Cellular and molecular mechanisms in graft-versus-host disease

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ABSTRACT

Graft-versus-host disease is a complication in patients undergoing hematopoietic stem cell transplantation. Graft-versus-host disease includes acute graft-versus-host disease and chronic graft-versus-host disease. Host APCs (e.g., dendritic cells and macrophages), effector T cells (e.g., Th1, Th17, and abnormal Th17: regulatory T cell ratio), B cells, and NK cells are implicated in graft-versus-host disease physiopathology. Proinflammation cytokines (e.g., IL-17, IL-1β, and TNF-α) are increased in graft-versus-host disease. Costimulatory molecules play an important role in inducing graft-versus-host disease. Pattern-recognition receptors, such as TLRs and nucleotide-binding oligomerization domain-like receptors, are critically involved in the pathogenesis of graft-versus-host disease. Complement system C3 mediates Th1/Th17 polarization in human T cell activation and skin graft-versus-host disease. Accumulation of CD26 T cells in graft-versus-host disease target organs was found. As a therapeutic target, soluble CD83 molecules or antibodies have been demonstrated to have therapeutic effects against graft-versus-host disease, and signaling molecules promote the inflammatory and immune process of graft-versus-host disease. These immune cells and molecules could be the predictors of graft-versus-host disease development and the drug targets of the treatments for graft-versus-host disease. This article focuses on major advances on cellular and molecular mechanisms in graft-versus-host disease. J. Leukoc. Biol. 99: 279–287; 2016.

Introduction

Allogeneic HSCT has been widely used for the treatment of hematologic malignant and nonmalignant hematologic diseases and other diseases. GVHD is a life-threatening complication in patients undergoing HSCT [1, 2]. GVHD includes aGVHD and cGVHD. The incidence of aGVHD ranges from 10 to 80%, with symptoms usually developing at 2-3 wk post-transplant [3]. The exact incidence after allo-HSCT is unknown in cGVHD patients. And the incidence rates of cGVHD range from 6 to 80%, depending on the presence of risk factors and diagnostic criteria used. With the use of U.S. National Institutes of Health consensus criteria, Socié and Ritz [4] reported that the 36-mo cumulative incidence of cGVHD was 53.7% [5, 6]. Historically, features occurring within 100 d were classified as aGVHD and those occurring beyond 100 d as cGVHD. However, it is now recognized that clinical features of cGVHD can occur within 100 d post-transplant and that features of aGVHD and cGVHD may coexist [7–9]. Immune cells and molecules are involved in the pathologic process of GVHD and could be the predictors of GVHD development, aGVHD is preceded by high counts of CD4 and CD8 T cells. cGVHD is generated by thymic damage, production of aberrant B cells, and defective function of T cells, along with cytokine dysregulation [9]. The pathologic process of GVHD is summarized in 3 sequential phases: 1) activation of APCs; 2) effector cells activation, proliferation, differentiation, and migration; and 3) target tissue destruction [10]. This article focuses on the major advances on immune pathologic mechanisms for GVHD.

IMMUNE CELLS IMPLICATED IN GVHD

Macrophages, as APCs, play critical roles in the initiation of GVHD

Antigen presentation is known to play a key role in aGVHD and cGVHD pathogenesis. The activation of donor and recipient APCs plays critical roles in the initiation of GVHD. Activation of host APCs occurs in the first phase of GVHD and before donor-cell infusion [11]. Before HSCT, the conditioning regimen (irradiation and/or chemotherapy) leads to the damage and activation of host tissues, especially the intestinal mucosa. This
allows microbial products to translocate to the circulation, which stimulates the secretion of proinflammatory cytokines from macrophages, such as IL-1 and TNF-α. Activated macrophages produce chemokines that activate neutrophils (Fig. 1). These proinflammatory cytokines increase the expressions of MHC and adhesion molecules on APCs, enhancing the antigen-presenting capacity of APCs [12].

Host macrophages could exacerbate GVHD through the high levels of proinflammatory TNF-α and IFN-γ and the low levels of anti-inflammatory IL-10 [15]. Donor macrophages deriving from donor bone marrow mediate the development of cGVHD and infiltrate into the skin. CSF-1-exacerbates macrophage infiltration and cutaneous pathology. Recipients of grafts from CsFlr⁻/⁻ mice had substantially less macrophage infiltration and cutaneous pathology compared with those receiving wild-type grafts. Depletion of macrophages that use an anti-CSF-IR mAb markedly reduced cutaneous and pulmonary cGVHD [14].

**DCs play a central role in the pathophysiology of GVHD**

Plasmacytoid DCs and myeloid DCs have the capability to stimulate Th17 responses through the release of cytokines. Potent activators of DC subsets are released upon tissue damage and microbial exposure during allo-HSCT. These motifs aggravate the Th17 response via the activation of various pattern recognition receptors, thereby initiating and perpetuating GVHD [15]. Donor conventional DCs are the primary APC responsible for indirect presentation of alloantigens following bone marrow transplantation. DCs take up antigens via the indirect pathway, predominantly via MHC-II to CD4 T cells, and this process commences almost immediately after transplantation (Fig. 1) [16–18]. Recipient CCR7⁺ DCs induced donor T cell to express chemokine receptors, and CCR7 regulated the migration of activated DC from tissue to draining lymph node. Depletion of CCR7⁺ host DCs can be an effective approach in prevention of aGVHD and preservation of GVL effects [19]. TDCs, which have low expressions of MHC and costimulatory molecules and express high levels of immunosuppressive cytokines, play a crucial role in reducing the severity of aGVHD by modulating cytokine secretion, expanding Foxp3⁺ Treg, and suppressing allo-CD4 T cell proliferation. In the mice treated with TDCs, the serum levels of IL-10 and TGF-β were elevated significantly and the percentage of Foxp3⁺ cells continually increased [20].

**T cells involved in GVHD through secreting proinflammatory cytokines**

aGVHD was preceded by high counts of CD4 and CD8 T cells [21]. The persistence of donor-derived alloreactive T cells and autoreactive T cells has been implicated in cGVHD pathogenesis [10]. Donor T cells firstly recognize host alloantigens and become activated. Th1 cells are considered the main triggers of the process (Fig. 1) [22]. Donor T cells that predominantly differentiate into Th1 cells and generate proinflammatory cytokines mediate GVHD [23]. As a subset of CD4 T cell, Th2 is also involved in GVHD pathogenesis. The manifestations and severity of GVHD are highly variable and are influenced by the proportions of naive cells maturing along Treg, Th1, Th2, or Th17 phenotypes [24].

Increasing evidence indicates the involvement of Th17 cells in GVHD. Th17 cells are suspected of initiating the Th1 response and aggravating tissue inflammation, resulting in full-blown GVHD. Adoptive transfer of Th17 cells is capable of inducing lethal aGVHD [25], and small numbers of Th17 cells could aggravate the lethality of GVHD in several allogeneic recipients [26]. Th17 cells, characterized by production of IL-17A, IL-17F, IL-22, and IL-21, are important mediators of skin GVHD because of the activation of STAT3 in keratinocytes, which results in epidermal hyperplasia [23, 27]. Patients presented with an increased number of Th17 cells compared with healthy individuals [28]. IL-17 favors GVHD development when purified CD4 T cells are transferred to allogeneic recipients, and IL-17A depletion reduced the disease severity [29–31].

Donor CD8 T cells could ablate or prevent the lupus syndrome, in part, by killing recipient B cells. Host CD8 T cells can reciprocally down-regulate donor CD8 T cells and thus, prevent them from suppressing the autoimmune process [32]. Donor CD8 T cells were more potent than CD4 T cells for inducing cGVHD. Donor CD8 T cells preferentially damaged recipient medullary epithelial cells and impaired negative selection, resulting in production of autoreactive CD4 T cells that perpetuated damage to the thymus and augmented the development of cGVHD [33]. Dual TCR T cells—a subset of peripheral T cells that naturally expresses TCR and contributes disproportionately to aGVHD from patients with aGVHD—demonstrate an activated phenotype and produce pathogenic cytokines. Human double TCR T cells are strongly activated and expanded by allogeneic stimulation in vitro and disproportionately contribute to the repertoire of T cells recognizing major and minor histocompatibility antigens [34].

An imbalance between Th17 cells and Treg contributes to GVHD [35]. Th17:Th1 and Th17:Treg ratios were increased significantly in liver cGVHD, as demonstrated by an increase in CCR6⁺, CD161⁺, and RORαt⁺ T cells [36]. The unbalanced loss of Treg is associated with the reciprocal increased secretion of proinflammatory cytokines by Th1 and Th17 cells [37, 38]. The Th17 percentage and RORαt expression were significantly higher, whereas Treg percentage and Foxp3 expression were significantly lower in severe aGVHD (grades 3–4) and mild aGVHD (grades 1–2) patients than in aGVHD (grade 0) patients and healthy donors. Parallel to those findings, Treg expansion has been shown to be capable of reducing the severity of aGVHD in murine models [39, 40]. The dynamic balance between the Th17 and Foxp3⁺ Treg and the changes of Th17-associated cytokines could be the indicators of the disease progression and prognostic biomarkers of aGVHD [41, 42]. Adoptive transfer of Treg can prevent GVHD in rodents, suggesting a therapeutic potential of Treg for GVHD in humans. Infusion of donor Treg has been reported to prevent aGVHD successfully in mice and more importantly, has shown promise in phase I clinical trials. Infusion
of clinical-grade-enriched Tregs could delay the occurrence of xenogeneic GVHD without causing any obvious toxicity or death in the murine model (Fig. 1) [43]. CD8hi Tregs controlled GVHD by reducing alloreactive T cell proliferation as well as decreasing inflammatory cytokine and chemokine secretion within target organs through a CTLA-4-dependent mechanism in humanized mice. These CD8hi Tregs could induce long-term tolerance effectively without compromising general immunity and graft-versus-tumor activity [44].

Immune dysfunction of B cell contributes to GVHD
cGVHD is characterized by the production of autoantibodies. The basic pathogenesis involves the cognate recognition of foreign MHC II of host B cells by donor alloreactive CD4 T cells (Fig. 1). B Cell-depleting therapy with Rituximab has been associated with beneficial effects in patients with cGVHD, which demonstrates the role of B cells in the pathogenesis of cGVHD. aGVHD in allos-H SCT patients with infiltrating donor T cells is associated with delayed B cell reconstitution and impaired antibody response. B Cell deficiency already existed before transplant in adult leukemic patients and was aggravated after transplant [45]. Inadequate reconstitution of naive B cells and high levels of BAFF have been found in patients with cGVHD. Treatment with exogenous BAFF could amplify cell size and survival in B cells from cGVHD patients. These changes might be associated with the expansion of activated CD27 B cells that produce autoantibody in cGVHD. Lowered BCR signaling threshold in cGVHD is associated with increased B cell proliferation and activation in response to antigen. cGVHD B cells exhibited increased BCR signaling components, B cell linker protein, and Syk phosphorylation compared with B cells from patients without cGVHD [46].

Donor B cells augmented Th2 cells expansion through producing IL-4, which mediates Th2 differentiation of donor T cells in GVHD recipients. The presence of Th2 cells is correlated with the cGVHD by mediating skin and lung damage. The mechanisms, whereby donor B cells contribute to pathogenesis of cGVHD, include (Fig. 1): 1) augmenting the clonal expansion of the residual autoreactive donor CD4 T cells and 2) augmenting donor CD4 T differentiation into proinflammatory Th2 cells. During cGVHD pathogenesis, donor B cells are activated by donor CD4 T cells to up-regulate MHC II and costimulatory molecules [47]. Mertk is important in MHC II-mediated B cell signaling and in mediating apoptotic...
cell-induced inhibition of DC activation/maturation. MerTK mediates apoptotic cell clearance and regulates activation and cytokine secretion. B6 congenic MerTK−/− mice were unresponsive in cGVHD induced by allogeneic T cells [48].

Bregs negatively regulate T cell immune responses through the secretion of regulatory cytokines, such as IL-10 and direct cell–cell contact. B Cells with immunoregulatory properties are enriched within the CD19+IgM+CD27+ memory and cell secretion of regulatory cytokines, such as IL-10 and direct cell–cell contact (Fig. 1). Moreover, Bregs from patients with cGVHD were less frequent and less likely to produce IL-10 than were Bregs from healthy donors and patients without cGVHD [49].

NK cells prevent GVHD

NK cells have been considered one of the main effector cells that mediate early GVL reactions. Alloreactive, donor-derived NK cells have also demonstrated killing recipient APCs and cytotoxic T lymphocytes, thus preventing GVHD and graft rejection and to contribute largely to the defense against cytomegalovirus infection in an early post-transplant period (Fig. 1). Donor-derived NK cells play a crucial role in the eradication of cancer cells, especially when there is a KIR ligand mismatched in the donor-recipient direction. KIRs represent a family of activating and inhibitory receptors expressed on NK cells that shape and regulate NK cell functions. Based on the number of extracellular domains, KIR proteins are classified as KIR2D (2 domains) and KIR3D (3 domains) receptors. KIR3DS1 represents the only activating receptor with 3 extracellular domains [50]. Gabriel et al. [51] showed that progression-free survival of patients with multiple myeloma after autologous stem cell transplantation was decreased significantly in patients carrying the KIR3DS1 gene. In contrast, a beneficial effect of KIR3DS1 in the context of T cell-depleted HSC transplantation has also been observed, showing a protective effect of donor KIR haplotype B against leukemic relapse and improved disease-free survival in patients undergoing HSCT [52]. Furthermore, Venstrom et al. [53] showed that transplantation of HSCs from KIR3DS1 donors was associated with decreased aGVHD. Sivori et al. [54] showed that in alloreactive NK cell responses, KIR2DS1 expression represents a remarkable advantage, as it allows efficient killing of DCs and T cell blasts. B/A haplotype donors offer clinical advantages compared with A/A donors [55]. NKp46 is a major killer receptor expressed by human and mouse NK cells. The choosing of NKp46-high donors for the treatment of different hematologic malignancies might lead to better tumor eradication, while minimizing GVHD. In the absence of NKp46, GVHD is greatly exacerbated, resulting in rapid mortality of the transplanted animals because of infection with commensal bacteria [56]. Pretransplant conditioning plays a dual role in promoting minor mismatch GVHD by depleting recipient NK cells and inducing intestinal barrier loss. Intestinal damage was required for the induction of minor mismatch (MHC-matched) GVHD. Recipient NK cells prevented minor mismatch GVHD by limiting expansion and target organ infiltration of alloreactive T cells via a perforin-dependent mechanism, revealing an immunoregulatory function of MHC-matched recipient NK cells in GVHD [57].

CYTOKINES INVOLVED IN GVHD

Proinflammatory cytokines are increased in aGVHD

TNF-α plays a role in all phases of GVHD pathophysiology from the early phase of host APC activation to the phase of direct and indirect tissue damage mediated by cytotoxic lymphocytes. There is strong evidence supporting the correlation between TNF-α levels and GVHD. Among aGVHD target organs, the gastrointestinal tract appears especially susceptible to damage from TNF-α. The increased level of TNFRI was able to be measured at 2–3 wk in advance of the onset of clinical symptoms. There is a strong correlation between TNFRI levels on d 7 and overall survival. Inhibition of TNF-α in experimental allo-HSCT models ameliorated the apoptosis characteristic of GVHD-related damage to the gastrointestinal tract [58]. Infliximab (an anti-TNF-α mAb) and Etanercept (a rhsTNF-α fusion protein) are effective for treatment of patients with steroid-refractory aGVHD [59, 60].

Th17-associated cytokines, including IL-6, IL-1β, IL-17, IL-21, IL-23, and IL-23R, were all increased in aGVHD patients and drive differentiation and expansion of Th17 cells. IL-1β is a potent inflammatory mediator involved in different inflammatory conditions and could be considered a useful predictor of aGVHD development. A significant association was identified between the IL-1β genotype of the recipient (CC) and high IL-1β levels in the saliva. The level of IL-1β in the saliva and blood was increased in the recipients with aGVHD [61]. IL-7 signals via the IL-7R and drives homeostatic T cell proliferation in patients after allo-HSCT. sIL-7R increased with time after HSCT and is higher at 2, 3, and 6 mo if the donor were a sibling compared with an unrelated donor. Low sIL-7R was associated with any grade of aGVHD at 2 and 6 mo. Patients with cytomegalovirus reactivation had increased IL-7 values at 2 and 3 mo after HSCT [62]. IL-21 exacerbated xenogeneic GVHD and resulted in rapid fatality. As early as 6 d after hPBMCs transplanted to BRG mice, a marked expansion of hCD19 B cells was observed in spleen of IL-21-treated mice. The inhibition of IL-21 signaling was a good therapy to reduce GVHD by impairing T cell functions. Compared with the control group, IL-21 induced robust Ig secretion, which was accompanied by increased accumulation of CD19+CD38high plasma cells in spleen [63]. IL-22, which produced by Th22, Th1, and Th17 cells, plays a pathogenic role in the GVHD process. The potential mechanism of IL-22 in GVHD may attribute to increased alloreactive effector Th1 and decreased inhibitory Treg. Compared with mice cotransferring with bone marrow and spleen cells without IL-22 administration, more serious tissue damage and higher GVHD clinical score were observed in IL-22 mice. IL-22 administration was a benefit to early recovery of thymus after irradiation-induced injury. Administration of IL-22 could promote Th1 cell expansion in mesenteric lymph nodes but reduce Treg number. Levels of systemic inflammatory cytokines (IFN-γ and TNF-α) were up-regulated, whereas the level of immunosuppressive cytokine IL-10 was down-regulated in recipients with IL-22 injection [64].
Regulatory cytokine suppresses GVHD

IL-10 is a regulatory cytokine with important roles during GVHD. Host and donor B cell-derived IL-10 provides a unique mechanism of suppression of aGVHD. With the use of IL-10−/− donor or host mice (BALB/c or C57BL/6, respectively) in an MHC-mismatched model for aGVHD, a strongly aggravated course of the disease with increased mortality when donor or host cells could not produce this cytokine was found. A lack of IL-10 resulted in increased alloimmune T cell responses and enhanced activation of host DCs in spleen and mesenteric lymph nodes. Remarkably, IL-10 was prominently produced by host- and donor-derived CD5int CD1dint T cell Ig and mucin domain 1int B cells in this disease, and consistent with this, allogeneic HSCT resulted in exacerbated GVHD when mice lacking IL-10 expression in B cells were used as donor or host compared with controls [65].

SURFACE MOLECULES ON IMMUNE CELLS INVOLVED IN GVHD

Costimulatory molecules play an important role in inducing aGVHD

ICOS, a member of the CD28 family of costimulatory molecules, is induced on activated CD4 and CD8 T cells. As an essential immune regulator, ICOS plays an important role in inducing aGVHD. ICOS expression was up-regulated significantly on T cells in dogs undergoing eGVHD [66]. ICOS potentiates TCR-mediated PI3K activation and intracellular calcium mobilization. ICOSSF T cells, which selectively lost the ability to activate PI3K, caused less-severe GVHD compared with ICOS wild-type T cells [67]. SOCS are regarded as pivotal negative-feedback regulators of cytokine signals released by T cells and could attenuate the activities of effector T cells (e.g., Th1, Th2, and Th17) in innate and adaptive immunity [27]. SOCS are also key regulators of aGVHD pathology via a cytokine storm and act to enhance Th1 cell activation [68]. Of particular note, SOCS genes have well-documented, therapeutic effects and are therefore, promising candidates for the treatment of hematologic malignancies, such as leukemia and solid-organ transplantation [69]. SOCS family containing SH2 contains 8 members (cytokine-inducible SH2 and SOCS1–7), each of which has a central SH2 domain, an N-terminal domain of variable length and sequence, and a 40-aa C-terminal module called the SOCS box. SOCS1 and -3 are differentially expressed in recipients following alloimmune HSCT, suggesting a prognostic correlation between SOCS genes and the development of GVHD. The level of SOCS1 was decreased in recipients with grades II–IV aGVHD and cGVHD compared with normal donors and non-GVHD recipients, and the expression of SOCS1 was more decreased significantly in cGVHD than in aGVHD recipients. In contrast, SOCS3 expression was similarly reduced in all of the recipients [2].

CD molecules are implicated in inducing aGVHD

CD26 is an activation marker of hCD4 T cells and is associated with T cell signal transduction processes as a costimulatory molecule. CD26 T cells are accumulated in inflamed tissues, such as rheumatoid synovitis and autoimmune thyroiditis. Accumulation of CD26 T cells in GVHD target organs was found. High expression of the CD26 cell on CD4 T cells is correlated with the production of Th1 cytokines, whereas CD26 Th cells stimulate antibody synthesis in B cells. CD26high CD8 T cells belong to the early effector memory T cell subset, and CD26-mediated costimulation of CD8 T cells exerts a cytotoxic effect preferentially via granzyme B, TNF-α, IFN-γ, and FasL. Therefore, the targeting of CD26 in T cells has the potential to be useful in studies of immune responses to new vaccine candidates, as well as serving as innovative therapy for immune-mediated diseases [70]. With intraperitoneal injection of hPBMC into nonobese diabetic SCID/γc−/− mice, the mice exhibited the onset of GVHD symptoms associated with the presence of CD26high human lymphocytes in peripheral blood and GVHD target tissues. Administration of humanized anti-hCD26 mAb decreased GVHD severity and prolonged survival in hPBL-nucleotide-binding oligomerization domain/Shi-scid/IL-2Rγnull mice without loss of engraftment of human T cells, whereas increasing doses of CTLA-4g fusion protein diminished engraftment of human lymphocytes [71].

CD83, which is expressed in activated lymphocytes and DCs, is regarded as a marker of mature DCs. CD83 belongs to the Ig superfamily and is a highly glycosylated type I transmembrane glycoprotein. Studies in CD83−/− mice reveal that CD83 is essential for thymic maturation, peripheral function, and longevity of CD4 T cells. CD83 is also involved in B cell maturation, peripheral B cell function, and homeostasis [72]. As a therapeutic target, scCD83 molecules or antibodies have been demonstrated to have therapeutic effects against GVHD in preclinical models. Transplant treatment with rsCD83 attenuates innate and adaptive immune responses and leads to the prevention of chronic rejection in a rat renal transplant model [73, 74].

The common γ-chain (CD132) is a subunit of the ILCs for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. Levels of these cytokines were shown to be increased in the serum of patients developing aGVHD and cGVHD. Inhibition of CD132 could have a profound effect on GVHD. Anti-CD132 mAb reduced aGVHD potently with respect to survival, production of TNF, IFN-γ and IL-6, and GVHD histopathology. Anti-CD132 mAb afforded protection in CD8 T cells, whereas exposure of CD8 T cells to IL-2, IL-7, IL-15, and IL-21 increased granzyme B production. Furthermore, T cells exposed to anti-CD132 mAb displayed a more naive phenotype and showed reduced JAK3 phosphorylation. Additionally, anti-CD132 mAb treatment of established cGVHD reversed liver and lung fibrosis and pulmonary dysfunction characteristic of bronchiolitis obliterans [75].

Significantly higher levels of sFasL and EMPs were detected in the patients at the time of aGVHD attack. In addition, sFasL exhibited significant elevation, but EMPs exhibited simultaneously decrease within the early phase (+21 d) after allo-HSCT in the pre-aGVHD group compared with the non-aGVHD group. At +21 d, there was a significant difference of sFasL and EMPs between the pre-aGVHD group and the non-aGVHD group. EMPs expressed higher Fas antigen [Fas(+)/EMP] in the patients with aGVHD. EMPs may play protective roles through Fas in endothelial cell damage of aGVHD after allo-HSCT. This
suggested that the monitoring of EMPs and sFasL in the early phase after HSCT may be useful for early diagnosis and forecast of aGVHD, and it also provided new clues in understanding the pathogenesis of endothelial cell injury during aGVHD after allo-HSCT [76].

**PRRs are critically involved in the pathogenesis of GVHD**

There is growing evidence that microbes and innate PRRs, such as TLRs and nucleotide-binding oligomerization domain-like receptors, are critically involved in the pathogenesis of aGVHD. Intensive chemotherapy and/or total-body irradiation during pretransplant conditioning results in tissue damage and a loss of epithelial barrier function. Subsequent translocation of bacterial components as well as release of endogenous danger molecules stimulate PRRs of host APCs to trigger the production of proinflammatory cytokines that modulate T cell alloreactivity against host tissues but eventually, also benefit the GVL effect [77]. Minor mismatch GVHD required MyD88-mediated TLR4 signaling on donor cells, and intestinal damage could be bypassed by parenteral LPS administration, indicating a critical role for the influx of bacterial components triggered by intestinal barrier loss. The severity of minor mismatch GVHD was diminished following transfer of MyD88−/− donor cells. These results provide further support for the therapeutic use of antibiotics or possibly, TLR4 antagonists in HSCT/bone marrow transplant to reduce GVHD severity [57].

**The complement system regulates T cell activation and alloimmune responses in GVHD**

Complement system C3 mediates Th1/Th17 polarization in human T cell activation and skin GVHD in patients. C3 deposition was detected in squamous epithelium and dermis, blood vessels, and damaged sweat glands and was associated with gland damage and regeneration. Mice deficient in C3 have significantly lower GVHD-related mortality/morbidity. The frequency of IFN-γ (Th1)-, IL-4 (Th2)-, IL-17 (Th17)-, IL-2, and TNF-α-producing cells was reduced significantly among activated CD4 T cells in the presence of the C3 inhibitor [78]. C3aR and C5aR drive Th1 maturation. The targeting of C3aR/C3aR and C5aR/C5aR interactions could facilitate iTreg-mediated tolerance to alloantigens. Genetic deficiency or pharmacological blockade of C3aR/C5aR signaling augments iTreg generation, stabilizes Foxp3 expression, resists iTreg conversion to IFN-γ/TNF-α-producing effector T cells, and as a consequence, limits GVHD [79].

**Protein kinases in the signaling pathway have an important role in transmitting signals from a variety of cell surface receptors**

Syk is expressed in most hematopoietic cells, fibroblasts, and endothelial cells. Syk is a protein tyrosine kinase that has an important role in transmitting signals from a variety of cell surface receptors. Allogeneic bone marrow transplantation increased Syk phosphorylation in T, B, and CD11b+ cells [83]. Activated JAKs are required for T effector cell responses in different inflammatory diseases. Inhibition of JAK1/2 signaling resulted in reduced proliferation of effector T cells and suppression of proinflammatory cytokine production in response to alloantigen in mice. Ruxolitinib, which is JAK1/2 inhibitor, represents a novel targeted approach in GVHD by promotion of tolerogenic Treg [84].

**Transcription factors play an essential role in GVHD**

Transcription factors of the Rel/NF-κB family are known to play different roles in immunity and inflammation. c-Rel plays an essential role in the induction of aGVHD. c-Rel−/− T cells have a dramatically reduced ability to cause aGVHD after allogeneic bone marrow transplantation using major and minor histocompatibility mismatched murine models and had a reduced ability to expand in lymphoid organs and to infiltrate in GVHD target
organs. In addition, c-Rel−/− T cells were defective in the differentiation into Th1 cells after encountering alloantigens but were enhanced in the differentiation toward Foxp3+ Treg. Furthermore, c-Rel−/− T cells had largely preserved activity to mediate the GVL response [85]. Inhibition of c-Rel activity reduced alloactivation without compromising antigen-specific cytotoxicity of T cells [86].

CONCLUDING REMARKS

In summary, APCs (DCs and macrophages), effector T cells (Th1, Th2, Th17, and abnormal Th17:Threg ratio), B cells, and NK cells are implicated in GVHD physiopathology. Proinflammatory cytokines (IL-17, IL-1β, and TNF-α) are increased in GVHD. Costimulatory molecules play an important role in inducing aGVHD. PRRs and complement system C3 mediate Th1/Th17 polarization in human T cell activation and skin GVHD, and signaling molecules promote the inflammatory and immune process of GVHD. The physiopathology process of GVHD involved in immune cells and molecules is complex and includes 3 phases (shown as Fig. 1). In the first phase, the conditioning regimen leads to damage of host tissues, especially the intestinal mucosa, and microbial products translocate from the intestinal lumen to the circulation, which can stimulate and activate APCs to secrete proinflammatory cytokines, such as IL-1 and TNF-α. These proinflammatory cytokines increase the expression of MHC and adhesion molecules on host cells, enhancing APC antigen-presenting capacity. The second phase is characterized by activation of donor T cells. Donor T cell activation further increases the expression of MHC and adhesion molecules, chemokines, and the expansion of host-specific cytotoxic CD8 T, CD4 T, and B cells. In the final step, these effector cells then migrate to the target organs and mediate tissue injury, which leads to multiorgan failure. These immune cells and molecules could be predictors of GVHD development, and the scientific advances on the role of the immune cells and molecules in the pathogenesis involved in GVHD and clinical investigation have led to an improved understanding of the physiopathology of GVHD and provided more effective therapeutic strategies for GVHD.

AUTHORSHIP

L.Z. designed the study. All authors contributed to data preparation and drafting and revising the manuscript.

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The authors disclose no conflicts of interest.

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