Stem cell mobilization with G-CSF analogs: a rational approach to separate GVHD and GVL?

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The separation of graft-versus-host disease (GVHD) and graft-versus-leukemia (GVL) remains the “holy grail” of allogeneic stem cell transplantation, and improvements are urgently needed to allow more effective therapy of malignant disease. The use of G-CSF–mobilized peripheral blood as a clinical stem cell source is associated with enhanced GVL effects without amplification of significant acute GVHD. Preclinical studies have demonstrated that G-CSF modulates donor T cell function before transplantation, promoting T<sub>H</sub>2 differentiation and regulatory T cell function. In addition, the expansion of immature antigen-presenting cells (APCs) and plasmacytoid dendritic cells (DCs) favors the maintenance of this pattern of T cell differentiation after transplantation. Although these patterns of T cell differentiation attenuate acute GVHD, they do not have an impact on the cytolytic pathways of the CD8<sup>+</sup> T cells that are critical for effective GVL. Recently, it has been demonstrated that modification of G-CSF, either by pegylation of the native cytokine or conjugation to Flt-3L, results in the expansion and activation of donor iNKT cells, which significantly augment CD8<sup>+</sup> T cell–mediated cytotoxicity and GVL effects after transplantation. Given that these cytokines also enhance the expansion of regulatory T cells and APCs, they further separate GVHD and GVL, offering potential clinical advantages for the transplant recipient. (Blood. 2006; 107:3430-3435)

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Pathophysiology of acute GVHD and GVL effects

During GVHD, the presentation of histocompatibility antigens by residual host antigen-presenting cells (APCs) stimulates naive donor CD8+ and CD4+ T cells, whereas the presentation of host antigens by repopulating donor APCs to donor CD4+ T cells amplifies alloreactivity. Critical to this process is the effective trafficking of donor T cells to the appropriate lymphoid environment, where they encounter professional APCs. Subsequently, donor T cells undergo type 1–biased differentiation and produce a range of proinflammatory cytokines, leading to a cascade of pathologic events. Monocytes/macrophages, primed by Treg cells and stimulated by LPS, release cytopathic quantities of inflammatory cytokines characteristic of the “cytokine storm.” Target tissue apoptosis is thus mediated in a major histocompatibility complex (MHC)–independent and –dependent fashion through inflammatory cytokines (TNF-α, IL-1, and nitric oxide) and CD8+ T cell cytotoxicity (Figure 1).12

Initiation of GVL reactions is dependent on a complex series of bidirectional interactions between DCs and cells of the innate and adaptive immune systems, including natural killer (NK) cells, natural killer T (NKT) cells, and CD4+ and CD8+ T cells. The result is enhancement of innate antitumor effects and licensing of DCs to allow the presentation of antigen to effector cells of the adaptive immune system. After MHC-matched allogeneic SCT, GVL effects are directed against a variety of targets, depending on the characteristics of the underlying malignancy. Targets may include ubiquitously expressed minor histocompatibility antigen (mHA), unique leukemia-associated proteins (such as BCR-ABL) or nonpolymorphic self-proteins (such as proteinase 3 or Wilms tumor 1) overexpressed by leukemic cells and presented directly by host APCs within MHC class I to donor CD8+ T cells. Alternatively, these antigens may be presented by host or donor APCs within MHC class II to donor CD4+ T cells. In addition, the loss or mismatch of inhibitory KIR ligands (particularly MHC class I) may be recognized by donor NK cells and trigger MHC-independent cytotoxicity. Thus, depending on the nature of the leukemia, donor CD8+ T cells, CD4+ T cells, or NK cells may be activated and may contribute to the effector phase of GVL, using perforin, TNF-related apoptosis inducing ligand (TRAIL), and TNF-α cytolytic pathways (Figure 2).

Separation of GVHD and GVL after G-CSF mobilization and allogeneic SCT

The mobilization of stem cells with G-CSF may have a number of immunomodulatory effects. First, the disruption of adhesion molecules by proteases released from myeloid precursors may influence the ability of T cells to traffic to lymphoid tissue and induce GVHD. Consistent with this, CD62L expression on donor T cells is profoundly reduced after G-CSF administration. Second, the distortion of the lymphoid environment by expanded myeloid precursors will alter the anatomic ability of T cells to interact with professional APCs and increase the frequency with which T cells encounter cytokine-expanded immature APCs. Third, hemopoietic tissue expanded by G-CSF and stromal tissue stimulated by G-CSF may release soluble immunomodulatory proteins, such as IL-10, TGF-β, and IFN-α, which may subsequently modulate donor T cell function.

Effects on donor T cells

Stem cell mobilization with G-CSF polarizes conventional αβ T cells toward a Th2 pattern of cytokine production, but this polarization is not associated with a reduction in CTL activity or GVL. Similarly, other interventions to induce Th2/Th1 bias, including mobilization with IL-18 or posttransplantation administration of IL-11, also prevent GVHD while maintaining GVL effects. Tayebi et al reported a reduction in the production of type 1 cytokines after mobilization of healthy donors with G-CSF. Consistent with this, Franzeke et al reported that the expression of GATA-3, a key Th2 transcription factor, was increased in CD4+ T cells after the administration of G-CSF.

Regulatory T cells (Treg) are important in the attenuation of GVHD, and clinical studies have shown that CD4+ T cells from G-CSF–mobilized donors secrete more IL-10 but less IL-4, IL-2, and IFN-γ than the same populations before G-CSF administration. Purified CD4+ T cells were able to suppress allopurinol responses of autologous T cells in an antigen-nonspecific manner, consistent with a regulatory Tr1-like phenotype. These effects have now been confirmed in vivo, where protection from GVHD after G-CSF mobilization with G-CSF is dependent on donor T cells and both IL-10 and TGF-β. Edinger et al demonstrated that despite reducing GVHD, Treg do not impair the ability of CD8+ CTLs to lyse tumor cells in vitro and that, at a 1:1 ratio, in vivo GVL effects are retained when sufficient numbers of effector T cells are transferred. When low numbers of effectors are transferred, however, GVL effects are impaired, giving rise to otherwise apparently contradictory data regarding the effects of Treg on GVL.

Because purified T cells from G-CSF–treated donors have altered cytokine and transcription profiles before transplantation, the alteration of donor T cell function occurs in the absence of alloreactivity and is likely to reflect modulation by cytokines, expanded myeloid cells, or both. G-CSF receptor (G-CSFR) mRNA is ubiquitously expressed in hemopoietic and nonhemopoietic tissue. Surprisingly, G-CSFR mRNA was detected in a small subset of purified CD4+ and CD8+ T cells from healthy donors before the administration of G-CSF. Culture of purified CD4+ T cells in the presence of G-CSF before stimulation with mitogen resulted in increased expression of IL-4 and reduced IFN-γ mRNA. Subsequent clinical studies have detected G-CSFR in T cells only after G-CSF administration. Although the contribution

Chronic GVHD

The incidence of extensive chronic GVHD is increased after G-CSF–mobilized SCT. The pathophysiology of chronic GVHD is poorly understood, but it is becoming increasingly clear that the process is fundamentally distinct from that of acute GVHD. Although donor T cells are critical in the initiation of chronic GVHD, it is now established that the effector pathways involve cells of the myeloid lineage and fibrogenic cytokines such as TGF-β, all of which are amplified by G-CSF. Leukemia relapse rates are markedly reduced in patients in whom chronic GVHD develops, reflecting ongoing effective CTL responses and, perhaps most important, the chronicity of the process, which is in contrast to that in patients in whom severe acute GVHD develops.

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of contaminating non–T cells cannot be excluded in these studies, it is possible that G-CSF may also modulate donor T cell function directly. The definitive mechanism of T cell modulation by G-CSF thus remains to be defined.

Effects on antigen-presenting cells

The presentation of alloantigen by host APCs is central to the initiation of GVHD and GVL.32 It is becoming clear, however, that APCs are also involved in the induction and maintenance of tolerance, particularly when the predominant APCs are immature or phenotypically distinct (eg, plasmacytoid DCs).33 APCs, therefore, represent an attractive explanation for the immunomodulatory effects of G-CSF on T cells.

G-CSF–mobilized peripheral blood stem cell grafts contain a 50-fold increase in CD14+ monocytes, which suppress alloantigen-induced T cell proliferation and CD28-signaling of CD4+ T cells in Figure 1. Stem cell mobilization with G-CSF attenuates acute GVHD through effects on T cells and APCs. After SCT, tissue injury and local inflammation (including IL-1, IL-6, and TNF-α release) are initiated by the conditioning regimen and promote the activation of host APCs. The interaction between activated host APCs (displaying disparate histocompatibility antigens) and naive donor CD4+ and CD8+ T cells preferentially drives type 1 differentiation, generating large amounts of IFN-γ that primes mononuclear phagocytes of donor and host origin. Donor CD4+ T cell responses are subsequently perpetuated by donor APCs presenting host antigens. After activation by LPS and other gut-derived immunostimulants, monocytes primed by Th1 cytokines secrete cytopathic quantities of proinflammatory cytokines (TNF-α, IL-1) and mediate tissue injury in the inflammatory effector pathway of GVHD. Concurrently, effector donor CD8+ T cells are expanded, gain cytolytic function, and mediate target tissue GVHD through their cellular cytolytic machinery (eg, perforin, granzyme, TRAIL) in the cytolytic effector pathway. This leads to the "cytokine storm" characteristic of acute GVHD, whereby target tissues are damaged in MHC-independent and –dependent fashion. After G-CSF mobilization of stem cell donors, however, 3 key immunomodulatory effects before transplantation lead to the attenuation of GVHD. First, donor T cells up-regulate GATA-3 expression and are biased toward TH2 differentiation, limiting TH1-dependent monocyte activation after SCT. Second, G-CSF induces the generation of Th1 regulatory cells (distinct from classical CD4+CD25+ Treg) through IL-10 production. Third, G-CSF expands regulatory APCs within the donor (immature myeloid precursors and plasmacytoid DCs) which, after transplantation, promote the generation of classical CD4+CD25+ IL-10–producing Treg. The generation of IL-10 and TGF-β from Th1 and Treg serve to further inhibit the inflammatory effector phase of GVHD, limiting target tissue damage.

Figure 2. Stem cell mobilization with potent G-CSF analogs activates iNKT cells with subsequent promotion of donor CTL function and GVL effects. After stem cell mobilization with potent G-CSF analogs, donor iNKT cells are expanded and functionally activated. These iNKT cells interact with residual host APCs and may be activated directly through CD1d-presented glycolipid or indirectly through cytokines (including IL-12 and IL-18). After activation, iNKT cells secrete large amounts of cytokine, including IFN-γ, which further primes host APCs and activates cellular effectors of the innate (NK cell) and adaptive (CD4+ and CD8+ T cell) immune systems. NK cells are activated by host APCs through activating receptor interactions (including NKG2D-NKG2Dl and CD70-CD27) and cytokines (including IFN-α/β, IL-12, IL-15, and IL-18). NK cells reciprocally enhance APC activation through the secretion of IFN-γ and TNF-α and directly mediate MHC-independent GVL through interactions with activating ligands, KIR mismatch, or the recognition of leukemic targets lacking MHC class 1. Donor CD4+ T cells, activated by host hemopoietic or leukemia-specific antigens presented by host (or donor) APCs, mediate GVL effects against MHC class 2+ leukemic targets expressing the relevant antigens. Donor CD8+ T cells activated by a similar range of antigens, presented by host APCs only, mediate GVL against leukemic targets expressing the relevant antigens within MHC class 1.
Effects of stem cell mobilization with novel G-CSF analogs

Stem cell mobilization with G-CSF modulates GVHD by promoting T_{H}2 and regulatory T cell function in the context of expanded regulatory or inhibitory APCs. This prompted us to question whether all G-CSF molecules behave similarly and whether differing moieties may be exploited to further separate GVHD and GVL. We have demonstrated that protection from GVHD is dependent on the G-CSF dose and can be maximized by pegylation of the native cytokine. Mobilization with pegylated G-CSF (Peg-G-CSF) results in enhanced expansion of tolerogenic APCs and augmentation of T_{reg} activity that in turn promotes tolerance. A second family of molecules, the progenipoietins (including progenipoietin-1 [ProGP-1]) are engineered chimeric G-CSF and Flt-3L proteins that have significantly greater ability to mobilize stem cells and APCs than either native molecule alone. Grafts mobilized by these cytokines have marked tolerogenic properties that reside in the T cell and APC compartments. Surprisingly, after stem cell mobilization with pegylated G-CSF or ProGP-1, GVL and GVHD are effectively separated, and maximal GVL effects are dependent on the presence of invariant NKT (iNKT) cells (Figure 2).

iNKT cells are increasingly recognized as having important roles in immunoregulation and tumor surveillance. iNKT cell recognition of glycolipid antigens, such as α-galactosylceramide (α-GalCer), characteristically leads to rapid production of immunomodulatory cytokines, particularly IFN-γ and IL-4. iNKT cell activation using α-GalCer has been shown to influence disease progression in a variety of experimental models of autoimmunity and inflammation (for a review, see Van Kaer). Although most studies suggest that α-GalCer prevents autoimmunity by promoting T_{H}2 responses, it is becoming clear that the induction of tolerogenic DCs or regulatory T cells may also have a crucial role. Administration of α-GalCer to recipients in a mouse model of allogeneic SCT after sublethal irradiation significantly reduced GVHD, subsequently shown to be associated with T_{H}2 polarization of donor T cells. Haraguchi et al. using a nonlethal irradiation BMT model, confirmed that residual host iNKT cells provided protection from GVHD. Protection could be conferred by adoptive transfer of additional host-type iNKT cells, whereas the presence of iNKT cells in the donor graft was associated with increased GVHD severity, demonstrating differential immunomodulatory effects of donor and host iNKT cells in allogeneic SCT. Stem cell mobilization with ProGP-1 expands and activates donor iNKT cells, resulting in enhanced responses to α-GalCer. After transplantation, donor iNKT cells promote the licensing of residual host DCs and enhance perforin-restricted CD8+ T cell cytotoxicity against host-type antigens. Enhanced cytotoxicity and GVL effects are not associated with Flt-3L signaling or effects on DCs but can be reproduced by prolonged G-CSF receptor engagement with pegylated G-CSF. The enhanced regulatory properties inherent in grafts mobilized using potent G-CSF analogs compensate for the increased CTL function that otherwise might be expected to result in increased GVHD. Studies to date predict that the separation of GVHD and GVL effects after stem cell mobilization with potent G-CSF analogs will be most marked when GVHD is CD4 dependent and GVL effects are mediated by CD8+ CTL and NK cells.

iNKT cells are expanded in G-CSF transgenic mice, and G-CSF receptor mRNA has been demonstrated in NKT-like cells. In clinical studies, however, mobilization with standard G-CSF is not associated with the modulation of donor iNKT cell function, and iNKT cells are not expanded in G-CSF–mobilized stem cell grafts from healthy donors. Consistent with this, stem cell mobilization with standard G-CSF does not alter GVL in murine models, suggesting that molecular alterations in the new G-CSF moieties result in the augmentation of signaling that influences iNKT cell expansion and function. ProGP-1 binds to the G-CSF and Flt-3L receptors with an affinity similar to that for native molecules. ProGP-1 is able to bind to G-CSF and Flt-3L receptors simultaneously and thus to colocalize and amplify signaling at the cell membrane. Although combined mobilization of donors with G-CSF and Flt-3L receptorsfailed to reproduce the enhanced GVL effects of ProGP-1, enhanced GVL effects were observed after mobilization with pegylated G-CSF. We hypothesize, therefore, that the predominant effect is likely to be through the G-CSF receptor. Interestingly, the pegylation of G-CSF not only enhances serum half-life (and
thus receptor occupancy), it also alters the cellular trafficking of G-CSF to enhance G-CSF receptor stimulation at a cellular level.50

Administration of G-CSF after BMT

G-CSF is often also administered to recipients after transplantation to hasten engraftment, though potential immunologic effects have recently become the source of considerable controversy. Analysis of data reported to the European Group for Blood and Marrow Transplantation (EBMT) has demonstrated that the administration of G-CSF after BMT increases the incidence of acute and chronic GVHD, with consequent reductions in overall survival.51 Although the underlying mechanism remains unclear, it is intriguing that detrimental effects were not seen in recipients of G-CSF–mobilized PBSC grafts. It is attractive to postulate that the administration of G-CSF after BMT may activate donor iNKT cells and augment alloresponses in an environment that is not balanced by the inhibitory effects of G-CSF on donor T cells and APCs during stem cell mobilization. We are studying this hypothesis in preclinical models; the literature cautions against the routine use of G-CSF after allogeneic BMT until further data are available.

Future directions and alternative strategies

The mobilization of stem cells with pegylated G-CSF in healthy donors will have to be closely monitored in well-designed clinical trials that focus on GVHD severity and relapse in the context of dose and differentiation. Biol Blood Marrow Transplant. 2004;10:373-385.


T lymphocytes do not necessarily mediate alloresponses, although they can be targets of alloreactive effector T cells. Thus, bystander alloresponses may also contribute to GVHD development.68 In preclinical models, the development of GVHD is dependent on either naive or memory T cells.36 This suggests that the induction of effective GVL may be dependent on different T-cell subsets.

References


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Although T-cell depletion in clinical allogeneic SCT is a concept that has largely come and gone, the ability of naive, but not memory, T cells to induce GVHD has ignited the idea of depleting T-cell subsets. In this regard, the relative ability of effector and central memory T cells to mediate GVL will have to be addressed in preclinical models before clinical trials that deplete naive T cells from the stem cell graft. Understanding the mechanism by which memory T cells fail to induce GVHD will provide important insights and may result in therapeutic approaches that block T cell adhesion molecules and subsequent trafficking of donor T cells to primary lymphoid organs.

Although the depletion of APCs to prevent GVHD initially appeared an attractive concept, it now seems clear that this approach will prevent the induction of effective GVL.32 However, the relative role of APC subsets in the induction of GVHD remains unknown. Although this is difficult to dissect with currently available reagents, it will be the next critical step in moving the field forward. Finally, it now seems important to focus on the modification of DC function in vivo by preventing activation and maturation because this approach may promote the induction of regulatory T cells and, in turn, may retain GVL effects early after transplantation.


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