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Review

Granulocyte-Macrophage Colony-Stimulating Factor in Allogenic Hematopoietic Stem Cell Transplantation: From Graft-versus-Host Disease to the Graft-versus-Tumor Effect

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Allogenic hematopoietic stem cell transplantation (allo-HSCT) is a widely used treatment for a broad range of hematologic malignancies because of its graft-versus-tumor (GVT) effect. Unfortunately, allo-HSCT is still associated with morbidity and mortality related to relapse and transplantation complications, namely graft-versus-host-disease (GVHD). In an era of therapies specifically targeting molecular pathways, transcription factors, and cytokines, a better understanding of GVHD physiopathology is essential for the development of new therapeutic approaches. In this review, we outline the current knowledge of the role of granulocyte- macrophage colony-stimulating factor (GM-CSF) in allo-HSCT. We first discuss the biology of GM-CSF and its signaling pathways, with a focus on the main producing cells, T cells. We discuss recent preclinical studies pointing to a pivotal role of GM-CSF in GVHD, in particular gastrointestinal GVHD. We then summarize the potential role of GM-CSF in the GVT effect, discussing some potential strategies for exploiting GM-CSF in the context of allo-HSCT.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a well-established therapy for a wide range of hematologic malignancies due to its graft-versus-tumor (GVT) effect. Graft-versus-host-disease (GVHD) is a common

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complication after allo-HSCT and remains an important cause of morbidity and mortality not related to relapse in transplant recipients. During GVHD, donor-derived alloreactive T lymphocytes are activated, proliferate, and migrate to target organs, including the skin, liver, and gut [1]. The cytokines produced in this process by T cells and antigen-presenting cells (APCs) are central players in GVHD pathogenesis [2,3] and might represent therapeutic targets for GVHD prophylaxis and treatment [4].

The roles of some of these cytokines, such as TNF-α and IFN-γ, have been studied extensively [3], and recent evidence points to the potential contributions of other potentially targetable cytokines in GVHD pathogenesis, including granulocyte-macrophage colony stimulating factor (GM-CSF) [5–7]. In this review, we summarize the available knowledge about the role of GM-CSF in the allo-HSCT context. In particular, we discuss the experimental and observational evidence supporting a role of GM-CSF in GVHD and GVT.

GM-CSF Biology

The cytokine GM-CSF is a member of the colony-stimulating factor (CSF) superfamily, so named after the ability of the members to stimulate the formation of hematopoietic cell colonies. GM-CSF is a small, 22-kDa glycoprotein of varying molecular weight depending on its glycosylation state [8]. It is noted to be involved in "emergency" conditions, as levels are nearly undetectable in the circulation at steady state but can quickly increase during inflammation or infection [9,10]. GM-CSF was isolated for the first time in mouse lung-conditioned medium in 1977 and identified as a molecule able to generate granulocyte and macrophage colonies in vitro [11]. Today, the known role of GM-CSF is much more pleiotropic than described previously. GM-CSF is involved in diverse processes, including growth and development of granulocytes, macrophages, and dendritic cells (DCs); stimulation and initiation of myeloblast or monoblast differentiation; and chemotaxis of eosinophils. GM-CSF also has been noted to induce the upregulation of the major histocompatibility complex (MHC) class II molecules on APCs, thereby influencing the interaction between these cells and CD4 T cells [12].

GM-CSF appears to have a nonessential role at the steady state. Mice lacking GM-CSF display limited perturbations of the myeloid system and mostly show an absence of alveolar macrophages, leading to impaired ability to clear pulmonary surfactant [13]. However, the deregulation of GM-CSF production leading to its overexpression is associated with radical changes in the immune system. Mouse models of GM-CSF overexpression show important abnormalities characterized by enhanced macrophage and DC recruitment/differentiation, leading to a pathologic phenotype including blindness [14] and desquamative interstitial pneumonitis secondary to massive macrophage accumulation [15]. Mice with GM-CSF overexpression in T cells showed an important extramedullary hematopoiesis associated with histiocyte infiltration [16]. Another mouse model with a specific and inducible overexpression of GM-CSF in CD4 T cells showed an important expansion of myeloid cells that could infiltrate different organs, mainly the central nervous system, causing neurologic deficits [17].

GM-CSF is produced by different cell types, including epithelial cells, endothelial cells, fibroblasts, B cells, and natural killer (NK) cells [18,19]. In addition, activated T lymphocytes are one of the major producers of GM-CSF. To study GM-CSF-producing cells in vivo during neuroinflammation, Komuczki et al. [20] generated a very elegant GM-CSF fate mapping mouse strain. Prior to the induction of inflammation, GM-CSF expression was almost undetectable, whereas after several days of inflammation, GM-CSF was produced by multiple lymphocytes in lymph nodes ($CD4^+$ and $CD8^+$ T cells, $\gamma\delta$ cells, and NK cells), with $CD4^+$ T cells as the major producer in the central nervous system [20]. In the periphery, depending on the cytokine environment, naïve $CD4^+$ T cells differentiate into different helper T (Th) cell subpopulations, such as Th1, Th2, and Th17. Interestingly, GM-CSF production is not considered specific to one Th subtype but clearly depends on the activation of $CD4^+$ T cells. Indeed, a new independent subset of cells induced by the IL-7-STAT5 pathway, termed Th-GM-CSF cells, has been identified as a major producer of GM-CSF [21]. A master transcriptional factor driving the development of Th-GM-CSF has not yet been identified. Interestingly, on IL-12 stimulation, these cells exhibit plasticity in vitro as they switch toward a Th1 phenotype and express T-bet, the Th1 master transcriptional factor, which does not seem to be required for the polarization of Th-GM-CSF cells [21,22].

Positive and negative regulators of GM-CSF expression have been described. The NFAT transcription factor is critical to initiate chromatin remodeling on *Csf2* enhancer and promoter [23]. Bhlhe40 (Basic Helix-Loop-Helix Family Member E40), a member of the basic helix-loop-helix

transcription factor family, is important for inducing the expression of GM-CSF [5,24]. Using Chip-seq, Lin et al. [24] showed that Bhlhe40 binds to a regulatory region of *Csf2*. In contrast, we and others have noted that the transcriptional factor Ikaros represses GM-CSF expression in T cells [25,26], potentially through its binding to the CNS downstream of *Csf2*, an important enhancer of *Csf2* transcription [26]. STAT5 signaling was identified as crucial for the generation of Th-GM-CSF cells [21,22,26,27]; however, the role of RORγt in GM-CSF production remains unclear [28,29]. Some authors have posited that *RoRc*-deficient mice cannot express GM-CSF [29], whereas others have suggested that RORγt can bind *Csf2* promoter and induce GM-CSF expression [28].

In humans, concomitant with TCR and CD28 activation, the addition of cytokines IL-6, IL-7, or IL-2 to the cell culture medium was sufficient to bias the T cell differentiation toward a Th-GM-CSF subtype with a distinct transcriptome signature [22]. In healthy donors, Th-GM-CSF cells represent approximately 10% of memory $CD4^+$ T cells and coexpress TNF-α, IL-2, CD127, and FASL [22,30].

GM-CSF acts on cells that express the GM-CSFR, constituted by a 60- to 80-kDa ligand-specific α-chain subunit (Csfra) and a 120- to 140- kDa β-chain subunit (Csfrb) that is common with IL-3 and IL-5 receptors [31]. Each α-chain subunit of the GM-CSFR binds GM-CSF with low affinity, and the β-chain subunit binds cytokines very poorly by itself. However, the presence of this β-chain subunit transforms the cytokine binding of the α-chain from low affinity to high affinity, causing heterodimerization of both chain subunits and thus forming a hexameric complex (2 α-chains, 2 β-chains, and 2 GM-CSF molecules) [32]. Then 2 hexamer complexes aggregate and form a dodecamer complex that can start the signaling cascade. GM-CSFR is expressed by DCs and their precursors, granulocytes, monocytes, macrophages, human intestinal epithelial cells, and microglia [10,33,34]; however, the status of GM-CSFR expression on T lymphocytes remains unclear. Only 2 studies report that the α-chain of GM-CSFR is expressed in both resting and activated regulatory T cells (Tregs) [35] and in invariant NK T (iNKT) cells [36]; however, several other studies did not detect any GM-CSFR expression on T cells [10,29,37,38]. Of note, a form of the soluble GM-CSFRα subunit produced by an alternative splicing that can bind GM-CSF and block its activity has been reported [39,40].

In the literature, GM-CSF historically has been reported to have a pathogenic role in murine models of autoimmune diseases and neuroinflammation [41]. Patients with multiple sclerosis (MS) have increased numbers of Th-GM-CSF cells in peripheral blood and cerebrospinal fluid [22,30]. An increased GM-CSF-producing lymphocyte pool also has been described in the peripheral blood of patients with active rheumatoid arthritis [42]. The expression and role of GM-CSF in GVHD have been brought to light only recently [5,43,44].

GM-CSF in GVHD

Over the years, GM-CSF has been associated with GVHD in 2 different and opposing ways (Figure 1). On the one hand, and probably the most extensively studied, GM-CSF is described to have a deleterious role by exacerbating GVHD; on the other hand, the cytokine seems to be able to exert a protective role by enhancing Treg proliferation.

Inflammatory side of GM-CSF in GVHD

The role of GM-CSF as an inflammatory cytokine has been widely described in the literature [45]. The inflammatory role of GM-CSF seems crucial to the development of gastrointestinal (GI)-GVHD.

In murine models of allo-HSCT, in two different studies using an anti-GM-CSF-blocking antibody in the recipient or using a $CD4^+$ T-cell-specific $Csf2^{-/-}$ graft, GI-GVHD severity and tissue damage were clearly reduced [5,43]. Regarding the source of GM-CSF, Tugues et al. [43] demonstrated the importance of GM-CSF production by $CD4^+$ T cells in GVHD induction. They showed that after transfer of wild type (WT) or $Csf2^{-/-}$ $CD4^+$ and $CD8^+$ T cells in different combinations, only the combinations in which *Csf2* was absent in $CD4^+$ T cells conferred protection from GVHD. Finally, using donor or host cells lacking the beta subunit of the GM-CSF receptor, they demonstrated that GM-CSFR deficiency in donor cells affects the severity of GVHD. These findings support the role of GM-CSF as a bridge cytokine able to connect the adaptive and innate immune systems.

In a model reported by Piper et al. [5], during GVHD, T cell-producing GM-CSF induces an indirect alloantigen presentation by enhancing the expression of costimulatory molecules in donor-derived conventional DCs. This model was endorsed by Gartlan et al. [6] using a different transgenic mouse model, who showed that the absence of GM-CSF production by donor T cells resulted in attenuation of antigen presentation and reduction in histopathologic signs of GI-GVHD. These studies in mice highlight the

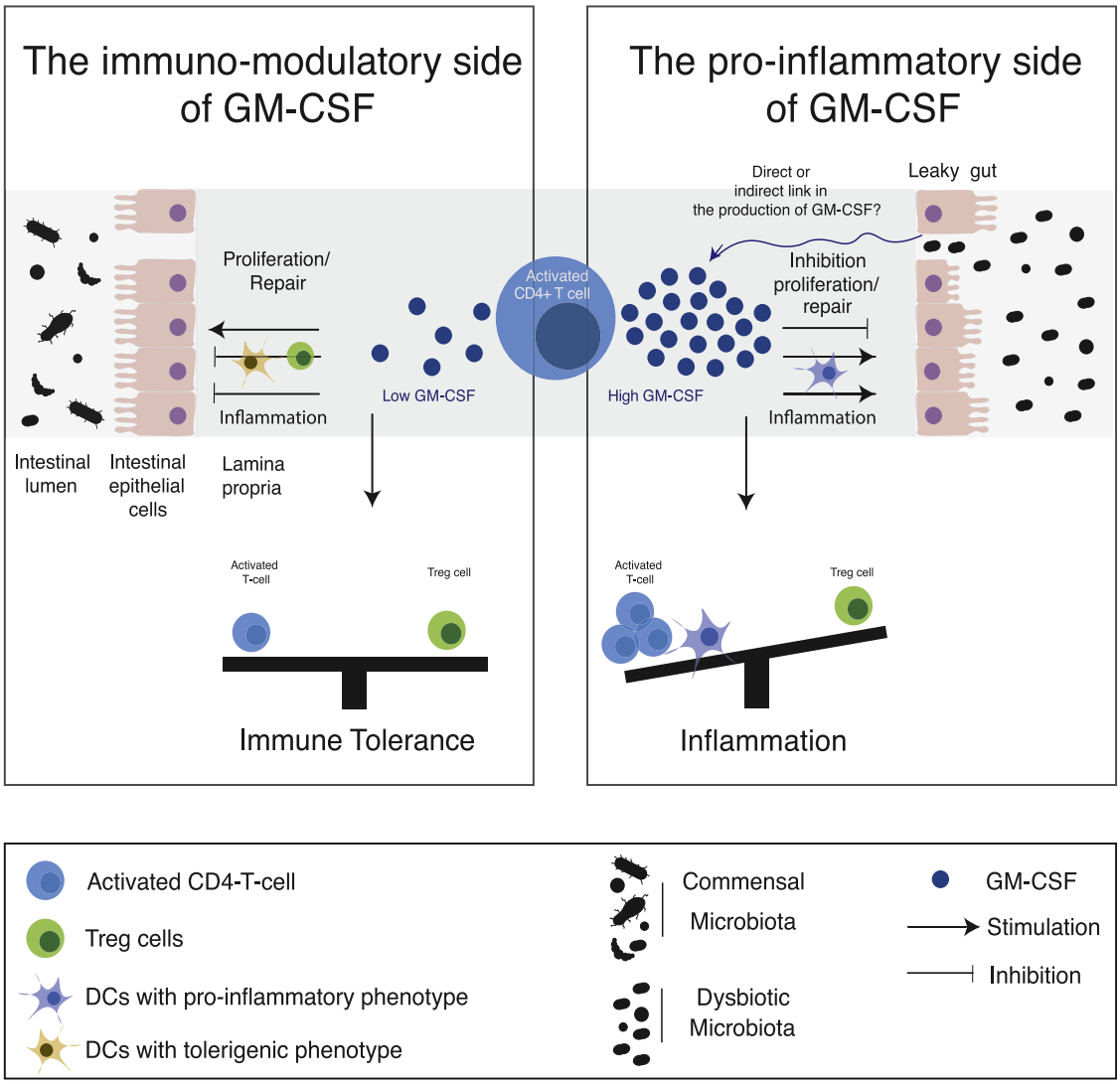

Figure 1. Focus on possible roles of GM-CSF in GI GVHD pathogenesis after allo-HSCT, showing GVHD-protective (left) and GVHD-promoting (right) effects of GM-CSF.

importance of GM-CSF specifically produced by $CD4^+$ T cells in the pathogenesis of GI-GVHD. GM-CSF also may contribute to CNS-GVHD by activating microglial cells, which produce TNF as a neurotoxic cytokine [46].

Three important studies have demonstrated the fundamental role of GM-CSF in GI-GVHD development and provided some mechanistic insights [5,7,44]. In the first study, Ellrich et al [44] pointed out the importance of the transcriptional factor Batf. Indeed, in both MHC- and minor histocompatibility complex-mismatched allo-HSCT GVHD mouse models, recipients of *Batf*$^{-/-}$ donor $CD3^+$ cells had milder GI-GVHD compared with recipients of WT donor $CD3^+$ cells. Interestingly, the differences observed were only in the severity of GI-GVHD; liver GVHD was not affected by the absence of Batf in donor $CD3^+$ cells. The absence of Batf was associated with reduced expression of GM-CSF by T cells, suggesting that Batf regulates, directly or indirectly, GM-CSF expression in these cells. Of note, the reduced expression of GM-CSF in recipients treated with *Batf*-deficient donor T cells was associated with reduced recruitment of myeloid cells. Importantly, injection of exogenous GM-CSF in recipients of *Batf*$^{-/-}$ donor $CD3^+$ cells exacerbated the signs of systemic and GI-GVHD, further supporting the central role of GM-CSF in GI-GVHD pathogenesis. Indeed, the transcription factor BATF is upregulated in GVHD colonic human tissues and associated with increased rates of apoptosis [44].

Piper et al. [5,7] provided some additional insights in two important studies. Their first study showed that when recipient mice were reconstituted with *Rag-1* bone marrow cells alone or with

$CD4^+$ T cells from WT or *Bhlhe40*$^{-/-}$ donors, mice that received $CD4^+$ *Bhlhe40*$^{-/-}$ T cells had better survival compared to those receiving WT $CD4^+$ cells. Moreover, they demonstrated that these animals had significantly decreased GM-CSF production [5]. In a second work, the authors investigated in more detail the $CD4^+$ GM-CSF^+ population in acute GVHD (aGVHD) target organs in mice and found that GM-CSF was produced in all the different organs tested (spleen, liver, lung, and colon) but with significantly more important production in the colon [7]. The existence of 2 transcriptionally distinct populations producing GM-CSF in the colon based on the presence or absence of IFN-γ coexpression was observed in a scRNA-seq analysis. At the protein level, IFN-γ production identified two subsets of GM-CSF-producing $CD4^+$ cells: double-positive IFN-γ^+ GM-CSF^+ cells and single-positive IFN-γ^- GM-CSF^+ cells. The coexpression of GM-CSF and IFN-γ in $CD4^+$ cells could indicate a subset with an increased pathogenicity or proinflammatory function; however, the relevance of these 2 different GM-CSF^+ populations in GVHD induction has remained unknown.

GM-CSF is expressed in the human GI tract at the steady state, mostly in the sigmoid colon, where innate lymphoid cells are the main producing cells [47]. GM-CSF expression regulates the proliferation of intestinal epithelial cells, maintaining the integrity of the intestinal epithelium, and stimulates the differentiation of DCs, controlling intestinal inflammation [48]. In particular, in a model of chemically induced colitis, GM-CSF has been shown to support the proliferation of crypt cells [49], a crucial compartment for intestinal epithelial regeneration during inflammation. GM-CSF expression increases during inflammatory processes such as ulcerative colitis [47,50], where GM-CSF amplifies the intestinal inflammatory loop and skews macrophage differentiation toward an M1 phenotype [47,51,52]. In patients with GI-GVHD, *CSF2* mRNA levels in the GI tract were correlated with the severity of GVHD, with higher *CSF2* transcription levels in GI biopsies seen in patients with grade IV GI-GVHD compared to those with grade I GI-GVHD [43]. Moreover, GM-CSF-$CD4^+$ T cell levels in peripheral blood were higher in GVHD patients compared to healthy donors [43]. Collectively, these data support a potential role of GM-CSF in GVHD pathogenesis in humans.

Immunosuppressive drugs used for the prevention and treatment of GVHD, such as glucocorticoids, cyclosporine A, and tacrolimus, are strong inhibitors of T cell activation thus reducing cytokine production. The immunosuppressive effect of glucocorticoids is attributed in part to the inhibited production of proinflammatory cytokines, including GM-CSF, in T cells via regulation of their transcription [53]. Through the inhibition of calcineurin, tacrolimus and cyclosporine A are responsible for inhibition of the NFAT signaling cascade, and GM-CSF expression is suppressed by the inhibition of NFAT and the reduction in *Csf2* enhancer activity [23,54,55]. These drugs also can modulate the production of cytokines by APCs [53,56,57]. The effect of ruxolitinib (an inhibitor of JAK1 and 2), which was recently approved to treat aGVHD and chronic GVHD (cGVHD), on GM-CSF has not been studied to date; however, given that GM-CSF production in T cells seems to be STAT5-dependent [26,27], we can hypothesize that ruxolitinib might efficiently inhibit GM-CSF production by T cells and APCs.

The immunomodulatory side of GM-CSF in GVHD

A role of GM-CSF as an immunomodulatory cytokine through modulation of APCs (specifically DCs) and Tregs has been reported. Several lines of evidence from different medical fields point to an immunomodulatory function of GM-CSF through the indirect stimulation of Treg proliferation [58–60]. In experimental mouse models, GM-CSF was seen to orient the differentiation of DCs toward a specific phenotype, $CD11c^+CD8^-$, with tolerogenic functions [61] able to induce and expand Tregs [59]. In an MHC-mismatched allo-HSCT mouse model, GM-CSF administration was reported to prevent and attenuate the progression of skin cGVHD [62]. GM-CSF injections for 3 days after engraftment were able to prevent or attenuate cGVHD by inducing in vivo Treg proliferation and by inducing the in vivo expansion of $CD11c^+CD8^-$ DCs [62,63]. At steady state, mice deficient for *Csf2* had significantly reduced numbers of DCs, macrophages, and Tregs in the colon; moreover, DCs isolated from these mice were clearly impaired in their ability to drive Treg differentiation [64]. The action of GM-CSF on Tregs seems crucial for modulating cGVHD, as the depletion of donor-derived Tregs at the time of transplantation leads to a loss of GM-CSF's protective effect on cGVHD development [62].

The specific mechanism that leads to the expansion of Treg cells by GM-CSF has not been fully elucidated. GM-CSF could stimulate the proliferation of Tregs indirectly in a DC-dependent manner or directly by binding to GM-CSFR. In a mouse model of aGVHD, GM-CSF produced by

$CD4^+$ T cells did not influence Treg reconstitution in terms of numbers [5]. In a study by Hotta et al. [62], the suppressive function of Tregs in mice that underwent allogeneic bone marrow transplantation was comparable in mice treated with GM-CSF and mice treated with phosphate-buffered saline. This suggests that in this mouse model, GM-CSF exerted its role in GVHD suppression through the induction of Treg proliferation rather than by affecting Treg function [62]. Conversely, in vitro murine GM-CSF-expanded Tregs displayed greater suppressive capacity than control Tregs expanded with CD3/CD28 beads only [35]. Several groups have reported that Treg adoptive transfer efficiently suppresses murine GVHD [65] through pleiotropic mechanisms [66], and very encouraging clinical experiences have been reported [67]. GM-CSF priming of Tregs before adoptive transfer as GVHD prophylaxis might improve their expansion and/or function similar to a previous study using TNF-α priming [68]. Human macrophages stimulated in vitro for 24 or 48 hours with GM-CSF and cocultured with autologous T cells were able to induce Treg differentiation [69], demonstrating how GM-CSF can indirectly modulate T cell fate.

There are no clear data in the literature on the role of GM-CSF in the direct expansion of human Tregs. In a retrospective study involving HLA-identical sibling donor transplantation for hematologic malignancies, Devine et al. [70] observed that the mobilization of peripheral blood stem cells in donors with GM-CSF compared to donors with G-CSF was associated with lower rates of moderate to severe aGVHD in recipients [70]. No differences in the engraftment time nor in the relapse incidence were described. However, the composition of the peripheral blood stem cell grafts differed markedly between the two groups, with lower numbers of $CD34^+$ cells, T cells, B cells, and NK cells in the GM-CSF group. No detailed information on the number or function of Tregs is provided.

GM-CSF also has been described as crucial during homeostasis for improving the resistance to bacterial translocation and for modulating the microbiota composition [48,71]. Motha et al. [64] observed that GM-CSF expression was absent in newborn mice and increased overtime. When mice were treated with broad-spectrum antibiotics to reduce the gut microbiota, the production of GM-CSF was drastically reduced [64,72]. In a non-HSCT setting, the overexpression of GM-CSF by transfecting GM-CSF-encoded lentivirus into a hepatocellular carcinoma cell line via subcutaneous orthotopic transplantation in a mouse model modified the microbiota composition, inducing the expansion of some bacteria, such as *Blautia*, that have been associated with a reduced risk of aGVHD, and the reduction of others, such as *Streptococcus*, that have been associated with an increased risk of aGVHD [73,74].

GM-CSF in GVT

Little is known about the role of GM-CSF in the GVT effect of allo-HSCT. In a murine MHC-mismatched HSCT model, the GVT effect was maintained even in the absence of GM-CSF, and mice receiving transplantation with $Csf2^{-/-}$ T cells showed similar control of tumor growth as mice that received WT T cells. The improved survival in mice that received $Csf2^{-/-}$ T cells compared to mice that received WT T cells was related mainly to the decreased severity of aGVHD in these mice [43]. These important findings suggest that, at least in mice, GM-CSF is dispensable for the GVT effect.

Whether exogenous GM-CSF can increase the GVT effect of the transplant procedure is an area of current investigation. GM-CSF has been used as an adjuvant in several experimental tumor vaccines with some conflicting results [4,10,33,75]. GM-CSF-based vaccines have been applied in experimental allo-HSCT as a strategy to boost the GVT effect and thereby reduce relapse. In mice after syngeneic bone marrow transplantation, a GM-CSF/B16 vaccine generated using B16-F10 melanoma cells genetically modified to express GM-CSF was able to induce antitumor responses that were stronger after reconstitution of the T cell compartment, as expected [76]. Conversely, GM-CSF/B16 vaccination after allogenic bone marrow transplantation failed to elicit detectable antitumor responses, probably as a consequence of delayed reconstitution of the T cell compartment in the allogeneic setting and/or of the presence of GVHD [76]. This experimental evidence demonstrates the complexity of immune reconstitution in the transplantation setting.

In human hematologic malignancies, a GM-CSF-based vaccine, known as GVAX, using irradiated autologous myeloblasts transduced to secrete GM-CSF, was originally tested in patients with chronic myeloid leukemia in chronic phase with persistent measurable disease despite imatinib treatment and shown to improve molecular responses [77]. In another pilot trial, the use of GVAX in 5 patients with myelodysplastic syndrome (MDS) reported encouraging results, with some degree of clinical response seen in 2 patients and no serious adverse events noted [78]. In the

transplantation setting, GVAX was administered to 15 patients with advanced MDS or high-risk acute myeloid leukemia after allo-HSCT in a phase 1 study. The data confirmed the safety of GVAX administration in this setting and suggested its ability to elicit responses, such as a durable complete remission in 9 out of 10 subjects [79]. However, in a phase 2 multicenter double-blind trial, the use of GVAX after allo-HSCT for MDS/acute myeloid leukemia did not show any beneficial effects in terms of progression-free-survival compared with placebo [80]. The authors speculated that low doses of GM-CSF are associated with better progression-free survival than higher doses; however, an insufficient number of patients precludes any solid subgroup analysis. Moreover, it is also possible that the delayed immune reconstitution could have negatively impacted the antitumor effect of GM-CSF vaccine in the allo-HSCT setting, similar to what was observed in the preclinical studies [76].

DISCUSSION

GM-CSF has a pleiotropic function. Although our understanding of GM-CSF has increased over the years, the underlying mechanisms determining the proinflammatory versus the regulatory effects of GM-CSF are not fully understood. The action of GM-CSF on a broad range of cellular targets might partially explain how this cytokine could have two opposing roles in GVHD pathogenesis (Figure 1).

GM-CSF function through intracellular signaling activation can be influenced and regulated by the concentration of GM-CSF itself. It is possible that low concentrations of the cytokine activate the immunomodulatory pathway, whereas high concentrations stimulate the inflammatory pathway. Low concentrations of GM-CSF were reported to induce tolerogenic DCs, whereas high doses develop into proinflammatory DCs [81]. It has been reported that at low GM-CSF concentrations, GM-CSFR is phosphorylated on the Ser585, leading to cell survival without proliferation, whereas at high concentrations, GM-CSFR is phosphorylated on Tyr577, leading to both cell survival and proliferation. Moreover, the STAT5 signaling pathway is activated only in the presence of high concentrations of GM-CSF [82,83]. The action of GM-CSF also seems to be dependent on its concentration when used as a vaccine adjuvant [75]. This could have therapeutic implications, as GM-CSF blockade could be used to reduce cytokine concentrations and to favor the immunomodulatory action of GM-CSF.

Another factor that could influence GM-CSF action is the environment. GM-CSF is already produced at low concentrations in the GI tract at steady state, supporting its immunomodulatory role. One hypothesis is that in transplant recipients, the presence of gut damage could lead to an increased GM-CSF production to favor repair of the damage by inducing the proliferation of epithelial cells. In GVHD patients, in whom gut damage is more important, deregulation of GM-CSF expression could occur, with increased production of the cytokine, which at this point would have mostly an inflammatory role. In this scenario, GM-CSF as a treatment for GVHD would not be a great choice, given the inflammatory environment. The kinetics of GM-CSF expression could be important; indeed, we could think about treating patients with GM-CSF before transplantation to reduce damage to the GI tract caused by the conditioning regimen by inducing epithelial proliferation and microbiota reconstitution. Recent studies have described an association between the microbiota and GVHD. It would be intriguing to explore whether GM-CSF expression could modify the microbiota composition.

Moreover, the action of GM-CSF could depend on the presence of other cytokines in the environment. Indeed, the coexpression of GM-CSF with other cytokines, such as IFN-γ or TNF-α, could potentiate its pathogenic and inflammatory effects; however, its coexpression with TGF-β could direct the immunomodulatory action through the stimulation of Tregs.

CONCLUSION

In the allo-HSCT field, it is important to find molecules that are selectively active against GVHD without impairing the GVT effect. In mice, GM-CSF appears to be crucial for the pathogenesis of GVHD and dispensable for the GVT effect, making it an interesting drug target candidate. Future studies providing further insights into the biology of GM-CSF in GVHD could pave the way to the development of new therapeutic strategies for GVHD prevention and treatment.

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