Gamelli RL, He LK, Liu H: **Recombinant human granulocyte colony-stimulating factor treatment improves macrophage suppression of granulocyte and macrophage growth after burn and burn wound infection.** *J Trauma* (1995) **39**(6):1141-1146.
• *Investigation into whether G-CSF substitution can reduce bone marrow suppression after sepsis.*

42. Arslan E, Yavuz M, Dalay C: **The relationship between tumor necrosis factor (TNF)-α and survival following granulocyte-colony stimulating factor (G-CSF) administration in burn sepsis.** *Burns* (2000) **26**(6):521-524.
• *Decreasing TNFα levels are found to be predictive of survival, and a patient group treated with G-CSF in addition to standard treatment also showed a survival benefit.*

43. Kragsbjerg P, Holmberg H, Vikerfors T: **Dynamics of blood cytokine concentrations in patients with bacteremic infections.** *Scand J Infect Dis* (1996) **28**(4):391-398.

44. Tanaka H, Ishikawa K, Nishino M, Shimazu T, Yoshioka T: **Changes in granulocyte colony-stimulating factor concentration in patients with trauma and sepsis.** *J Trauma* (1996) **40**(5):718-725.
• *This paper compares serum concentrations of G-CSF with those of other cytokines during the course of trauma and sepsis.*

45. Endo S, Inada K, Inoue Y, Yamada Y, Takakuwa T, Kasai T, Nakae H, Kuwata Y, Hoshi S, Yashida M: **Evaluation of recombinant human granulocyte colony-stimulating factor (rhG-CSF) therapy in granulopoietic patients complicated with sepsis.** *Curr Med Res Opin* (1994) **13**(4):233-241.