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Potential of colony-stimulating factors to improve host defense in organ transplant recipients

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Purpose of review

Although immunosuppressive drugs prevent graft rejection, they also predispose patients to infection, representing a major complication in organ transplantation. It would thus be highly desirable to attain true immune tolerance without increasing the risk of infections and malignancies. Before the background of current strategies in the management of infections, the novel concept of differential reactivation of immunity, *ie*, boosting the innate immune response while continuing suppression of the adaptive immune response, is introduced.

Recent findings

Present clinical experience, feasibility, and potential risks of applying factors that potentially display such dichotomal properties (*eg*, granulocyte colony-stimulating factor [G-CSF] or granulocyte-macrophage colony-stimulating factor [GM-CSF]) are discussed.

Summary

In dexamethasone-treated PBMC isolated from control patients, or in cells obtained from immunosuppressed liver transplant patients, GM-CSF was found to selectively restore the innate immune response, without activating the specific immune response implicated in graft rejection. Moreover, GM-CSF efficiently restored the immune response against an otherwise lethal bacterial infection in immunosuppressed mice, without inducing the rejection of a skin transplant. These recent data could have implications for clinical practice and suggest a more detailed evaluation of agents with similar actions to GM-CSF in the restoration of innate immunity in organ transplant patients.

Keywords

GM-CSF, G-CSF, transplantation, immunity, infection

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Abbreviations

CSF	colony-stimulating factor
G-CSF	granulocyte colony-stimulating factor
GM-CSF	granulocyte-macrophage colony-stimulating factor
IFN	interferon
IL	interleukin
IL-1ra	interleukin-1 receptor antagonist
LPS	lipopolysaccharide
MAP	mitogen-activated protein kinases
PBMC	peripheral blood mononuclear cells
TNF	tumor necrosis factor

Introduction

Solid organ transplantation is a therapeutic option for many human end-stage diseases [1]. More than 600,000 solid-organ transplantations have been performed worldwide since the first renal transplantation in 1954, and the numbers are steadily increasing [2•]. The quality of life and survival rates after transplantation have improved because of advances in immunology, new drugs, and surgical techniques. The ultimate goal remains the development of donor-specific tolerance, which is hard to achieve because almost all transplant recipients continue to require immunosuppressive drugs throughout life [3–6]. In spite of challenges to tolerance induction and to steroid withdrawal, immunosuppressive therapy therefore remains the mainstay treatment in organ transplantation [7,8,9•,10–13]. This however impairs the host immune defense against infections and malignancies, which remain a major cause of morbidity and mortality [14]. Therefore, effective prevention or treatment of infection is still a primary goal in organ transplantation [15].

Current strategies to control infection after organ transplantation

Generally, either cyclosporine or tacrolimus is used together with steroids to prevent graft rejection, with the consequence of similar patterns of increased risk of infection after surgery. Opportunistic infections occur with the highest frequency after the first month to approximately 6 months after transplantation [14–17]. The risk of infection is determined primarily by the intensity of the exposure and the net state of immunosuppression, the duration of immunosuppression having more impact than the intensity [14,17]. The diagnosis of infections is difficult because the symptoms are often blunted by the immunosuppressive therapy [17].

Therefore, prevention is the optimal approach to handling infection in organ transplant recipients. Immunizations are efficient interventions, provided their use is based on a careful risk-benefit analysis, in which the effectiveness of the vaccine is weighed against possible

adverse reactions, including graft rejection [2•]. Because immunosuppressive drugs often cause leukopenia, which represents a risk factor for infection, the use of colony-stimulating factors (CSF) was considered early to increase leukocyte counts in these patients.

Antimicrobial strategies in organ transplantation have various drawbacks, including drug resistance and the risk of graft rejection (Table 1). The art of exogenous immunosuppression is to keep the balance between the risk of infection and the risk of rejection [17].

A new strategy for the treatment of transplant recipients is to boost the innate immune response while continuing the suppression of the adaptive immune response with the following preclinical background: in an alymphoid murine model, the robust innate immune response to the acute injury within the first day of transplantation was not shown to elicit allograft rejection [18–20]. Moreover, in studies using T cell–deficient mice, rejection occurred only after T cell reconstitution, even when skin or allografts were allowed to recover [21]. However, stimulation of the innate immune system leads to increased expression of, for example, cytokines, which may activate the adaptive immune system and thus promote the injury of the graft [18]. Importantly, the results from animal experiments and clinical studies indicate that restoration of innate immunity without restoration of, or in the absence of, adaptive immunity, might be beneficial for resistance to infection [22,23••,24]. In the context of pharmacologic intervention, this requires a preferential reactivation of the effectors of the innate immune response, such as macrophages, while the T cell response remains suppressed [25–27].

Potential of granulocyte colony-stimulating factor in organ transplantation

Granulocyte colony-stimulating factor (G-CSF) has been used routinely to reverse neutropenia, an important risk factor for infection in oncology patients. The treatment of leukopenia in transplant recipients was an obvious progression. However, transplant patients present a unique circumstance in that their immunosuppression is intentional and of critical importance to graft and patient survival.

Although the main and strongest effect of G-CSF treatment lies in the increase of neutrophil counts, the production of monocytes and lymphocytes is also increased, the latter after approximately 5 days of daily treatment [28,29]. The increase in lymphocyte counts stems from an increased production of naïve cells in the bone marrow, not from peripheral proliferation, and the expression of activation or proliferation markers remains stable [29]. This, combined with the fact that lymphocytes do not express G-CSF receptor, indicates that G-CSF does not activate lymphocytes [30]. Contrary to its activating effects on mature neutrophils, G-CSF treatment leads to a decrease in the release of proinflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1β, IL-12, and interferon (IFN)-γ in the blood of volunteers *ex vivo*, and it increases the production of the antiinflammatory soluble TNF receptors p55 and p75 as well as IL-1 receptor antagonist (IL-1ra) and prostaglandin E_2 [28,31,32].

Animal experiments have indicated that G-CSF may improve heterotropic graft survival. Rats given G-CSF in addition to tacrolimus for 14 days after heterotropic heart transplantation showed better graft survival above the single-treatment groups [33]. The suppression of IL-12p35 expression effected by G-CSF, combined with the suppression of IL-2 production by tacrolimus, was proposed as the mechanism [34]. The perioperative use of G-CSF in addition to tacrolimus also reduced the inflammation score in heterotropic rat heart transplants with simultaneous intravenous infusion of donor bone marrow [35]. In another approach, G-CSF treatment of rat donors before heart extraction prolonged graft survival in the recipient. This was improved even more by the additional transfusion of blood from the G-CSF–treated donors. This antirejection effect of the blood was attributed to G-CSF–mobilized CD11b/c+ monocytes [36,37]. The infusion of G-CSF–treated allogeneic blood seemed to be more effective than the transfusion of isogeneic blood

Table 1. Current antimicrobial strategies in organ transplantation patients

	Effect		
Current strategies	Antibacterial	Antiviral	Potential problems
Antibiotics	+	–	Antibacterial resistance, side effects
Antiviral drugs	–	+	Antiviral resistance, recurrent episodes of viral diseases, side effects
Antifungal drugs	Antifungal		Antifungal potency, side effects
Pathogen-specific immunity induction	–	+	Efficiency of culture, simulation and expansion conditions, antiviral potency
Immunosuppressant withdrawal	+	+	Chronic graft rejection, further infections, malignancies
Vaccination	+	+	Immunogenicity, safety, graft rejection
Differential immune reactivation	+	?	Graft rejection, antibacterial potency

Table includes pharmacologic interventions in major infections; not included are some facets of preemptive therapy (eg, pretransplant infection risk analysis) and parasitic infections.

because of the differential expression of IL-12 subunits, downregulating p35 and upregulating p40 [38]. The pretransplant injection of G-CSF and tacrolimus increased graft survival and downregulated the expression of both IL-12 subunits as well as of IFN-γ, whereas TNF, IL-1, IL-6, IL-18, IL-10, and transforming growth factor-β expression were not suppressed [39]. G-CSF may also exert an indirect influence on graft survival by inducing type 2 immune cells, which downregulate type 1 cells, producing rejection-associated cytokines such as IFN-γ, IL-2, TNF, and IL-12 [34]. Thus, G-CSF treatment seems to suppress the rejection reaction by downregulating IL-12 production.

Also, G-CSF was beneficial in infection prophylaxis in transplant recipients. In blood drawn from immunosuppressed liver allograft recipients between the 5th and 15th days postoperatively, *in vitro* priming with G-CSF significantly increased the neutrophil respiratory burst—a central function in host defense against bacterial or fungal infections [40].

Granulocyte CSF has been used to treat severe leukopenia stemming from immunosuppressants, cytomegalovirus infection, cytomegalovirus infection in combination with the treatment ganciclovir, viral infection, or sepsis in transplant recipients, where it raised the leukocyte count and allowed better adherence to chemotherapeutic regimens and therefore to better results. No adverse effects regarding graft rejection or graft-*versus*-host reactions were reported in kidney and liver transplant recipients and a heart transplant patient [41–47]. A significant reduction in the incidence of infections was observed in comparison with a control population [43].

Granulocyte CSF was also administered perioperatively to three pediatric liver transplant recipients with severe hypersplenism, with successful restoration of leukocyte counts above 5000/μL within 3 to 10 days [48]. Foster *et al.* [49] administered G-CSF to 37 primary liver allograft recipients for the first 7 to 10 days after transplantation and compared the outcomes with those of the previous 49 allograft recipients who did not receive G-CSF. The G-CSF–treated patients had a decreased number of sepsis episodes per patient, a lower percentage of sepsis-related deaths, and fewer acute rejection episodes (22% *vs* 51%) during a follow-up time of 4 to 28 months. However, in a randomized, placebo-controlled, double-blind multicenter trial in 172 patients receiving placebo or G-CSF preoperatively until up to 21 days after treatment, the incidence of infections, the number of treatments for rejection, the length of stay in the intensive care unit or hospital, and the percentage of deaths were not different between the placebo and the G-CSF groups, although increases in leukocyte counts were achieved, as expected [50•]. The reasons proposed to explain the differences in outcome were improvement of care of transplant patients since the previous trial regarding the introduction of tacrolimus and infection prophylaxis, the use of diverse immunosuppressive regimens, and the length of G-CSF treatment.

In essence, G-CSF is safe and reverses leukopenia in transplant patients. Preclinical data indicate that G-CSF treatment may enhance the antirejection effects of tacrolimus, which might be exploited to reduce the dose of tacrolimus and to prevent leukopenia in a combination therapy approach. However, the multicenter study could not find clear benefits of continual short-term application of G-CSF in the prevention of infections, though alternative dosage regimens or application at later stages after transplantation when the opportunistic infections occur with highest frequency (1–6 months after transplantation) may prove more successful.

Granulocyte-macrophage colony-stimulating factor for the improvement of antibacterial resistance

Granulocyte-macrocyte CSF (GM-CSF) was first identified on the basis of its ability to stimulate the clonal proliferation of myeloid precursors *in vitro*. Although the results from GM-CSF–deficient mice demonstrated that endogenous GM-CSF is more critical in pulmonary defense than in basal hematopoiesis, the exogenous application of this drug has revealed its more diverse biologic effects on the immune system, enhancing the functional activities of neutrophils, monocytes, macrophages, and dendritic cells [51–55].

Recombinant human GM-CSF is used to reverse leukopenia, as prophylaxis or adjunctive treatment of infection in immunosuppressed patients, or as a vaccine adjuvant [53]. GM-CSF was found to be safe and to reduce the incidence of infections, the use of aggressive antibiotic therapy, the duration of hospital stay and mortality in seven renal transplant patients, in comparison to historical control patients [56]. This was also true in children after orthotopic liver transplantation [57]. Because GM-CSF not only increases the numbers of granulocytes and monocytes but also primes both cell populations for increased function, thus it was uncertain whether GM-CSF might cause graft rejection through concomitant activation of lymphocytes.

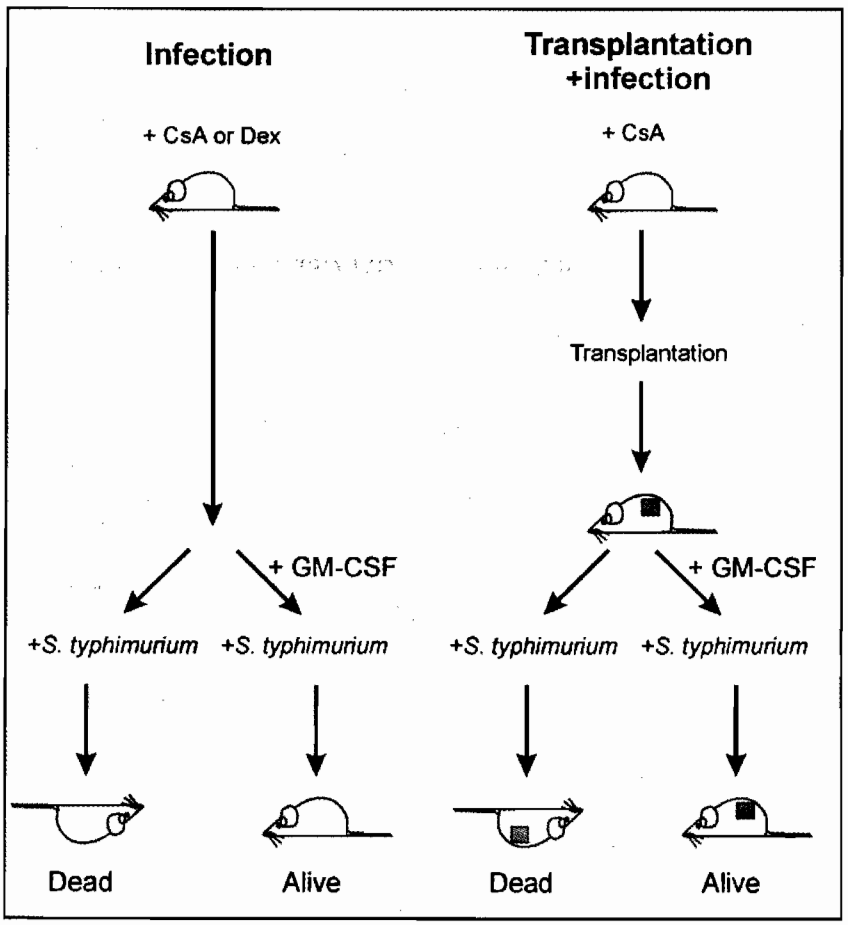
In contrast to G-CSF, GM-CSF has a proinflammatory profile. It potentiates lipopolysaccharide (LPS) toxicity and enhances LPS-induced TNF and IL-1 production, both in healthy mice and in LPS-tolerant mice [58–60]. GM-CSF treatment reversed the suppression of TNF production in macrophages from rats in hemorrhagic

shock [61]. Incubation with GM-CSF restored the capacity of whole blood from patients with multiple injury, cardiac surgery, and severe sepsis to respond to LPS stimulation with TNF release *in vitro*. Concomitantly, the expression of HLA-DR, a marker for immunosuppression, was increased on these patients' monocytes. Moreover, upon GM-CSF treatment, TNF release was also elevated in blood from healthy volunteers in the presence of the antiinflammatory mediators IL-10, transforming growth factor-β, and prostaglandin E_2 [62].

Granulocyte-macrophage CSF was shown to counteract dexamethasone-induced inhibition of superoxide anion release by monocytes and to increase their fungicidal activity against the opportunistic mold *Aspergillus fumigatus* [63]. GM-CSF given *in vivo* was able to restore dexamethasone-suppressed killing of *Aspergillus fumigatus* conidia by bronchoalveolar and peritoneal macrophages to normal values *ex vivo* but did not affect the dexamethasone-mediated reduction of spleen weight and spleen cellularity or the suppression of lymphocyte responses to concanavalin A [64–66]. However, this was only so when GM-CSF was given before dexamethasone treatment or after discontinuation of dexamethasone treatment [64–66]. The release of TNF, IL-1α, and MIP-1α in response to *Aspergillus* conidia was restored by GM-CSF [67].

We investigated whether the potential capacity of GM-CSF to restore the innate immune response also held true for immunosuppressive drug treatment. In fact, we found a recovery of the proinflammatory TNF response to LPS stimulation in glucocorticoid-immunosuppressed blood from healthy donors as well as in blood from immunosuppressed liver transplant patients. Notably, the T cell response in terms of IL-2 and IFN-γ production and proliferation was not reactivated [68••]. Regarding the *in vivo* relevance of these findings, we found that GM-CSF restored the survival of dexamethasone-suppressed or cyclosporine A–immunosuppressed mice from an otherwise lethal infection with *Salmonella typhimurium*. However, GM-CSF did not induce graft rejection of a skin allotransplant in cyclosporine A–immunosuppressed mice (Fig. 1) [68••]. This selective immune restoration potential of GM-CSF thus suggests a therapeutic value in improving resistance against infections upon organ transplantation. Data from *in vitro*, *ex vivo*, and *in vivo* experiments on the use of G-CSF or GM-CSF in transplantation are summarized in Table 2.

Figure 1. Schematic showing treatment of infection by granulocyte-macrophage colony-stimulating factor (GM-CSF)



This treatment allows immunosuppressed mice (CBA/Ca, 4 to 6 mice per group) to survive a lethal bacterial infection *(Salmonella typhimurium)* without inducing graft rejection of a previous skin allotransplant. CsA, cyclosporine A; Dex, dexamethasone.

Table 2. Experience with colony-stimulating factors in transplantation settings or models

Granulocyte colony-stimulating factor	
ex vivo	Improves defense functions in blood from immunosuppressed transplant patients
in vivo	Bolsters Th-1-specific immune suppression by tacrolimus in rats
	Safe and efficacious in reversing leukopenia in transplantation patients
	Positive effects on morbidity parameters and sepsis-related mortality in Phase II study
	No effect on morbidity or mortality in Phase III multicenter study
Granulocyte-macrophage colony-stimulating factor	
in vitro	Restores impaired immune response in refractory human monocytes
	Reactivates anergic monocytes from sepsis patients
	Overrides hyporesponsiveness of whole blood, induced by trauma, sepsis, or cardiac surgery
	Selectively reconstitutes gene expression related to innate immunity rather than that related to adaptive immunity (gene-array)
ex vivo	Reconstitutes tumor necrosis factor production without activating the adaptive immunity of the T cell response in dexamethasone-suppressed blood and blood from immunosuppressed liver transplant recipients
	Increases the respiratory burst of human neutrophils after liver transplantation
in vivo	Potentiates immune responses to lipopolysaccharide
	Restores impaired immune responses in lipopolysaccharide-desensitized mice
	Restores the survival of immunosuppressed mice from an otherwise lethal bacterial infection without effect on previously transplanted graft acceptance
	Safety shown in organ transplant patients with leukopenia

Mechanisms of differential immune reactivation by granulocyte-macrophage colony-stimulating factor

The GM-CSF receptor is found on progenitor cells and mature neutrophils, monocytes, and macrophages and is composed of an α subunit, unique to the GM-CSF receptor, and a β (βc) subunit, which is shared with the receptors for IL-3 and IL-5 [69]. Receptor binding of GM-CSF initiates at least two distinct signaling pathways, culminating in the induction of c-*myc*, the activation of Janus kinase-2, and DNA replication, or the activation of *ras* and mitogen-activated protein kinases (MAP), with subsequent induction of the genes c-*fos* and c-*jun*, both of which are involved in the regulation of hematopoietic differentiation [53,70,71].

By cDNA expression array of dexamethasone-suppressed PBMC, we found that various transcription factors, including NF-κB p65, a critical regulator of many cytokine genes, were upregulated by GM-CSF [68••]. NF-κB is one of the central targets of the suppressive action of corticosteroids, explaining their anti-inflammatory effect [72–74]. Therefore, it seems likely that the partial reconstitution of NF-κB expression by GM-CSF may contribute to the restored expression of some cytokine genes as well. Given the fact that glucocorticoids inhibit LPS-induced TNF translation, by inhibiting JNK/SAPK and MAP38, a reconstitution of the transcription of these factors by GM-CSF might also represent a possible strategy to restore TNF, IL-8, IL-6, and PAF-R production in macrophages, which are pivotal in mediating the inflammatory response [75–77]. Intriguingly, GM-CSF did not increase the gene or protein expression of IL-1, which is able to induce an IL-2–independent proliferation of T cells, but rather stimulated the production of IL-1ra, which neutralizes the bioactivity of the cytokine [68••]. Interestingly, a study measuring the serum cytokine profile in cancer patients treated subcutaneously with rhu-GM-CSF for 7 days reported increased levels of IL-1ra, together with TNF, IL-10, IL-12, neopterin, and macrophage colony-stimulating factor, whereas GM-CSF and G-CSF levels decreased after an initial peak. Moreover, in whole blood samples of these GM-CSF–treated patients, the LPS-stimulated release of TNF, IL-6, and IL-1ra increased initially, whereas IL-1β, IL-10. and IL-12 decreased [78]. In our study, specific lymphocyte responses, such as the expression of CD27 and T cell specific RANTES, were not upregulated by GM-CSF [68••]. Also, other factors critical for T cell activation such as linker for activation of T cell (LAT) and trans-acting T cell–specific transcription factor (GATA3) were not restored by GM-CSF [79–81].

First trials of GM-CSF for prophylaxis against infections have so far not been successful. In patients with HIV infection, GM-CSF treatment did not prevent the development of opportunistic infections, and perioperative treatment (8 days) of patients undergoing oncologic surgery did not reduce infection rates [82,83]. These studies emphasize that the treatment window for GM-CSF is critical and must be well defined, especially because the induction of graft rejection is one of the major speculated risks. This means that further preclinical studies, such as long-term follow-up after organ transplantation in rats or nonhuman primates, are mandatory. There are good reasons to evaluate GM-CSF as a candidate for the reactivation of innate immunity against infections while continuing the suppression of the adaptive immune response to prevent graft rejection.

Conclusion

An alternative strategy to trying to attain true immune tolerance without increasing the risk of infections and malignancies in organ transplant patients consists of the specific restoration of the innate immune while keeping the adaptive immune response, which is implicated in graft rejection, suppressed. Recent findings provide good reasons to evaluate GM-CSF as a candidate molecule for such treatment. However, several studies investigating the use of GM-CSF for prophylaxis against infections have indicated that the treatment window for GM-CSF is critical and must be well defined, especially because the induction of graft rejection is one of the major spec-

ulated risks. This means that further pre-clinical studies, such as a long-term follow-up after organ transplantation in rats or non-human primates, are mandatory.

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