

# Growth Factors G-CSF and GM-CSF: Clinical Options

Thomas Hartung, Sonja von Aulock, and Albrecht Wendel

The body's immediate response to bacterial infection becomes clinically evident as an inflammatory reaction accompanied by an acute-phase response in the liver and hyperthermia. Activation of the immune system must be counterregulated to curb these processes and to prevent (or at least minimize) damage to the host tissue. The major part of this intricate regulation is performed by the cytokine mediator network, a relay of glycoprotein signals that first activate proliferation and host defense functions of immune cells and then control the return to a state of readiness when the infection is under control.

Septic shock encompasses fulminant, and self-destructive activation of the defense system that is now understood as a systemic inflammatory reaction followed by multiple organ failure. This extreme activation of the nonspecific immune system is followed, provided the patient can be stabilized by intensive medical care, by corresponding massive counterregulation. The patient, whose immune system is exhausted, is left almost defenseless in a state termed immune paralysis, or anergy. A patient in this state is particularly susceptible to life-threatening secondary infections.

Granulocyte (G-CSF) and granulocyte/macrophage colony-stimulating factors (GM-CSF) (Table 64.1), central mediators of the endogenous response to infection and inflammation, have been cloned and are commercially available in forms approved for clinical use (Table 64.2). Both stimulate the proliferation and release of immune cells from the bone marrow, and so they were originally approved for the treatment of leukopenia. G-CSF, when given prophylactically or as substitution in situations of deficiency, has also been attributed with improved host defense paired with antiinflammatory effects. GM-CSF, on the other hand, is considered a potent immunostimulator and proinflammatory agent.

Evidence from many animal studies and some clinical studies suggests that prophylactic treatment with G-CSF at the time a risk can be anticipated, such as before an operation, may offer protection from infections and lower the incidence of sepsis. GM-CSF therapy may find a place in reactivating the immune system of patients in a state of immune paralysis following septic shock, thereby reinforcing the patients' impaired defense system

against secondary infections. (See Table 64.3 for approved and experimental indications.)

## G-CSF

### Endogenous G-CSF Response to Infection

The glycoprotein G-CSF is present at low concentrations (around 25 pg/ml) in the serum of healthy volunteers.<sup>1</sup> A significant increase in G-CSF secretion may be detected during the acute phase of a bacterial infection.<sup>4-6</sup> Patients may have peak concentrations as high as 200 ng/ml at the onset of septic shock.<sup>7</sup> These levels decrease significantly within a few days in survivors, but in nonsurviving sepsis patients the G-CSF levels remain persistently elevated.<sup>8,9</sup> Furthermore, patients who do not respond to infection with increased G-CSF production have a worse prognosis than patients who respond with G-CSF production.<sup>10</sup> These studies showed that patients with the best outcome are those who are able to respond appropriately to an infectious agent by increasing G-CSF levels and then decreasing them upon resolution of the infection. However, in patients with a fatal outcome, G-CSF levels tend to remain elevated, indicating an inability of the host to respond to circulating G-CSF, continued signaling for G-CSF production, or failure of the host to mount a sufficient G-CSF response.<sup>11</sup> The observation that injection of pharmacologic doses of G-CSF (10 µg/kg) into healthy volunteers elevated the serum G-CSF to levels in the upper range of those reached by endogenous production during infection<sup>12</sup> supports the hypothesis that administration of G-CSF under the conditions discussed above may be beneficial to the host by increasing or accelerating the response to infection.

### Role of G-CSF in the Cellular Immune System

The central role of endogenous G-CSF lies in the maintenance and control of granulopoiesis, which is essential for efficient host defense, as demonstrated in knockout mice<sup>13</sup> or in normal mice injected with anti-murine G-CSF antiserum.<sup>14</sup> Both sets of mice were severely neutropenic and unable to recruit additional

TABLE 64.1. Common Abbreviations and Synonyms for G-CSF and GM-CSF.

Abbreviation	Synonym
<b>G-CSF</b> , CSF-G	Granulocyte colony-stimulating factor
CSF- $\beta$ , CSF-3	Colony-stimulating factor $\beta$ or 3, respectively
MGI-1G, MGI-2	Macrophage/granulocyte inducer 1G or 2, respectively
G/M-CSA	Granulocyte/macrophage colony-stimulating activity
DF	Differentiation factor; pluripoietin
pCSF	Pluripotent colony-stimulating factor
<b>GM-CSF</b> , CSF-GM	Granulocyte/macrophage colony-stimulating factor
CSF- $\alpha$ , CSF-2	Colony-stimulating factor $\alpha$ or 2, respectively
MGI-1GM	Macrophage/granulocyte inducer 1GM
Eo-CSF	Eosinophil colony-stimulating factor
HCGF	Hematopoietic cell growth factor
KTGF	Keratinocyte-derived T cell growth factor
NIF-T	T cell-derived neutrophil migration inhibition factor

Modified from Ibelgaufs.<sup>1</sup>

TABLE 64.2. Commercial Formulations of G-CSF and GM-CSF.

Generic name	Trade name	Country
<b>G-CSF</b>		
Filgrastim	Neupogen	Europe, US, Canada, Australia
	Gran	Japan, Taiwan, Korea, China
Lenograstim	Neutrogin	Japan, China
	Granocyte	Europe, Australia
Nartograstim	Neu-Up	Japan
<b>GM-CSF</b>		
Molgramostim	Leukomax	Europe, Canada
Sargramostim	Leukine	US

Modified from Root and Dale,<sup>2</sup> with permission.

neutrophils from the bone marrow in response to an infectious challenge and were therefore more susceptible than controls to sublethal doses of infective agents.

Doses of G-CSF ranging from 1 to 60  $\mu\text{g}/\text{kg}/\text{day}$  given to human volunteers for 6 days produced dose-dependent 1.8- to 12-fold increases in the absolute neutrophil count.<sup>15</sup> In a volunteer study, we found a dose-dependent increase of the polymorphonuclear neutrophil (PMN) count with a plateau

lasting throughout the whole 12 days of treatment with G-CSF (filgrastim). Counts equal to those prior to treatment were seen 72 hour after the last injection, (unpublished observations, submitted for publication). Lesser increases in monocyte and lymphocyte counts have also been reported.<sup>16</sup> In addition to increasing the pool of circulating neutrophils, G-CSF primes these immune cells for enhanced effector functions by improving the oxidative burst, phagocytosis, and chemotaxis and by extending their lifetime by delaying apoptosis.<sup>17-19</sup> Thus G-CSF promotes the migration of increasing numbers of immunocompetent and highly potent neutrophils to the focus of infection in an effort to eradicate invading microbes.

Neutrophils pose the first line of defense against all particles identified as foreign; but they may cause damage to host tissues if they are activated prematurely or damaged or if the inflammatory reaction is not terminated on time.<sup>20</sup> Thus during the exploration of possible indications for G-CSF, it was necessary to consider whether the newly recruited neutrophils were functional or preactivated and whether application of G-CSF might unbalance the cytokine mediator network, resulting in exacerbation of the inflammatory reaction.

The *in vitro* studies discussed above showed that G-CSF does not directly activate but, rather, primes PMNs for increased responsiveness to subsequent stimulation. The implication of this priming effect of G-CSF on PMNs is that their functions are potentiated only in the case of stimulation by exogenous signals.

### Effects of G-CSF on the Humoral Response

The inflammatory response is driven primarily by the cytokines tumour necrosis factor- $\alpha$  (TNF $\alpha$ ), interleukin-1 (IL-1), and interferon- $\gamma$  (IFN $\gamma$ ), which are produced by monocytes/macrophages and lymphocytes. Endogenous countermeasures include a reduction in their production or secretion and the release of their respective antagonists: soluble TNF receptors (sTNF-R) or IL-1 receptor antagonist (IL-1ra).

*In vitro* and *ex vivo* experiments indicated that G-CSF adjusts the response of rodent and human mononuclear cells to immunostimulatory agents, such as the gram-negative cell wall

TABLE 64.3. Approved and Experimental Indications of G-CSF and GM-CSF.

Indication	G-CSF	GM-CSF
Aplastic anemia	Approved in some countries	
Acute leukemia	Approved in some countries	Approved in some countries
Bone marrow transplantation	Approved	Approved
Chemotherapy-induced neutropenia	Approved	Approved
Diabetic foot infection	Experimental (phase II)	
Fungal infection, candidemia	Experimental (phase II)	
Myelodysplastic syndrome	Approved in some countries	
Nonneutropenic infection	Experimental (phase II-III)	
Neutropenia in HIV infection	Approved in some countries	Approved in some countries
Peripheral blood progenitor cell transplantation	Approved in most countries	Approved in some countries
Severe chronic neutropenia	Approved	

Modified from Frumkin and Dale,<sup>3</sup> with permission.

component lipopolysaccharide (LPS), by causing a decrease in the production of proinflammatory cytokines and an increase in antiinflammatory cytokine release.<sup>21-23</sup> These results were substantiated in a series of ex vivo human volunteer studies: Whole blood from G-CSF-treated volunteers responded to a variety of immunostimuli, such as LPS, preparations from gram-positive bacteria, superantigens, or phorbol esters, with reduced TNF activity in comparison to blood from placebo-treated controls.<sup>24,25</sup> Neutrophils from these G-CSF-treated volunteers showed increased ex vivo LPS-inducible IL-1ra release, whereas the shedding of soluble TNF receptors was unaffected when calculated per PMN, though of course these cells were present in significantly higher numbers in the blood of the G-CSF-treated subjects.<sup>25</sup> Furthermore, the release capacity of the chemoattractive leukotriene B<sub>4</sub>, when expressed per neutrophil, was decreased significantly. This finding can be interpreted as a further antiinflammatory effect of G-CSF unrelated to the cytokine network (unpublished observations). In addition, IFN $\gamma$  formation by lymphocytes was attenuated in whole blood incubated in the presence of LPS. Thus the overall antiinflammatory effect of a single G-CSF injection consisted of the attenuated release of proinflammatory mediators by monocytes and lymphocytes and concomitant augmented formation and secretion of the respective antagonists by neutrophils.

In another study we examined the effects of daily G-CSF treatment for 12 days in 24 healthy volunteers. Compared to a placebo group, TNF $\alpha$ , IL-12, and IFN $\gamma$  release in whole blood samples in response to ex vivo stimulation by LPS was reduced in the verum groups compared with the control group throughout treatment. Thus the antiinflammatory effect of G-CSF is also maintained under sustained treatment regimens. The in vitro addition of IL-12 to LPS-stimulated blood lessened the attenuation of IFN $\gamma$  and TNF $\alpha$  release capacity, indicating that suppression of IL-12 release is pivotal in the antiinflammatory activity to G-CSF.

To assess the time window during which treatment with exogenous G-CSF might improve the course of an infection, a volunteer trial was held in which G-CSF was injected 2 or 24 hours before challenge with LPS in vivo.<sup>26</sup> Administration of G-CSF shortly before LPS boosted the levels of TNF, IL-6, IL-8, IL-1ra, and both kinds of sTNF-R. In comparison, G-CSF injection 1 day prior to challenge significantly decreased IL-8 levels and moderately attenuated the release of TNF and IL-6. The release of IL-1ra and sTNF-R had increased prior to LPS injection. Administration of LPS resulted in a further increase in the sTNF-R I and II levels, whereas IL-1ra release remained unaltered. Despite the different effects on cytokine release patterns, the two treatment regimens resulted in similar positive effects on neutrophil activation and similar changes in surface molecule expression. Moreover, both G-CSF pretreatments blocked LPS-induced granulocyte accumulation in the lung.

### G-CSF in Sepsis Models

Many studies have been performed in diverse animal models to explore the relevance of these observations during sepsis and

septic shock and to determine whether unacceptable side effects are associated with G-CSF treatment of these conditions. The efficacy of G-CSF, alone or in combination with antibiotics, has been explored in a wide variety of nonneutropenic infectious disease models including neonatal sepsis, pneumonia, infections complicated by ethanol intoxication, burn wound infection, intraabdominal sepsis, and intramuscular infection.<sup>18,27</sup> Results from these studies and those discussed below, in which the survival rate was increased significantly by G-CSF in most cases, indicated that prophylactic administration of G-CSF as a pretreatment when an increased risk of infection is foreseeable, (e.g., before an operation) may be beneficial prophylactically.

In all the animal studies discussed below, G-CSF treatment was initiated prior to or simultaneous with the infectious challenge. Results pertaining to the activity of neutrophils under G-CSF treatment in defense against infection were mostly uniformly positive: recruitment of neutrophils to the site of infection was improved in a pneumonia model<sup>28</sup> and infections seemed to remain more localized in infections with *Escherichia coli*, through cecal ligation and puncture (CLP) or with subcutaneous injections, than in control animals<sup>29,30</sup> as determined by histological evidence, myeloperoxidase activity, or glucose uptake in tissue adjacent to the site of infection. However, in a murine model where radionuclide-labeled *E. coli* were injected, no differences in the translocation through tissues were observed.<sup>31</sup> However, improved bactericidal activity was reported in each of these cases and in models of pneumonia and peritonitis.<sup>32,33</sup>

Regarding cytokine levels during infection, TNF $\alpha$  serum levels in G-CSF-treated animals were reported to be decreased compared to levels in controls in infection models in mice, rats and dogs.<sup>21,34-36</sup> G-CSF-treated galactosamine-sensitized mice exhibited reduced IL-2 serum levels without an effect on TNF $\alpha$  release.<sup>37</sup> Rabbits with immune complex colitis had lower levels of the proinflammatory leukotriene B<sub>4</sub> and thromboxane B<sub>2</sub>, but levels of the antiinflammatory prostaglandin E<sub>2</sub> were not affected.<sup>38</sup>

Apart from the general improvement in the course and outcome of infections, there is evidence that prophylactic treatment with G-CSF would be beneficial in the specific treatment of septic shock. G-CSF was shown to protect rodents against endotoxin-induced hepatotoxicity and shock<sup>21,39</sup> and peritonitis-induced multiple organ failure and death.<sup>6,40</sup> It also improved cardiovascular function, endotoxin clearance, and survival in two canine models of septic shock.<sup>6,41</sup>

Neutrophils have been implicated as key mediators in the pathogenesis of acute lung injury due to sepsis or endotoxemia. Accordingly, deliberate augmentation of neutrophil production and activity might be deleterious in patients with sepsis. Several studies have addressed this issue by examining the effects of G-CSF treatment on acute lung injury in guinea pigs,<sup>42</sup> pigs,<sup>29,43</sup> and sheep<sup>44</sup> challenged with LPS. The data from these preclinical studies have been consistent in showing no evidence of exacerbation of lung injury as a consequence of treatment with G-CSF.

## Use of G-CSF in Clinical Trials

The benefits of G-CSF treatment in nonneutropenic animal models of infection provided a basis for clinical studies on the effects of G-CSF in regard to the incidence and course of infections that might result in sepsis. G-CSF proved to be safe in intensive care unit (ICU) and septic patients,<sup>10,45,46</sup> who apparently also benefited from the therapy. Generation and function of neutrophils was improved in 20 postoperative/posttraumatic patients at risk of sepsis or with sepsis who were given continuous infusions of G-CSF (filgrastim) for 7 days. Furthermore, IL-8 decreased in all six patients whose initial IL-8 values were >90 pg/ml. IL-1ra increased in 10 patients, though there was no effect on the levels of TNF $\alpha$  or sTNF-R type I.<sup>47</sup>

When G-CSF treatment was commenced before an operation it significantly reduced the incidence of infectious complications in 19 cancer patients undergoing esophagectomy compared with 77 control patients.<sup>7</sup> G-CSF reduced the incidence of multiple organ failure in 756 pneumonia patients<sup>48</sup> and 37 liver allograft recipients.<sup>49</sup>

In conclusion, G-CSF showed antiinflammatory effects combined with improved host defense, not only in preclinical models but also in the clinical setting. Previous clinical experience has shown that the use of G-CSF is associated with a very low incidence of side effects, except mild bone pain. Therefore further trials of G-CSF given for prophylaxis of sepsis and septic complications are ratified, and widespread use of G-CSF in this setting might be considered a clinical option in the near future.

## GM-CSF

The initial phase of the systemic inflammatory response syndrome (SIRS) is characterized by excessive production of proinflammatory cytokines by monocytes/macrophages and is therefore termed the hyperinflammatory phase.<sup>50</sup> Here, antiinflammatory therapy (e.g., anti-TNF antibodies, IL-1ra, IL-10) was proposed as the appropriate measure.<sup>51</sup> However, it has been found that this initial phase is followed by a so-called hypoinflammatory phase, also termed immune paralysis.<sup>52,53</sup> Many patients who survive the acute hyperinflammation owing to intensive medical care succumb to subsequent infections. In such patients a drastic change in monocyte activity was observed (i.e., in vitro): The monocytes are no longer able to respond to an inflammatory stimulus such as LPS with secretion of proinflammatory cytokines (e.g., TNF $\alpha$ ).<sup>52,54-59</sup> The longer this state of immune paralysis continues, the more adverse is the prognosis.<sup>52,56</sup> Secondary infections during the condition of immune paralysis (i.e., when the organism's state of defense is insufficient) often determine the fate of a patient during septic multiple organ failure.

Therefore a therapeutic goal consists in the reconstitution of immune competence during the late phase of septic shock. For such an indication, immune stimulation with GM-CSF seems to represent a promising pharmacologic therapeutic

principle. GM-CSF is a pluripotent hematopoietic growth factor involved in regulating the proliferation, differentiation, and mature functions of granulocytes and monocytes/macrophages,<sup>60</sup> the two key cell types of the nonspecific immune system. GM-CSF has been used to accelerate recovery of the granulocyte and monocyte counts after chemotherapy or bone marrow transplantation, thereby reducing the risk of infections from bacterial or fungal sources due to leukopenia.<sup>61-64</sup>

## Role of Endogenous GM-CSF

The role of GM-CSF in vivo became evident in knockout animals. Mice with homozygous mutations of the GM-CSF gene showed no major deficits in hematopoiesis until 12 weeks of age, but they developed abnormal lungs and some suffered from subclinical bacterial or fungal infection.<sup>65</sup> These observations indicate that GM-CSF is not essential for maintenance of hematopoietic cells and their precursors but, rather, for normal pulmonary physiology and resistance to local infection. This conclusion was supported by the finding that the administration of neutralizing monoclonal antibodies specific for GM-CSF to *Cryptococcus neoformans*-infected normal mice increased mortality and induced rapid progression of the disease.<sup>66</sup>

To explore further the in vivo role of GM-CSF in infection, GM-CSF knockout mice were treated with endotoxin (LPS). Hypothermia and loss of body weight were markedly attenuated in LPS-treated GM-CSF-deficient mice compared with similarly treated control mice. Moreover, the levels of the circulating proinflammatory cytokines IFN $\gamma$ , IL-1 $\alpha$ , and IL-6 were lower in LPS-treated GM-CSF-deficient mice than in LPS-treated control mice. Peak levels of TNF $\alpha$  in response to LPS treatment were the same in the serum of all the mice, but TNF $\alpha$  persisted longer in GM-CSF-deficient mice. LPS-stimulated peritoneal macrophages from GM-CSF-deficient mice produced significantly less IL-1 $\alpha$  and nitric oxide than macrophages from wild-type mice, although there was no difference in TNF $\alpha$  production in vitro. These results indicate that GM-CSF contributes to cytokine production in LPS-mediated septic shock and that the attenuated production of these secondary cytokines (IFN $\gamma$ , IL-1 $\alpha$ , and IL-6) may contribute to the endotoxin-resistant phenotype of GM-CSF-deficient mice.<sup>67</sup>

## GM-CSF as an Immunostimulatory Drug

The initiation of host defense in the form of an inflammatory reaction is mediated primarily by the cytokines TNF $\alpha$  and IL-1.<sup>68-74</sup> GM-CSF was found to potentiate LPS-induced TNF $\alpha$  and IL-1 production of murine and human monocytic cells.<sup>58,75-77</sup> We also found that GM-CSF is a potent enhancer of LPS-induced TNF $\alpha$  production in vivo in normal and experimentally immunocompromised (LPS-desensitized) mice.<sup>78</sup> Furthermore, in vitro and ex vivo experiments revealed that LPS-induced IL-1 release from bone marrow or spleen cells was also enhanced in GM-CSF-treated mice.<sup>107</sup>

## GM-CSF Production: Strictly Controlled

GM-CSF is not detectable in the circulation of healthy animals or humans, though it may be found in the major organs at low concentrations.<sup>79</sup> Only small amounts of GM-CSF were measured in the serum of mice infected with *Listeria monocytogenes*.<sup>80</sup> Patients with experimental endotoxemia,<sup>81</sup> neutropenic fever,<sup>82</sup> or even sepsis<sup>83</sup> also do not normally have elevated serum GM-CSF levels. In patients with meningococemia GM-CSF concentrations higher than 1 ng/ml were only briefly present in subjects with life-threatening septic shock and were strongly associated with fulminant infection.<sup>6</sup>

As no systemic GM-CSF levels can be detected in patients with infection, endogenous GM-CSF is thought to play its physiologic role in the immediate vicinity of the cells by which it is secreted.<sup>84,85</sup> The hypothesis is supported by the observation that in patients with meningitis only cerebrospinal fluid contained a measurable concentration of GM-CSF.<sup>86</sup>

In summary, it appears that the body highly restricts production of the powerful immunostimulator GM-CSF. Therefore it is not surprising that rats who underwent CLP with sepsis-induced organ injury, when given rmGM-CSF, showed no increased survival rates but, rather, earlier deaths than the control group. Early leukosequestration to the peritoneal cavity was inhibited, and severe liver injuries were observed,<sup>87</sup> that might have resulted from the stimulating activity of GM-CSF on the expression of TNF. One study found that survival in two mouse models of gut-derived sepsis was improved by pretreatment with GM-CSF because of better gut barrier function and better bacterial clearance.<sup>88</sup> However, we found that prophylactic administration of rmGM-CSF neither augmented leukocyte numbers nor protected mice from lethal fecal peritonitis.<sup>14</sup> Consequently, systemic application of GM-CSF may be detrimental if given before or during the proinflammatory phase of sepsis.<sup>108</sup>

## GM-CSF in Models of Impaired Immune Competence

A number of studies have been performed in which neonatal rats (which are more vulnerable to infection than older animals) or animals first made susceptible to infection by trauma, burn, or myelosuppression were treated with exogenous GM-CSF and subsequently challenged by CLP or inoculation of infective agents. These studies may be considered models for the diminished status of the immune system experienced at the hypoinflammatory stage of sepsis.

Neonatal rats were found to have deficient PMN production and function during infection. Prophylactic rmGM-CSF given intraperitoneally 6 hours before a 90% lethal dose challenge with *Staphylococcus aureus* significantly improved survival in a neonatal rat model of infection.<sup>89</sup> In another study, neonatal rats with streptococcal sepsis were given rhGM-CSF after infection. A higher survival rate than in control animals not given rhGM-CSF was reported, apparently due to phagocyte

priming or cellular influx into the peritoneum (or both),<sup>90</sup> even though human GM-CSF is generally believed not to be bioactive in mice. Furthermore, GM-CSF administered in conjunction with penicillin to neonatal rats with established group B streptococcal infection decreased the mortality rate substantially in comparison to penicillin alone.<sup>91</sup>

Mice made susceptible to infection by trauma were treated with GM-CSF for 5 days before induction of peritonitis. These mice had a significantly higher survival rate than control mice, which underwent the same regimen but received placebo instead of GM-CSF. Peritoneal cell yields were increased in the GM-CSF group, and harvested macrophages stimulated with phorbol ester released larger amounts of both superoxide anion and TNF and less nitric oxide than mice in the control group.<sup>92</sup>

In a murine model 20% surface burns plus CLP were applied. Survival was significantly better on day 10 after injury in animals treated with GM-CSF on days 5–9 after the burn.<sup>93</sup> Concanavalin A-stimulated T cell proliferation and IL-2 production, which were suppressed after burn injury, were also improved by treatment with GM-CSF.<sup>93</sup>

The infection of GM-CSF-pretreated, myelosuppressed mice with normally lethal doses of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, or *Candida albicans* resulted in a significant dose-dependent improvement of survival.<sup>94,95</sup> There has also been research on combinations of GM-CSF with IL-6 or leukemia inhibitory factor (LIF), which are both potent inducers of the acute-phase response and can induce an increase in the platelet count.<sup>95</sup> The rationale for these combinations was that the synergism of the induction of opsonization of microorganisms by acute-phase proteins with activation of phagocytes by GM-CSF should increase resistance to infections. This hypothesis was proven correct when myelosuppressed mice were treated with either of the combinations and infected with *Pseudomonas aeruginosa*.<sup>96</sup>

Preclinical studies with recombinant human GM-CSF are limited by the lack of cross-species reactivity in mice.<sup>97</sup> The protein sequence homology between human and murine GM-CSF is only 60%.<sup>98</sup> Human GM-CSF does not affect canine PMNs in vitro;<sup>99</sup> and even in monkeys only short-term studies can be undertaken because antibodies develop to human GM-CSF.<sup>62</sup> However, because of the dangers associated with immune stimulation, volunteer studies may be considered unethical. We have taken advantage of a dose-finding study of GM-CSF for wound healing of basalioma, where we investigated the change in white blood cell count and the cytokine production pattern ex vivo in blood from patients treated with low doses of GM-CSF. Patients responded to the treatment with a general leukocytosis, though only the eosinophil fraction was significantly increased relative to the other populations. Cytokine secretion from GM-CSF-treated patients in response to either LPS or lipoteichoic acid was characterized by decreased IFN $\gamma$  and increased IL-10 and IL-1ra secretion and can therefore basically be considered an antiinflammatory reaction. Side effects were also relatively mild (unpublished observation; submitted for publication).

These results indicate that leukocytosis can be initiated by low doses of GM-CSF, with which the proinflammatory priming, observed *in vitro* and in animal models at higher doses, is replaced by a trend toward an antiinflammatory cytokine pattern.

### Potential Application of GM-CSF for Human Sepsis

Although monocytes from septic shock patients exhibit greater baseline respiratory burst activity than monocytes from healthy subjects, the response to secondary stimulation with bacterial stimuli is attenuated.<sup>100</sup> GM-CSF restored the ability of monocytes to respond appropriately to secondary stimulation. Expression of certain integrin adhesion molecules, CD62L, and Fc $\gamma$  receptors was increased on monocytes of septic shock patients; expression of CD11c was reduced. GM-CSF upregulated integrin expression and decreased CD62L, CD32, and CD16 expression. Priming monocytes with GM-CSF accelerated tissue factor activation following stimulation with LPS and bacterial culture supernatant.<sup>100</sup>

When a high dose (750  $\mu\text{g}/\text{m}^2$  day IV) of GM-CSF was administered to sarcoma patients with neutropenia for 2 weeks, no increase in basal release of TNF $\alpha$  or IL-1 $\beta$  by monocytes *ex vivo* was found, though the LPS-stimulated release of both factors reached 8-fold and 10-fold their respective values on day 0.<sup>101</sup> A single dose (2.5, 5.0, or 10.0  $\mu\text{g}/\text{kg}$ ) of GM-CSF resulted in a significant increase of *in vivo* plasma levels of IL-1ra and a trend toward increased IL-8 levels in cancer patients.<sup>102</sup> A case has been reported where a patient in the ICU with acquired agranulocytosis and sepsis experienced rapid neutrophil recovery and resolution of a clinical infection when treated with GM-CSF.<sup>103</sup>

### Possible Adverse Effects

In humans, systemic administration of GM-CSF at doses sufficient to produce plasma levels comparable to endogenous levels seen during severe meningococcal septic shock induced vasodilatation, hypotension, and hypoxia.<sup>6</sup> In a model where GM-CSF was highly expressed in rat lung after intrapulmonary transfer of the gene coding for murine GM-CSF using an adenoviral vector, a sustained but self-limiting accumulation of eosinophils and macrophages was associated with tissue injury in the lung followed by varying degrees of irreversible fibrotic reactions observed at later stages, suggesting that GM-CSF plays a role in the development of respiratory conditions characterized by eosinophilia, granuloma, or fibrosis.<sup>104</sup> Increases in plasma GM-CSF in patients with inflammatory disorders such as asthma<sup>86</sup> or granulocytosis due to infection<sup>105</sup> indicate that GM-CSF should be used with caution in patients with respiratory diseases. However, in a patient with T lymphocytosis with granulocytopenia and severe perianal infection, the eosinophilia initiated by GM-CSF (12.5  $\mu\text{g}/\text{kg}$  for 8 days) correlated with improvement of the perianal ulceration.<sup>106</sup>

## Conclusions

A number of studies have indicated that the immunostimulatory properties of GM-CSF may be beneficial in reconstituting the compromised immune system, thereby improving the outcome after secondary infections or sepsis.

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