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The Effects of Granulocyte Colony-Stimulating Factor (G-CSF) in Pre-Clinical Models of Infection and Acute Inflammation

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Introduction

The biologic activity of a group of proteins termed *colony stimulating factors* was first described three decades ago when it was demonstrated that the growth of bone marrow cells in culture could be promoted by soluble factors derived from other host cells [5,28]. Subsequent work revealed bacterial lipopolysaccharide to be a potent stimulus for colony stimulating factor activity, and led to the isolation of granulocyte colony-stimulating factor (G-CSF) from the lungs of mice that had been challenged with endotoxin [46]. In contrast to other colony-stimulating factors, G-CSF only promoted the neutrophilic differentiation of hematopoietic stem cells.

Biologic Properties of Human G-CSF

Human G-CSF is encoded by a single gene located on chromosome 17q and released as an 18 kD glycosylated protein comprising 174 amino acids [9]. G-CSF is part of a larger family of hematopoietic cytokines that includes interleukin 3 and granulocyte-macrophage colony-stimulating factor (GM-CSF). It is synthesized by a variety of cells including macrophages, fibroblasts, and endothelial cells, and gene expression is upregulated by key pro-inflammatory cytokines such as TNF and IL-1 [45].

In health, G-CSF is undetectable in the circulation, or present at levels of 30 pg/ml or less. Acute increases in plasma G-CSF concentration occur during acute infections, with circulating levels attaining nanogram values [8,32].

The biologic effects of G-CSF result from the interaction of the circulating cytokine with its specific cell surface receptor, a homodimeric protein with a molecular weight of 140 kD. The G-CSF receptor is a member of a large family of cytokine receptors that includes receptors for interleukins 2 through 7, 9, 11, and 12, as well as the receptors for GM-CSF, erythropoietin, growth hormone, ciliary neurotrophic factor, and leukemia inhibitory factor [3]. The receptor is a single transmembrane protein of 813 amino acids. The extracellular region of the molecule contains an immunoglobulin-like sequence, a cytokine receptor homologous region, and three fibronectin domains. The intracellular tail contains a sequence homologous to the gp130 protein that transduces IL-6 signals, and two distinct regions called boxes that transduce the mitogenic activity of the cytokine [3]. There are approximately 300 to 1000 G-CSF receptors on each human neutrophil [45].

Physiologic and Pharmacologic Effects of G-CSF

G-CSF exerts multiple overlapping biologic activities whose net effect is the stimulation of the release of polymorphonuclear neutrophils from the bone marrow, their activation for enhanced phagocytosis and killing, and the prolongation of their survival through the inhibition of spontaneous neutrophil programmed cell death (Table 1).

The distinctive biologic activities of G-CSF in vivo have been revealed by analysis of mice in which the gene for G-CSF has been deleted in the embryonic stem cells. These G-CSF knockout mice show circulating neutropenia, and reduced numbers of granulocytic precursors in the bone marrow. Their lifespan is shorter than that of their wild-type littermates, and they are prone to the development of systemic amyloidosis [39]; their ability to withstand an infectious challenge is severely compromised [39,58]. Similarly mice in which the G-CSF receptor has been deleted manifest defects in the expression of granulopoiesis and in the mobilization of hematopoietic precursors from the bone marrow [39]. Nonetheless the phenotypic defect is a survivable one, whose consequences are limited to the activation of a neutrophilic response.

Recombinant G-CSF has been produced in both a glycosylated (lenograstim) and a non-glycosylated (filgrastim) form [2]. More recently, a synthetic non-protein small molecule having high affinity for the murine G-CSF receptor, and producing the biologic effects of native -CSF has been developed [64]. Recombinant G-CSF can be given intravenously or subcutaneously; maximal levels are observed 2 hours after administration, however the cytokine is cleared slowly, and can be

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Table 1. *Biologic Activities of G-CSF*

Augmentation of Neutrophil Numbers
Stimulation of proliferation of granulocyte precursors (myelocytes and promyelocytes
Acceleration of maturation
Prolongation of neutrophil survival by inhibition of apoptosis
Enhancement of Neutrophil Function
Augmentation of respiratory burst
Mobilization of secretory vesicles
Increased phagocytosis and killing
Increased local trafficking in response to inflammatory stimulus
Upregulation of β2 integrin expression
Effects of In Vivo Administration
Increased levels of IL-1ra, soluble TNF receptors, II-6, IL-8, IL-10
Reduced release of TNF, interferon γ, and GM-CSF
Mild reduction in hemoglobin and platelet counts
Elevated LDH and alkaline phosphatase

detected in the circulation 24 hours after administration.

G-CSF has found a clinical niche in the management of the neutropenic patient. Its evaluation in infection and acute inflammation is ongoing. In a small non-randomized trial of twenty septic patients, Ishikawa et al reported increased numbers of circulating neutrophils, but a reduction in levels of C reactive protein, IL-6, and IL-8 in G-CSF-treated patients [30]. A recent phase II randomized trial of 61 patients reported that prophylactic administration of G-CSF to patients with head injury or intracerebral bleeds resulted in increases in neutrophil count, and a reduction in primary bacteremias, with no effect on rates of pneumonia or urinary tract infection, and no impact on mortality or length of stay in the ICU [24]. Despite the predicted toxicity in the patient with neutrophilia, worsening of clinical inflammation has not generally been observed, although a published case report suggests deterioration of ARDS during G-CSF therapy [56].

The experimental profile of the effects of G-CSF would suggest that the cytokine would be of most therapeutic benefit in clinical conditions in which neutrophil numbers were reduced, or where neutrophil activation and microbicidal function were impaired. In contrast, it might be predicted that G-CSF would induce tissue injury and clinical harm in disorders in which local activation of neutrophils was pathologic, for example rheumatoid arthritis, inflammatory bowel disease, or ARDS. Thus it would appear intuitively desirable that the presence of infection be documented, and the neutrophil activational state be measurable and monitored during therapy with G-CSF. These predictions, based on the biology of G-CSF, were evaluated through a systematic review of the G-CSF pre-clinical literature to define the impact of G-CSF on outcome in infectious and non-infectious pre-clinical models of sepsis, and in neutropenic and non-neutropenic hosts.

Methods

The Medline database from 1990 to 1998 was searched using the keyword, "Granulocyte Colony-Stimulating Factor", and restricting the search to animal studies published in the English literature. Studies were considered as mortality studies if mortality figures were quoted, and mortality in at least one of the study groups exceeded 30%, and were considered to provide organ function data if objective results of the physiologic function of at least one organ system (excluding the hematopoietic system) was recorded. Studies were included only if the experimental challenge was a micro-organism (other than a virus or a protozoa) or microbial product, traumatic or burn injury, or pancreatitis.

Results

The literature review identified fifty-eight experimental reports published between 1990 and 1998. There was considerable variability with respect to the animal species used, the nature of the acute challenge, and the use of adjuvant measures, as well as in the methodologic quality of the studies [51]. For those studies that used a mortality endpoint, treatment with G-CSF improved survival in 34 reports, increased mortality in two, and had no impact in seven. It is entirely probable that negative studies are under-represented in the preclinical literature as a result of a well-recognized publication bias that favours the publication of positive studies. Sixteen papers reported studies in animals that had been rendered neutropenic, whereas 43 reported the results of studies in animals with normal circulating neutrophil numbers (one paper evaluated G-CSF under both circumstances).

G-CSF in Microbial Challenge Models

Models were considered to represent a model of systemic microbial challenge if an inoculum of live organisms was administered by the intravenous, intraperitoneal, or intratracheal route in animals that had not been previously exposed to an acute insult.

Systemic Challenge Twenty-two reports evaluated the effects of G-CSF on the response to a systemic challenge with a pathogen; in most cases, animals were pre-treated with the cytokine prior to challenge (Figure 1). Beneficial effects of G-CSF administration were reduced or eliminated when G-CSF was given following microbial challenge. For example, Haberstroh studied pigs that received a chronic infusion of *Pseudomonas* and showed that pre-treatment with G-CSF resulted in reduced temperature, pulmonary artery pressure, and plasma levels of TNF and LPS when compared to saline controls when given prior to bacterial infusion [20], but not when delayed until three hours following bacterial infusion [21].

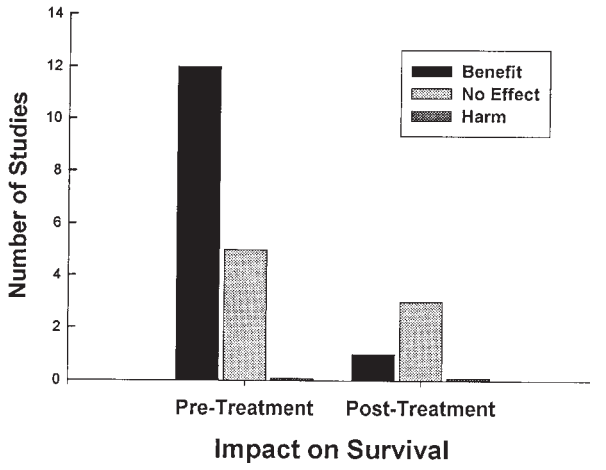

Fig. 1. *The effects of G-CSF in models of systemic challenge with viable micro-organisms. The strongest evidence of survival benefit is seen when G-CSF is administered prophylactically prior to infectious challenge; the single report of benefit for delayed therapy described a model of Tricosporon challenge in the mouse [44]. In none of the studies was there evidence that G-CSF resulted in harm.*

The effects of G-CSF in murine Candidiasis were evaluated in seven reports [10,19,22,37,52,62,66]; in all animals were pre-treated with G-CSF prior to fungal challenge. Survival was increased in six studies; in the seventh, G-CSF afforded protection only when given in association with stem cell factor [62]. The effects of G-CSF on survival were synergistic when used in conjunction with the antifungal agents amphotericin [22] or fluconazole [19]. The survival benefit was evident in both neutropenic and non-neutropenic mice, and occurred in association with a reduction in numbers of viable *Candida*, and with a reduction in circulating levels of TNF and IL-1 [37].

G-CSF was evaluated in three studies of Group B Streptococcal infections in neonatal rats. In two of these, the cytokine improved outcome when given to the neonatal animals [7] or to the mother prior to parturition [47]. No benefit was seen in the third report, despite an increase in circulating neutrophil numbers; intravenous gamma globulin was protective in this study [29]. Pretreatment with G-CSF was also shown to be beneficial in experimental infections with *Listeria* [6,33,57,59], and in a variety of experimental infections with clinically relevant Gram positive and Gram negative organisms [25,43,68].

In summary, animal models using systemic challenge with a fixed inoculum of organisms generally show that G-CSF reduces microbial numbers and improves survival when given prior to, or concomitant with microbial challenge (Figure 1). It is not clear that delayed administration of the agent improves outcome. The beneficial effects of G-CSF include attenuation of clinical manifestations of sepsis, as well as a reduction in pro-inflammatory cytokine levels.

Pulmonary Challenge Models The findings from the eight studies of the effects of G-CSF in models of pneumonia induced by tracheal instillation of live organisms are more complex. Survival was improved by G-CSF in 5 studies, and worsened in 2; in 3 circumstances no effect was seen (Figure 2).

Preheim and colleagues found that treatment of rats with G-CSF improved outcome in experimental pneumococcal pneumonia [54]. On the other hand, mortality was increased in rats challenged intratracheally with *E. coli* that had received G-CSF [15], and in mice pretreated with G-CSF that were challenged with *Klebsiella pneumoniae* [26]. In the latter study, the increased mortality resulting from G-CSF therapy was shown to be a consequence of stimulation of *Klebsiella* capsular polysaccharide production by the cytokine.

By restoring neutrophil numbers in mice that had been rendered neutropenic by cyclophosphamide, G-CSF improved survival rates following pulmonary challenge with *Pseudomonas aeruginosa* to those seen in non-neutropenic controls [60]. In this model, G-CSF failed to restore normal phagocytic and killing activities in neutrophils of cyclophosphamide-treated animals; rather its beneficial effects appeared to be a result of enhanced TNF release from alveolar macrophages, and concomitant treatment with anti-TNF antibody caused increased mortality [60].

Three papers have evaluated the effects of G-CSF in rodents with alcohol-induced liver injury. G-CSF was shown to enhance neutrophil influx into the lung of alcohol-treated rats challenged with *Klebsiella*, leading to improved survival [38]. On the other hand, G-CSF was without effect in rats with liver injury that

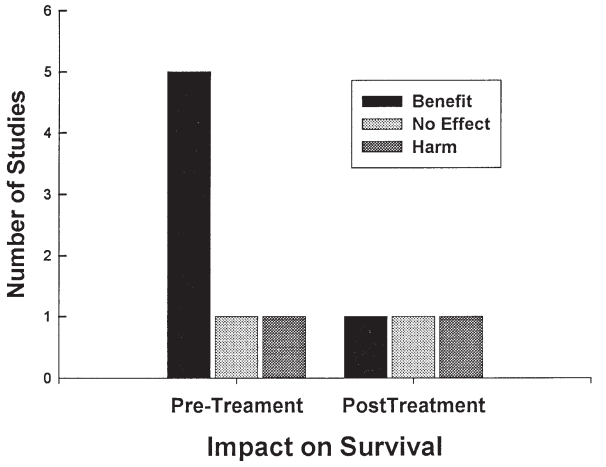

Fig. 2. *The effects of G-CSF in animal models of pneumonia. While prophylactic administration of G-CSF was associated with survival benefit in 5 studies, therapeutic administration equally resulted in benefit and harm.*

were challenged with the Gram positive organism, S. *pneumoniae* [54],[38].

Finally two studies have evaluated the effects of G-CSF in combination with appropriate antibiotic therapy in animal models of pneumonia. G-CSF increased neutrophil numbers and histologic evidence of tissue inflammation, but failed to improve mortality or prevent organ injury in a rabbit model of *Pasteurella* pneumonia [61]. However in a canine model of pulmonary challenge with *E. coli*, pretreatment with G-CSF in association with antibiotics and fluid resuscitation resulted in lower levels of LPS and TNF, attenuation of cardiac dysfunction, and augmented survival; no beneficial effects on bacterial clearance or lung function were evident.

In summary, G-CSF has inconsistent effects in the published studies of its effects on survival following intrapulmonary bacterial challenge (Figure 2). Divergent results may reflect the nature of the challenging species (Gram positive versus Gram negative), the existence of immune compromise, or the concomitant use of antibiotics, however a compelling explanation for the divergent results cannot be ascertained from the small number of published reports.

Intraperitoneal Challenge Three studies have shown that pretreatment of mice [27], rats [11], or dogs [13] with G-CSF consistently improves survival in *E. coli* peritonitis. G-CSF treatment is associated with reduced systemic, but increased local concentrations of TNF, along with increased neutrophil influx, and an improvement in cardiovascular function, reflected in improved left ventricular function and an elevated mean arterial pressure [13].

G-CSF in Models of Endotoxin Challenge

Pre-treatment of mice with recombinant human G-CSF has been reported to reduce the lethality of intravenous challenge with LPS (5 mg/kg), and to protect against the development of hepatitis in D-galactosamine-sensitized animals [17]. The beneficial effects of G-CSF appear to be a consequence of suppression of LPS-stimulated TNF production, and occur despite intact G-CSF-induced priming of neutrophils. Two reports from a single group showed that administration of G-CSF prior to, or concomitant with LPS infusion could attenuate lung leak in a sheep model of endotoxin-induced acute lung injury [23,36]. In a porcine model of endotoxin challenge, pre-treatment with G-CSF resulted in increased numbers of circulating neutrophils, but had no effect on arterial pressure, oxygenation, or lung leak [14]. Vollmar and co-workers found that, although G-CSF increases leukocyte-endothelial interactions, pre-treatment of rats with G-CSF reduced the LPS-induced adhesion of neutrophils to endothelial cells in the hepatic sinusoids, and blunted the release of pro-inflammatory cytokines by hepatic Kupffer cells [67]. In a canine endotoxin challenge model, Freeman and colleagues found that pretreatment with G-CSF reduced circulating endotoxin levels, and attenuated endotoxin-induced cardiovascular dysfunction, without reducing TNF levels [16].

Three reports describe the effects of G-CSF on the pulmonary response to endotoxin challenge. Zhang et al found that G-CSF pre-treatment of rats increased lung neutrophil recruitment and phagocytic capacity in response to endotoxin, without evidence of aggravating lung injury [69]; similar observations were made by Kanazawa and colleagues in a study performed in guinea pigs rendered neutropenic by pretreatment with cyclophosphamide [31]. In contrast, Terashima reported that lung injury was exacerbated in neutropenic guinea pigs that had been given G-CSF prior to endotoxin challenge [63].

In summary, endotoxin challenge studies generally do not show evidence of aggravation of local or systemic injury following G-CSF pre-treatment; there are no published reports evaluating the effects of administration of G-CSF following endotoxin challenge (Figure 4). The data suggest that G-CSF can exert an inhibitory effect on the initiation of a pro-inflammatory cytokine response; further studies are needed to determine whether this inhibitory influence occurs once inflammatory gene transcription has been initiated.

G-CSF in Polymicrobial Peritonitis

Eight studies were identified that evaluated G-CSF in models of complex polymicrobial peritonitis induced by cecal ligation and puncture [18,48,50,65], cecal perforation without ligation [41,42] or intraperitoneal inoculation of feces [4,40]. All seven studies that involved the administration of G-CSF (5 in rats, 1 each in mice and pigs) demonstrated a favourable impact on survival. Improved survival was evident when administration of G-CSF was delayed as long as four hours after infectious challenge [41,42]. Lorenz et al described additional survival benefit when G-CSF was added to systemic antibiotics [40], a finding reproduced by Goya [18], but not by O'Reilly [48]. Barsig and co-workers found that G-CSF levels are increased in a murine model of fecal peritonitis, and that neutralization of G-CSF increases, while administration of recombinant G-CSF reduces mortality [4].

In addition to the salutary effects of G-CSF on neutrophil numbers and function, reductions in circulating TNF and endotoxin were observed [41,50,40]. Toda further showed that G-CSF treatment led to improved renal and hepatic function, and to reduced pathologic evidence of lung injury [65]. One report found benefit for G-CSF in a combined model of hemorrhage and CLP [50].

In summary, studies of the role of G-CSF in models of polymicrobial peritonitis show a highly reproducible beneficial effect, reflected in mortality, reduced concentrations of pro-inflammatory mediators, and reduced

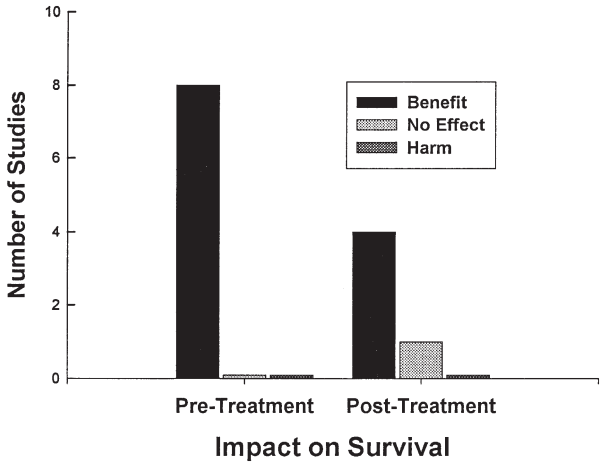

Fig. 3. *The effects of G-CSF in models of monomicrobial or polymicrobial peritonitis. Benefit was evident whether the cytokine was administered before or after infectious challenge. Moreover none of the studies suggested the potential for harm.*

organ injury. Moreover benefit is also seen when therapy is delayed (Figure 3).

Multiple Challenge Models

Infections in the critically ill patient often arise as a complication of another life-threatening process, and it has been shown that the response to a second stimulus can be altered by prior exposure to a primary stimulus [35]. Three authors evaluated G-CSF in a 'two-hit' model; all showed outcome similar to those seen in comparable single challenge models. Patton [50] found that animals subjected to hemorrhage and CLP were benefited by treatment with G-CSF, while Abraham and Stevens showed that G-CSF improved survival in rats challenged with *Pseudomonas* following hemorrhage [1]. Freeman and colleagues found that G-CSF worsened outcome for rats challenged with intrapulmonary *E. coli*, with or without prior hyperoxic lung injury [15].

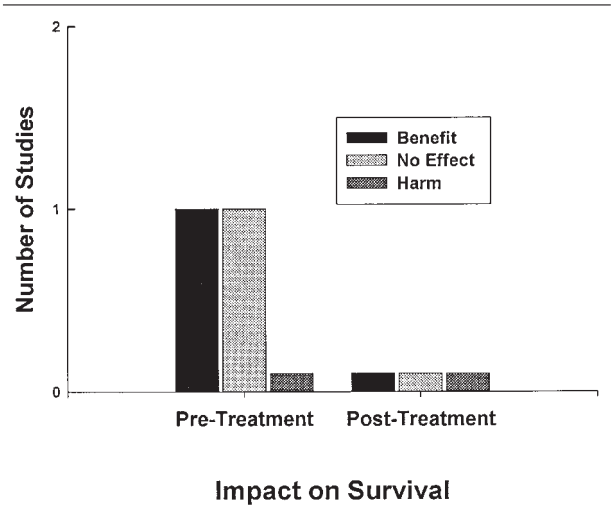

Fig. 4. *The effects of G-CSF in animal models of endotoxemia. Most endotoxemia studies did not evaluate the effects of G-CSF on mortality, and benefit was seen in only one study. Combined with a strong suggestion of harm in non-infectious inflammatory challenge models, these observations suggest that the most promising clinical role for G-CSF is in clinical disorders where uncontrolled or inadequately controlled infection occurs.*

Non-Infectious Models of Inflammatory Challenge

The modulatory role of G-CSF has been evaluated in four studies in which the acute challenge was a non-infectious stimulus. In a canine model of acute pancreatitis, Rao et al showed that treatment with G-CSF induced neutrophilia and reduced the number of positive bacterial cultures at distant sites, however it had no effect on rates of translocation to mesenteric lymph nodes [55]. In a murine model of burn injury, pretreatment with G-CSF led to improved survival for animals that had been both burned and given *E. coli* by gavage; rates of bacterial translocation were not altered [12]. Two studies of G-CSF in rat models of acute lung injury showed that G-CSF worsened survival following lung injury resulting from hydrochloric acid or ANTU [34], and was without effect in hyperoxic lung injury [5].

In summary, although G-CSF exerts anti-bacterial effects in models of sterile inflammation where secondary infection is a prominent feature, it does not help, (and may cause further harm,) in sterile inflammatory tissue injury.

Human Endotoxemia

Two reports have evaluated the effects of G-CSF in healthy human volunteers given an intravenous bolus of purified endotoxin [49,53]. Polmächer and colleagues found that the administration of rG-CSF to healthy volunteers 12 hours prior to a bolus of endotoxin increased the LPS-induced release of both pro-and anti-inflammatory cytokines [53]. Pajkrt evaluated the influence of the timing of administration of G-CSF on the LPS-triggered cytokine response [49]. Administration of G-CSF 24hours prior to LPS challenge resulted in reduced levels of TNF and IL-8, and increased levels of IL-1ra and soluble TNF receptors, whereas administration 2 hours prior to LPS challenge was associated with an exaggerated release of both pro- (IL-8, TNF) and anti-inflammatory (soluble TNFr, IL-1ra) mediators, and increased subjective symptoms and tachycardia. Both regimens reduced the trafficking of neutrophils to the lung following endotoxin challenge.

Conclusions

A systematic review of the extensive literature evaluating the used of granulocyte colony-stimulating factor

in pre-clinical models of infection and acute inflammation provides a number of insights int its in vivo activity that are of potential relevance to the design of human trials of this agent.

G-CSF reliably induces neutrophilia and increases local neutrophil trafficking to the site of an infectious challenge in both neutropenic and non-neutropenic models of sepsis; neutrophil phagocytosis and killing of bacteria also is improved by G-CSF therapy. When administered prior to the inflammatory challenge, G-CSF has been shown to exert an anti-inflammatory influence, reducing systemic levels of pro-inflammatory mediators such as endotoxin [41,50], tumour necrosis factor [41], and interleukin-1 [37]. This effect may be a consequence of better compartmentalization of the inflammatory response, since levels of TNF at the site of the infectious challenge are increased [27,60].

The beneficial effects of G-CSF are also seen as improvements in the organ dysfunction resulting from the experimental challenge [13,65], and its anti-inflammatory effects are mirrored in a reduction in acute lung injury [36].

The most consistent evidence of benefit for G-CSF is found in models of intra-abdominal infection, induced either by the intraperitoneal injection of a defined inoculum of organisms, or by the production of fecal peritonitis. Benefit is seen whether G-CSF is administered prior to, or at a clinically relevant interval following the infectious challenge, and mechanistic data are consistent with the hypothesis that G-CSF improves local peritoneal defenses, and minimizes systemic bacterial spread. On the other hand, evidence of potential harm is greatest in non-infectious models of acute inflammation. Findings in models of pneumonia are inconsistent, with evidence of harm as well as of benefit; harm may be a consequence of unexpected interactions with particular bacterial strains [26] or of inhibition of TNF release from alveolar macrophages [60].

The pre-clinical literature on G-CSF, therefore, suggests that the most promising area for clinical evaluation may be in patients with bacterial peritonitis, in the neutropenic host, or in the immunocompromised patient with disseminated fungal infection. In contrast, current clinical trials with this agent have focussed on patients with community-acquired or nosocomial pneumonia. Optimal makers of the biologic activity of G-CSF are not clear, however improvements in organ dysfunction, increases in neutrophil counts, evidence of bacterial clearance, improvements in neutrophil phagocytosis and killing, and reductions in circulating levels of TNF and LPS all predict a favourable effect on mortality in animal models.

References

1. Abraham E, Stevens P. Effects of granulocyte colony stimulating factor in modifying mortality from *Pseudomonas aeruginosa* pneumonia after hemorrhage. *Crit Care Med* 1992;20:1127–1133.
2. Anderlini P, Przepiorka D, Champlin R, Körbling M. Biologic and clinical effects of granulocyte colony-stimulating factor in normal individuals. *Blood* 1996;88:2819–2825.
3. Avalos BR. Molecular analysis of the granulocyte colony-stimulating factor receptor. *Blood* 1996;88:761–777.
4. Barsig J, Bundschuh DS, Hartung T, Bauhofer A, Sauer A, Wendel A. Control of fecal peritoneal infection in mice by colony-stimulating factors. *J Infect Dis* 1996;174:790–799.
5. Bradley TR, Metcalf D. The growth of mouse bone marrow cells in vitro. *Aust J Exp Biol Med Sci* 1966;44:287–294.
6. Buisman AM, Langermans JA, Van Furth R. Effect of granulocyte colony-stimulating factor on the course of infection with Gram positive bacteria in mice during granulocytopenia induced by sublethal irradiation or cyclophosphamide. *J Infect Dis* 1996;174:417–421.
7. Cairo MS, Plunkett JM, Mauss D, Van de Ven C. Seven-day administration of recombinant human granulocyte colony-stimulating factor to newborn rats: Modulation of neonatal neutrophilia, myelopoiesis, and Group B Streptococcus sepsis. *Blood* 1990;76:1788–1794.
8. Cebon J, Layton J, Maher D, Morstyn G. Endogenous hematopoietic growth factors in neutropenia and infection. *Br J Haematol* 1994;86:274.
9. Dale DC, Liles WC, Summer WR, Nelson S. Review: Granulocyte colony-stimulating factor role and relationships in infectious diseases. *J Infect Dis* 1995;172:1075.
10. DeMarsh PL, Sucolski SK, Frey CL, Bhatnagar PK, Koltin Y, Actor P, Petteway SR. Efficacy of the hematoregulatory peptide SK&F 107647 in experimental *Candida albicans* infections in normal and immunosuppressed mice. *Immunopharm.* 1994;27:199-206.
11. Dunne JR, Dunkin BJ, Nelson S, White JC. Effects of granulocyte colony stimulating factor in a nonneutropenic rodent model of *Escherichia coli* peritonitis. *J Surg Res* 1996;61: 348–354.
12. Eaves-Pyles T, Alexander JW. Granulocyte colony-stimulating factor enhances killing of translocated bacteria but does not affect barrier function in a burn mouse model. *J Trauma* 1996;41:1013–1017.
13. Eichacker PQ, Waisman Y, Natanson C, Farese A, Hoffman WD, Banks SM, Macvittie TJ. Cardiopulmonary effects of granulocyte colony-stimulating factor in a canine model of bacterial sepsis. *J Appl Physiol* 1994;77:2366–2373.
14. Fink MP, O'Sullivan BP, Menconi MJ, Wollert SP, Wang H, Youssef ME, Bellelsle JM. Effect of granulocyte colony-stimulating factor on systemic and pulmonary responses to endotoxin in pigs. *J Trauma* 1993;34:571–578.
15. Freeman BD, Correa R, Karzai W, et al. Controlled trials of rG-CSF and CD11b-directed MAb during hyperoxia and *E. coli* pneumonia in rats. *J Appl Physiol* 1996;80:2066–2076.
16. Freeman BD, Quezado Z, Zeni F, et al. rG-CSF reduces endotoxemia and improves survival during *E. coli* pneumonia. *J Appl Physiol* 1997;83:1467–1475.
17. Gorgen I, Hartung T, Leist M, et al. Granulocyte colony-stimulating factor treatment protects rodents against lipopolysaccharde-induced toxicity via suppression of systemic tumor necrosis factor-alpha *J Immunol* 1992;149:918– 924.
18. Goya T, Torisu M, Doi F, Yoshida T. Effects of granulocyte colony-stimulating factor and monobactam antibiotics (Aztreonam) on neutrophil functions in sepsis. *Clin Immunol Immunopathol* 1993;69:278–284.
19. Graybill JR, Bocanegra R, Luther M. Antifungal combina-

tion therapy with granulocyte colony-stimulating factor and fluconazole in experimental disseminated Candidiasis. *Eur J Clin Microbiol Infect Dis* 1995;14:700–703.
20. Haberstroh J, Breuer H, Lucke l, et al. Effect of recombinant human granulocyte colony-stimulating factor on hemodynamic and cytokine response in a porcine model of *Pseudomonas* sepsis. *Shock* 1995;4:216–224.
21. Haberstroh J, Wiese K, Geist A, et al. Effect of delayed treatment with recombinant human granulocyte colony-stimulating factor on survival and plasma cytokine levels in a non-neutropenic porcine model of *Pseudomonas aeruginosa* sepsis. *Shock* 1998;9:128–134.
22. Hamood M, Bluche PF, De Vroey C, Corazza F, Bujan W, Fondu P. Effects of recombinant human granulocyte colony-stimulating factor on neutropenic mice infected with *Candida albicans:* acceleration of recovery from neutropenia and potentiation of anti-*C. albicans* resistance. *Mycoses* 1994;37:93–99.
23. Hanaoka M, Kubo K, Miyahara T, Hayano T, Koizumi T, Kobayashi T, Sekiguchi M. Effect of post-treatment with granulocyte colony-stimulating factor on endotoxin-induced lung injury in sheep. *Exp Lung Res* 1998;24:15–25.
24. Heard SO, Fink MP, Gamelli RL, et al. The Filgrastim Study Group. Effect of prophylactic administration of recombinant human granulocyte colony-stimulating factor (filgrastim) on the frequency of nosocomial infections in patients with acute traumatic brain injury or cerebral hemorrhage. *Crit Care Med* 1998;26:748–754.
25. Hebert JC, O'Reilly M, Barry B, Shatney L, Sartorelli K. Effects of exogenous cytokines on intravascular clearance of bacteria in normal and splenectomized mice. *J Trauma* 1997; 43:875–879.
26. Held TK, Mielke ME, Chedid M, Unger M, Trautmann M, Huhn D, Cross AS. Granulocyte colony-stimulating factor worsen the outcome of experimental *Klebsiella pneumoniae* pneumonia through direct interaction with the bacteria. *Blood* 1998;91:2525–2535.
27. Hustinx W, Benaissa-Trouw B, Van Kessel K, et al. Granulocyte colony-stimulating factor enhances protection by anti-K1 capsular lgM antibody in murine *Escherichia coli* sepsis. *Eur J Clin Invest* 1997;27:1044–1048.
28. Ichikawa Y, Pluznik DH, Sachs RJ. In vitro control of the development of macrophage and granulocyte colonies. *Proc Natl Acad Sci USA* 1966;56:495.
29. Iguchi K, Inoue S, Kmar A. Effect of recombinant human granulocyte colony-stimulating factor administration in normal and experimentally infected newborn rats. *Exp Hematol* 1991;19:352–358.
30. Ishikawa K, Tanaka H, Matsuoka T, Shimazu T, Yoshioka T, Sugimoto H. Recombinant human granulocyte colony-stimulating factor attenuates inflammatory responses in septic patients with neutropenia. *J Trauma* 1998;44:1047–1054.
31. Kanazawa M, Ishizaka A, Hasegawa N, Suzuki Y, Yokoyama T. Granulocyte colony-stimulating factor does not enhance endotoxin-induced acute lung injury in guinea pigs. *Am Rev Respir Dis* 1992;145:1030–1035.
32. Kawakami M, Tsutsumi H, Kumakawa T, et al.. Levels of serum granulocyte colony-stiulating factor in patients with infections. *Blood* 1990;78:3254–3258.
33. Kayashima S, Tsuruu S, Hata N, Rokutanda M. Therapeutic effect of granulocyte colony-stimulating factor (G-CSF) on the protection against Listeria infection in SCID mice. *Immunology* 1993;80:471–476.
34. King J, Deboisblanc BP, Mason CM, et al. Effect of granulocyte colony-stimulating factor on acute lung injury in the rat. *Am J Resp Crit Care Med* 1995;151:302–309.
35. Koike K, Moore FA, Moore EE, Poggetti RS, Tuder RM, Banerjee A. Endotoxin after gut ischemia/reperfusion causes irreversible lung injury. *J Surg Res* 1992;52:656–662.
36. Koizumi T, Kubo K, Shinozaki S, Koyama S, Kobayashi T, Sekiguchi M. Granulocyte colony-stimulating factor does not exacerbate endotoxin-induced lung injury in sheep. *Am Rev Respir Dis* 1993;148:132–137.
37. Kullberg B-J, Netea MG, Curfs JH, Keuter M, Meis JF, van der Meer JW. Recombinant murine granulocyte colony-stimulating factor protects against acute disseminated *Candida albicans* infection in nonneutropenic mice. *J Infect Dis* 1998;177:175–181.
38. Lister PD, Gentry MJ, Preheim LC. Granulocyte colony-stimulating factor protects control rats but not ethanol-fed rats from fatal pneumococcal pneumonia. *J Infect Dis* 1993; 168:922–926.
39. Liu F, Poursine-Laurent J, Link DC. The granulocyte colony-stimulating factor receptor is required for the mobilization of murine hematopoietic progenitors into peripheral blood by cyclophoshamide or interleukin-8 but not flt-3 ligand. *Blood* 1997;90:2522–2528.
40. Lorenz W, Reimund K-P, Weitzel F, et al. Granulocyte colony-stimulating factor prophylaxis before operation protects against lethal consequences of postoperative peritonitis. *Surgery* 1994;116:925–934.
41. Lundblad R, Nesland JM, Giercksky KE. Granulocyte colony-stimulating factor improves survival rate and reduces concentrations of bacteria, endotoxin, tumor necrosis factor, aned endothelin-1 in fulminant intra-abdominal sepsis in rats. *Crit Care Med* 1996;24:820–826.
42. Lundblad R, Wang MY, Kvalheim G, Lingaas E, Giercksky KE. Granulocyte colony-stimulating factor improves myelopoiesis and host defense in fulminant intra-abdominal sepsis in rats. *Shock* 1995;4:68–73.
43. Matsumoto M, Matsubara S, Yokota T. Effect of combination therapy with recombinant human granulocyte colony-stimulating factor (r G-CSF) and antibiotics in neutropenic mice unresponsive to antibiotics alone. *J Antimicrob Chemother* 1991;28:447–453.
44. Muranaka H, Suga M, Nakagawa K, Sato K, Gushima Y, Ando M. Effects of granulocyte ad granulocyte-macrophage colony-stimulating factors in a neutropenic murine model of trichosporonosis. *Infect Immun* 1997;65:3422–3429.
45. Nelson S, Bagby GJ. Granulocyte colony-stimulating factor and modulation of inflammatory cells in sepsis. *Clin Chest Med* 1996;17:319–332.
46. Nicola NA, Metcalf D, Matsumoto M, Johnson GR. Purification of a factor inducing differentiation in murine myelomonocytic leukemia cells. *J Biol Chem* 1983;258:9017–9023.
47. Novales JS, Salva AM, Modanlou HD, Kaplan DL, del Castillo J, Anderson J, Medlock ES. Maternal administration of granulocyte colony-stimulating factor improves neonatal rat survival after lethal group B Streptococcal infection. *Blood* 1993;81:923–927.
48. O'Reilly M, Silver GM, Greenhalgh DG, Gamelli RL, Davis JH, Hebert JC. Treatment of intra-abdominal infection with granulocyte colony-stimulating factor. *J Trauma* 1992;33: 679–682.
49. Pajkrt D, Manten A, van der Poll T, et al. Modulation of cytokine release and neutrophil function by granulocyte colony-stimulating factor during endotoxemia in humans. *Blood* 1997;90:1415–1424.

50. Patton JH Jr, Lyden SP, Rgasdale DN, Croce MA, Fabian TC, Proctor KG. Granulocyte colony-stimulating factor improves host defense to resuscitated shock and polymicrobial sepsis without provoking generalized neutrophil-mediated damage. *J Trauma* 1998;44:750–758.
51. Piper RD, Cook DJ, Bone RC, Sibbald WJ. Introducing *Critical Appraisal* to studies of animal models investigating novel therapies in sepsis. *Crit Care Med* 1996;24:2059–2070.
52. Polak-Wyss A. Protective effect of human granulocyte colony-stimulating factor (h G-CSF) on Candida infections in normal and immunosuppressed mice. *Mycoses* 1991;34:109–118.
53. Polmächer T, Korth C, Mullington J, et al. Effects of granulocyte colony-stimulating factor on plasma cytokine and cytokine receptor levels and on the in vivo host response to endotoxin in healthy men. *Blood* 1996;87:900.
54. Preheim LC, Snitily MU, Gentry MJ. Effects of granulocyte colony-stimulating factor in cirrhotic rats with pneumococcal pneumonia. *J infect Dis* 1996; 174:225–228.
55. Rao R, Prinz RA, Kazantsev GB, et al. Effects of granulocyte colony-stimulating factor in severe pancreatitis. *Surgery* 1996;119:657–663.
56. Schilero GJ, Oropello J, Benjamin E. Impairment in gas exchange after granulocyte colony stimulating factor (G-CSF) in a patient with the adult respiratory distress syndrome. *Chest* 1995;107:276–278.
57. Serushago BA, Yoshikai Y, Handa T, Mitsuyama M, Muramori K, Nomoto K. Effect of recombinant human granulocyte colony-stimulating factor (rh G-CSF) on murine resistance against *Listeria monocytogenes. Immunology* 1992; 75:475–480.
58. Seymour JF, Lieschke GJ, Grail D, Quillici C, Hodgson G, Dunn AR. Mice lacking both granulocyte colony-stimulating factor (CSF) and granulocyte macrophage CSF have impaired reproductive capacity, perturbed neonatal granulopoiesis, lung disease, amyloidosis, and redced long term survival. *Blood* 1997;90:3037–3049.
59. Shinomiya N, Tsuru S, Katsura Y, Kayashima S, Nomoto K. Enhanced resistance against *Listeria monocytogenes* achieved by pretreatment with granulocyte colony-stimulating factor. *Infect Immun* 1991;59:4740–4743.
60. Shirai R, Kadoto T, Tomono K, Ogawa K, lida K, Kawakami K, Kohno S. Protective effect of granulocyte colony-stimulating factor (G-CSF) in a granulocytopenic mouse model of *Pseudomonas aeruginosa* lung infection through enhanced phagocytosis and killing by alveolar macrophages through priming tumor necrosis factor α (TNF-α) production. *Clin exp Immunol* 1997;109:73–79.
61. Smith WS, Sumnicht GE, Sharpe RW, Samuelson D, Millard FE. Granulocyte colony-stimulating factor versus placebo in addition to penicillin G in a randomized blinded study of gram-negative pneumonia sepsis: Analysis of survival and multisystem organ failure. *Blood* 1995;86:1301–1309.
62. Steinshamn S, Bergh K, Waage A. Effects of stem cell factor and granulocyte colony-stimulating factor on granulocyte recovery and *Candida albicans* infection in granulocytopenic mice. *J Infect Dis* 1993;168:1444–1448.
63. Terashima T, Kanazawa M, Sayama K, et al. Granulocyte colony-stimulating factor exacerbates acute lung injury induced by intratracheal endotoxin in guinea pigs. *Am J Resp Crit Care Med* 1994;149:1295–1303.
64. Tian S-S, Lamb P, King AG, et al. A small, nonpeptidyl mimic of granulocyte colony-stimulating factor. *Science* 1998;281: 257–259.
65. Toda H, Murata A, Matsuura N, Uda K, Oka Y, Tanaka N, Mori T. Therapeutic efficacy of granulocyte colony stimulating factor against rat cecal ligation and puncture model. *Stem Cells* 1993;11:228–234.
66. Uchida K, Yamamoto Y, Klein TW, Friedman H, Yamaguchi H. Granulocyte colony-stimulating factor facilities the restoration of residence to opportunistic fungi in leukopenic mice. *J Med Vet Mycol* 1992;30:293–300.
67. Vollmar B, Messner S, Wanner GA, Hartung T, Menger MD. Immunomodulatory ation of G-CSF in a rat model of endotoxin-induced liver injury: An intravital microscopic analysis of Kupffer cell and leukocyte response. *J Leuk Biol* 1997; 62:710–718.
68. Wakiyama H, Tsuru S, Hata N, et al. Therapeutic effect of granulocyte colony-stimulating factor and cephem antibiotics against experimental infections in neutropenic mice induced by cyclophosphamide. *Clin Exp Immunol* 1993;92: 218–224.
69. Zhang P, Bagby GJ, Stoltz DA, Spitzer JA, Summer WR, Nelson S. Modulation of the lung host response by granulocyte colony-stimulating factor in rats challenged with intrapulmonary endotoxin. *Shock* 1997;7:193–199.