GVHD Pathophysiology and MDSCs

Subjects: Others

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The backbone of conventional treatment for aGVHD relies on the use of corticosteroids. Corticosteroids, as the gold standard of initial therapy, result in diverse complete responses (25–69%). As aGVHD severity increases, so does mortality, which is indicated by mortality rates reaching 95% in grade III-IV aGVHD. Despite the research community's efforts to establish novel targeted strategies against GVHD, no optimal treatment regimen has been described.

Keywords: GVHD ; MDSC ; GVL

1. GVHD Pathophysiology and Implications for MDSCs

The pathophysiology of aGVHD has been connected to a 3-phase process: (1) *initial tissue damage* from the conditioning regimen, which activates host antigen-presenting cells (APC) by danger-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), (2) *afferent phase* characterized by the stimulation and proliferation of donor T cells in response to alloantigen expressed either on host APCs, labeled as direct antigen presentation, or on donor APCs, labeled as indirect presentation, and the (3) *effector phase* represented by generated donor T cell-mediated cytotoxic damage against host cells through Fas–Fas ligand interaction, perforin–granzyme, and TNF-α [1].

Through their capacity to attack the recipient's tissues, donor alloreactive T cells are considered a potential target to suppress aGVHD reactions and reduce organ injury. Recently, there has been increasing interest in the contribution of donor MDSCs on GVHD management due to their immunosuppressive effects on alloreactive T cell priming and expansion and induction of T Regs. As it has been demonstrated, MDSCs can be mobilized from normal BM in a relatively short amount of time and have the ability to inhibit GVHD as well as allograft rejection efficiently [2].

More than two decades ago, Mielcarek et al. showed that mononuclear cells from mobilized blood obtained from healthy donors after G-CSF initiation were less responsive (31.5% ± 9.2% response, $p = 0.003$) compared to mononuclear cells collected from the peripheral blood before the administration of G-CSF. This study also demonstrated that G-CSF-mobilized peripheral blood CD14+ mononuclear cells had the ability to suppress alloantigen-induced proliferation of CD4+ T cells by more than 50% [3]. An inverse relationship between suppressor cell activity and CD4 T cell apoptosis was further documented following autologous stem cell transplantation [4]. Subsequent studies by Luyckx et al. aimed to characterize these cells. Flow cytometry on individual peripheral blood samples from six different G-CSF-treated PBSC-donors identified a MDSC population (Lin−, HLA-DR−, CD11b+), reaching a median value of 90% (range 83.5–94.5%) among a CD45+ cell population, whereas, in the control individuals, this was only 35% (range 21.7–58.0%). The expanded MDSC population comprised monocytic (CD33high, CD14high, SSClow, CD15−) and granulocytic (CD33int, CD14low, SSChigh, CD15high) subpopulations with the ability to suppress T cell alloreactivity [5].

2. Early Studies in Murine Models

Early murine studies identified enhanced activity of lymphocyte suppressor myeloid cells and concomitant appearance of these cells in the spleen of thymectomized, irradiated, bone marrow-reconstituted mice after systemic bacillus Calmette–Guerin (BCG) administration [6]. Subsequent research confirmed transient accumulation of suppressor cells, deficient of surface markers for macrophages, NK cells, B cells, or T cells, in the lymphoid tissues of neonatal or irradiated mice [7][8]. To further elucidate the function of these "null" cells or natural suppressor cells (NS), more studies were performed in murine GVHD models. These cases involved rapidly proliferating stem cell populations, including NS cells capable of suppressing alloreactive T cells in an antigen-independent manner [9], raising questions about whether an implication in GVHD could exist [9][10]. NS cells were demonstrated to arise during the first weeks after bone marrow transplantation in lethally irradiated mice [11]. NS cells were also identified in normal bone marrow with comparable characteristics to that in spleens of early bone marrow transplant recipients [12], providing a possible source for obtaining NS-enriched populations for adoptive transfer studies after in vitro expansion [12]. The myeloid origin of NS cells was confirmed later, as they were quantified as progenitors of monocyte lineage by colony-forming assays in soft agar [13] before the term "MDSC" was

introduced [14]. More recent murine studies showed that MDSCs (Gr-1+ and CD11b+ population) seem to accumulate during the first 2 weeks after allo-HCT. This phenomenon is probably driven by the proinflammatory cytokine storm mediated by the preparative regimen. Involved cytokines include IFN-γ, G-CSF, IL-1β, and IL-6, creating an ideal stroma for accumulation and activation [15][16]. In the absence of aGVHD, MDSC percentage returns to basal level by week 6, parallel to the decline of cytokine levels. On the other hand, the development of GVHD is related to a constant enhancement of MDSC in a severity-dependent manner ($p < 0.05$, Student's t-test) either due to stimulation by cytokines or due to interaction with the bone marrow and spleen microenvironments [17].

Interestingly, the treatment of donor mice with CpG and incomplete Freund's adjuvant (IFA) increased MDSCs (CD11b+ Gr-1+) that abrogated T cell alloreactivity in vitro and GVHD in vivo. This model also demonstrated increased levels of IL-6 and IL-10 after treatment with CpG; however, a direct association of increased cytokine levels with T cell anergy has not been demonstrated [18]. On the other hand, interleukin-13 (IL-13) production enabled a Ly6C+, CD11b+, Arg1+ (MDSC-IL13) population, promoting metabolic stress and T cell dysfunction [19]. Messmann and colleagues reported that MDSCs generated in vitro after culture of bone marrow cells with G-CSF and GM-CSF inhibited GVHD-induced death by 80%, preferentially by inducible nitric oxide synthase (iNOS) and attenuated histologic GVHD through Th2 induction. In contrast, the anti-tumor cytotoxicity of alloantigen-specific T cells was maintained ($p \leq 0.05$, Student's *t*-test) [20]. The immunosuppressive function of MDSCs, obtained after administration of G-CSF on GVHD models, has been elucidated further by other in vivo studies [21]. Wang and colleagues acquainted the ability of G-CSF to proliferate MDSCs and equip HLA-DR−/low, CD33+, CD16− eMDSCs with regulatory properties against the expansion of autologous CD3+ T cells in a TGFβ-dependent manner. Meanwhile, eMDSCs managed to promote T Reg upregulation and polarization of Th1/Th17 cells to Th2 cells [21]. These findings were translated into an attenuation of aGVHD and increased survival [21]. In studies performed by Messmann et al., skewing of T cells toward Th2 cells and attenuation of intestinal and cutaneous GVHD manifestations were independent of MHC class I expression and antigen presentation [20]. Interestingly, allogeneic T cell proliferation and homing were not influenced under these circumstances, thus maintaining anti-tumor activity [20]. The previous year, a novel approach utilizing systemic infusion of combined donor-derived-MDSCs and T Regs obtained after G-CSF has successfully demonstrated ameliorated inflammation in an aGVHD mouse model [22]. According to histopathologic analysis, the extent of tissue inflammation and lymphocyte infiltration were significantly reduced in all primary target organs of aGVHD, such as the skin, intestine, and liver [22].

To pinpoint the mechanism by which MDSCs protect from GVHD, Joo and colleagues evaluated whether indoleamine 2,3-dioxygenase (IDO) is implicated in T cell suppression, thus mimicking immunomodulation observed between maternal T cells and fetal tissue during pregnancy. Apart from confirming the regulatory effects of G-CSF in allo-HCT, they showed that this impact was mediated by IFN-γ-induced 2,3-IDO functional activity [23]. Moreover, the suppressive functions of MDSCs are assumed to be reactive oxygen species (ROS) dependent [17].

Further studies validated the immunosuppressive role of ARG1 after HCT and inhibition of alloreactive T cell responses [17][19]. L-arginine (l-Arg) is a nonessential amino acid with a central role in T cell proliferation and function. l-Arg is catabolized by ARG1, an enzyme produced by MDCSs, resulting in reversed T cell function by suppressing T cell expression of the CD3ζ chain and the cell-cycle regulators cyclin D3, and cyclin-dependent kinase 4 [24].

As previously mentioned, iNOS is involved in MDSC-related immunoregulation. NOS is a known antiproliferative for T lymphocytes, B lymphocytes, and NK cells [25][26]. The leading MDSC group capable of producing NO is believed to be the monocyte-MDSC subset (CD11b+ Ly6Chigh Ly6Glow) and, more specifically, a highly immunosuppressive subpopulation expressing CD34+. The local production of NO, in response to IFN-γ produced by activated T cells, was able to reduce T cells activation and proliferation and protect from GVHD in vivo [27].

Although the potency of MDSCs generated ex vivo in aGVHD was well documented, in vivo activity was limited as MDSCs lost their suppressive ability after setting into a highly inflammatory environment within the HCT recipient. Previous studies in aGVHD emphasized the critical role of the NLRP3 inflammasome pathway in MDSC alloimmune stimulation [28]. This limitation was related to cell-intrinsic inflammasome upregulation, production of inflammatory mediators, and myeloid differentiation [2]. The inflammasome is an intracellular multiprotein complex that controls the induction of inflammatory caspases such as caspase-1 and -11 [28]. To elucidate the environmental and intrinsic mechanisms of MDSC activation, Koehn et al. evaluated the implication of the NLRP3 inflammasome pathway in an aGVHD major histocompatibility mismatch murine model [29]. Factors related to inflammasome activation are produced during allo-HCT (e.g., preparative regimen, aGVHD), including the intestinal release of bacterial products and danger-associated molecules from dying cells that translocate into the internal milieu [30]. In their studies, Koehn and colleagues showed that preparative regimen-induced adenosine triphosphate (ATP) relocation is a main driver of MDSC dysfunction mediated by ATP receptor (P2x7R) engagement and NLR pyrin family domain 3 (NLRP3) inflammasome stimulation.

Taking it one step further, they also revealed that P2x7 knockout or inhibition of ATP association with P2x7R receptor inhibited inflammasome activation. The latter was exhibited with extracellular ATP exhaustion via apyrase and pharmacologically via treatment with A-438079, a highly selective P2x7R inhibitor [29].

The Toll-Like Receptor 4 (TLR4)–Myeloid Differentiation primary response gene 88 (MyD88) pathway is also contributing to the insufficient expansion of donor MDSCs and initiation of aGVHD [31][32][33], whereas pharmacological induction of TLR4 aggravates GVHD lethality [34]. In the context of allo-HCT, the activation of TLR4/MyD88 results from intestinal microbiota products, such as lipopolysaccharides (LPS), which are released after intestinal injury from the conditioning. In T cell-depleted BM murine transplant, the expression of MyD88, but not lack of it, was essential for protection against fatal intestinal aGVHD as observed after repeated LPS injections. MyD88 favored the presence of CD11b+ Gr-1+ cells in target organs, whereas, at the same time, the degree of intestinal T cell infiltration was inferior [31].

mTOR pathway inhibitors, such as sirolimus and rapamycin, are increasingly used in the prophylaxis and management of aGVHD. Their multiple activities include immunosuppressive actions through T cell suppression while promoting T Regs, inhibiting antigen presentation and dendritic cell maturation, antifibrotic properties, antineoplastic, and antiviral activities [35]. In vivo studies showed that rapamycin could induce G-MDSCs accumulation with an enhanced immunosuppressive role in the presence of aGVHD via upregulation of ARG1 and iNOS and induction of regulatory T cells. Graft-versus-tumor effect was maintained [36][37].

Another mechanism through which MDSCs are involved in aGVHD is the upregulation of T Regs. The cytokine-driven polarization of T cells from Th1/Th17 to Th2 secondary to increased IL-4/INF-γ and IL-4/IL-17 ratio has been proposed [21]. Il-10, produced by granulocyte-MDSCs, might also have a central role in this. As demonstrated, the treatment of donor mice with ProGP-1 and G-CSF expanded a CD11bhighGr-1low population that induced IL-10-producing regulatory T cells, likely via the indirect presentation of host antigens within the context of donor MHC class II, and prevented GVHD, while the GVL effect was preserved ($p < 0.0001$, Kaplan–Meier and compared by log-rank analysis) [38]. Moreover, MDSCs upregulate T Regs through the enhanced expression of ligands for T cell co-stimulatory molecule NKG2D, such as RAE-1 and MULT-1 [39].

While many studies delineated the role of MDSCs on aGVHD, the knowledge concerning that association with cGVHD is limited. Recently, Lim et al. showed in a preclinical model that ex vivo-generated human cord blood MDSCs (CD14+, HLA-DRlow, CD11b+, CD33+) limited clinical and pathologic cGVHD severity by alleviating thymic damage and attenuating Th 17 and Th 2 differentiation, proposing a possible therapeutic strategy for the clinical application of MDSC infusion [40].

3. Studies in Humans

Before introducing the term “MDSCs”, studies documented an increase of NS cells in the apheresis products following mobilization compared to bone marrow and cord blood products [41][42][43]. In particular, Mills and colleagues demonstrated an increased frequency of natural suppressor cells in GM-CSF-mobilized products and bone marrow cells of patients with NHL, compared to cord blood products and unmobilized apheresed mononuclear cells from healthy volunteers [41]. Moreover, Talmadge et al. were the first to report high levels of suppressor cell activity in the peripheral blood of the patients after transplantation [42]. These studies involved patients with solid or hematological malignancies, and all documented an inverse relationship between NS cells and T cell number and function [41][42]. Concerning products derived from healthy donors and besides the anticipated recruitment of hemopoietic progenitors, rhG-CSF also managed to produce an unexpected modification of lymphocyte subsets [43]. In accordance, more recent data provided evidence that cell populations representing M-MDSC and G-MDSCs increase in healthy donors’ peripheral blood during G-CSF mobilization [5][44], irrespective of age and sex [41]. Research confirmed the inverse correlation between the incidence of aGVHD and the presence of M-MDSCs [27][44], and eMDSCs in the graft [21]. A lower incidence of aGVHD was observed both in the haploidentical and HLA-matched allo-HCT setting [45]. Furthermore, Lv and colleagues correlated negatively the presence of MDSC with cGVHD, documenting no significant effect on relapse and survival [45]. Individuals receiving higher numbers of G-MDSCs and M-MDSCs in grafts displayed a lower incidence of grade II-IV aGVHD and severe cGVHD after haploidentical HCT [45]. The accumulation of M-MDSCs in patients after allo-HCT, especially during higher grade aGVHD, was associated with the suppression of CD3ζ-chain expression on T cells via 2,3-IDO [46]. Although the proportion of M-MDSCs cells correlated significantly with G-CSF administration in donors [5][44], G-CSF administration in patients at any time-point after allo-HCT did not influence CD14+HLA-DRlow frequency [46]. However, retrospective data showed improved anti-leukemic effect after administering G-CSF–donor lymphocyte infusions (G-CSF-DLI) compared to regular–DLIs in relapsing patients post allo-HCT. G-CSF-DLI were enriched with G-MDSCs and M-MDSCs and, remarkably, they did not increase the cumulative incidence of GVHD [47].

The role of ECP as an alternative treatment approach in GVHD has been well established [48][49][50]. The infusion of ECP-treated leukocytes in steroid-refractory GVHD has been demonstrated to mobilize P-MDCS [51] or CD33+CD11b+ MDSC subsets [52]. MDSC levels were particularly enhanced in aGVHD patients compared to patients with cGVHD, and they seem to possess a vital role in the immunomodulatory modality of ECP [52].

Primary target organs of aGVHD include the skin, the liver, and the intestine [53][54]. Unfortunately, limited information could be recovered during the literature review regarding the association between GVHD manifestation and MDSCs. Of note, a prospective clinical study by Vendramin et al. demonstrated that G-CSF induced the expansion of M-MDSCs capable of abrogating aGVHD by reducing tissue damage and inflammation in all target organs [44]. More data were obtained from studies involving the administration of G-CSF mobilized MDSCs in xenogeneic models of GVHD. A study from D'Aveni et al. showed lower GVHD histopathological scores, mainly in the colon, in humanized mouse models after the infusion of G-CSF-mobilized CD34+ monocytes [27]. A similar effect was demonstrated by Wang and colleagues, who showed that G-CSF mobilized HLA-DR−/lowCD33+CD16− cells resulted in improved histopathological score in hepatic and intestinal tissues [21].

M-MDSCs and invariant natural killer (iNKT) cells were shown to expand shortly after allo-HCT [55]. The delayed recovery of both M-MDSCs and iNKT cells following transplantation was associated with an increased occurrence of grade III-IV aGVHD. Nevertheless, the combination of lower M-MDSCs and higher iNKT cells correlated with enhancement of GVL effect and decreased the rate of leukemia relapse [55]. A major obstacle facing the field of GVHD management through suppressor cells is deregulation of the immune system, delayed reconstitution, and increased susceptibility to infections. The conditioning regimen in conjunction with stem cell infusion and subsequent tissue damage creates an inflammatory milieu that might induce an exaggerated expansion of M-MDSCs [56]. In such circumstances, increased M-MDSC levels predict for higher non-relapse mortality [56].

The experimental information from animal models and observations from patients undergoing allo-HCT indicates that MDSCs illustrate a promising tool for preventing and managing GVHD in the clinic. On the other hand, limited data were derived from human studies. Generating MDSCs from human is difficult due to low concentration. More than one month of cultivation is required to produce sufficient MDSCs which might be too long for their application as a treatment for severe acute GVHD, whereas the ideal method for their processing has not yet been identified [57].

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