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Immunomodulation and pharmacological strategies in the treatment of graft-versus-host disease

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Abstract

Background—Allogeneic hematopoietic stem cell transplantation offers great promise for the treatment of a variety of diseases including malignancies and other diseases of hematopoietic origin. However, morbidity and mortality due to graft-versus-host disease (GVHD) remains a major barrier to its application.

Objective—This review will provide an overview of the pathophysiology of GVHD and discuss the recent advances in GVHD management in both preclinical and clinical studies.

Methods—An extensive literature search on PubMed from 1995 to 2008 was performed.

Results/conclusion—There has been much progress in our understanding of GVHD and finding new means to control acute GVHD. While these approaches hold promise, as of yet, none have yet to replace the standard methods that we may use routinely to decrease the incidence of GVHD.

Keywords

Allogeneic hematopoietic stem cell transplantation; graft-versus-host disease

Allogeneic hematopoietic stem cell transplantation (HSCT) has developed very quickly since its first application in the 1960s. Now approximately 8000 allogeneic HSCT procedures are performed annually in the United States [1]. Allo HSCT offers great promise for the treatment of a variety of diseases ranging from leukemia and aplastic anemia to other diseases of hematopoietic origin. Allogeneic HSCT is especially advantageous in cancer patients because it can offer graft-versus-tumor (GVT) effects. However, its wide application is hampered by graft-versus-host disease (GVHD), in which donor immune cells attack the immunocompromised recipient cells resulting in multi-organ damage. GVHD may occur in acute or chronic form. Acute GVHD usually manifests in 20 to 40 days after allogeneic HSCT [1], however it can also occur in days or as late as 2 months following transplantation [2]. Chronic GVHD has a later onset, usually after 100 days following transplantation, and may develop as an extension of acute GVHD, or *de novo*. The morbidity of GVHD is increasing, which may be due in part to the increased use of unrelated or mismatched donors and the rising age of transplant recipients [3]. In this review, we will mainly discuss the approaches for the prevention and treatment of acute GVHD.

Incidence/symptoms of acute GVHD

Acute GVHD mainly affects the skin, gastrointestinal tract, lung and liver. The stage of GVHD is determined by a system that quantifies the extent of skin involvement (rash), diarrhea, and serum bilirubin level, ranging from grade I for minimal to grade IV for severe disease [3].

Despite prophylaxis, acute GVHD (grade II–IV) occurs in 30–60% of patients after allogeneic HSCT from HLA-identical sibling donors [4]. Its incidence increases when grafts come from HLA-matched unrelated or HLA-mismatched donors, and when recipients are older or positive for cytomegalovirus (CMV). The mortality from acute GVHD can reach to as high as 50% [4].

Pathophysiology of acute GVHD

The occurrence of acute GVHD can be conceptualized into three sequential phases: (1) effects of conditioning regimens in inducing proinflammatory cytokines as well as immune suppression in the recipient; (2) donor T cell activation; and (3) the effector phase resulting in organ attack [5]. Conditioning regimens to reduce tumor burden and prepare the recipient for the graft usually include total body irradiation (TBI) and/or chemotherapy. Underlying disease, infection in a host and conditioning, can all damage the host and result in the secretion of proinflammatory cytokines, such as TNF- α , IL-1, and IL-6. The presence of these inflammatory cytokines increases the expression of adhesion molecules, costimulatory molecules and MHC in the host. These cytokines help the recipient's dendritic cells to mature and then activate donor T cells. Increased risk of GVHD associated with advanced stage leukemia, intensive conditioning regimens and histories of viral infections are consistent with the theory that the milieu from the damaged host tissues promotes an alloreaction. The second phase of acute GVHD involves activation and proliferation of alloreactive donor T cells. In this phase, donor T cells that can recognize MHC or/and minor histocompatibility antigen differences (miHA) will be activated and then proliferate and secrete some proinflammatory cytokines. The central role of host antigen-presenting cells (APCs) in donor T cell activation has been demonstrated by murine models. Host APCs are required and sufficient for GVHD occurrence [6•], while donor APCs are required for maximal GVHD [7].

The effector phase of acute GVHD is mediated by cellular effectors (primarily cytotoxic T lymphocytes) and inflammatory cytokines such as TNF- α , IL-1 and IL-6. Proinflammatory cytokines play a pivotal role in the pathophysiology of acute GVHD. This has been demonstrated in a murine model in which allogeneic donor T cells were transplanted into either MHC class I or MHC class II deficient bone marrow chimeric mice transplanted with syngeneic wild type bone marrow cells [8•]. In CD4⁺ T cell-mediated GVHD, GVHD did not require epithelial MHC class II expression and the neutralization of the inflammatory cytokines, TNF- α and IL-1 β , significantly prevented acute GVHD. In CD8⁺ T cell-mediated GVHD, MHC class I absence on host epithelium significantly delayed the onset of acute GVHD mortality, but induced equally severe target organ damage. Similarly, the neutralization of the inflammatory cytokines, TNF- α and IL-1 β , prevented acute GVHD [8•]. TNF- α may be implicated in the occurrence of GVHD in several ways. TNF- α activates APCs and promotes donor T cell activation [2]. TNF- α also recruits effector T cells and monocytes into target organs through the induction of adhesion molecules and chemokines [2]. Finally, TNF- α causes direct organ and tissue damage by inducing apoptosis and necrosis [2].

Prevention and treatment of acute GVHD in murine models

1. Molecular targeting of intracellular signaling pathways

The proteasome plays a critical role in the degradation of proteins including inhibitory kappa B (I κ B) which binds and inhibits NF κ B activity. NF κ B plays a critical role in T cell biology, particularly with respect to cytokine responses. Thus proteasome inhibition may indirectly result in suppression of NF κ B activity. One proteasome inhibitor, bortezomib, has been shown to have direct anti-tumor effects and has been approved for the treatment of multiple myeloma [9]. We have found that bortezomib can also sensitize tumor cells to immune mediated killing [10]. We also demonstrated that bortezomib given immediately after allogeneic HSCT in mice can reduce acute GVHD lethality [11•]. Bortezomib can inhibit alloreactive T cell proliferation and directly induce alloreactive T cell apoptosis *in vitro* and *in vivo*, and decrease proinflammatory cytokine production [11•]. Importantly, GVT effects were preserved in advanced tumor-bearing recipients. Clinical studies evaluating the efficacy of bortezomib in allogeneic HSCT in multiple myeloma patients are currently underway.

The peroxisome proliferator-activated receptor (PPAR), a member of the nuclear hormone receptor superfamily, acts as ligand-sensitive transcription factor and regulates gene transcription by binding as a heterodimer with retinoid X receptors to specific response elements (PPREs) in the promoter regions of target genes [12]. A new synthetic triterpenoid, 2-cyano-3,12-dioxolean-1,9-dien -28-oic acid (CDDO), is a ligand of PPAR γ and has been found to have anti-tumor functions through PPAR γ -dependent and -independent pathways [13,14]. CDDO also can inhibit nitric oxide production in mouse macrophages and prevent ileitis by inducing TGF- β production by intraepithelial lymphocytes [15]. We have recently demonstrated that CDDO given immediately after allogeneic HSCT in mice protected the mice from acute GVHD [16]. CDDO can inhibit alloreactive T cell proliferation and directly induce alloreactive T cell apoptosis *in vitro* and decrease proinflammatory cytokine TNF- α production *in vivo*, which is associated with protection from GVHD [16].

Histone deacetylase (HDAC) removes acetyl groups from histone tails, causing the DNA to wrap more tightly around the histones and interfering with the transcription of genes by blocking access of transcription factors. Suberoylanide hydroxamic acid (SAHA) is a HDAC inhibitor that has been demonstrated to inhibit tumor cell growth [17]. It has recently been approved by FDA for the treatment of advanced primary cutaneous T cell lymphoma (CTCL) [18]. SAHA has also been found to be capable of inhibiting the production of proinflammatory cytokines IL-1, TNF- α , IFN- γ and IL-12 *in vivo* [19]. Based on these biological functions, Reddy and colleagues assessed the effects of SAHA on GVHD and GVT in a murine model. The administration of SAHA resulted in significant protection from GVHD in two different murine HSCT models. Although a reduction of proinflammatory cytokines was observed, no direct inhibition of T cell responses was found and GVT was spared [20•].

Janus kinase 3 (JAK3) plays a pivotal role in cytokine signaling of T cells. Mutations that abrogate JAK3 function result in severe combined immunodeficiency (SCID). WHI-P131/JANEX-1, an inhibitor of JAK3, was found to significantly increase mouse survival from lethal GVHD when administered after allogeneic HSCT, and what is more, GVT effects were maintained [21].

2. Blockade of cytokine pathways

Proinflammatory cytokines play a pivotal role in the pathophysiology of acute GVHD. Thus, there is much interest in blocking cytokines to control GVHD. Blocking TNF- α and IL-2 pathways has been applied clinically, which will be discussed in the section of the treatment of clinical GVHD.

IL-7 is required for T and B cell development in mice and also highly modulates mature T cell function. Thus it is no surprise that there is much interest in targeting the IL-7 pathway to prevent GVHD. In a lethally irradiated MHC-haploidentical GVHD model, *in vivo* administration of recombinant human IL-7 (5 µg/day intraperitoneally for 28 days following HSCT) was shown to worsen GVHD [22]. In a lethally irradiated full MHC-mismatched GVHD model, the administration of anti-IL-7Rα antibody significantly decreased GVHD-related morbidity and mortality [23]. In a similar model, this research group found that no evidence of GVHD was detected in IL-7-deficient host mice and adding IL-7 back significantly increased GVHD-related mortality and morbidity [24]. However, the contrary result was also found. Alpdogan et al found that administration of IL-7 after allogeneic HSCT in lethally irradiated MHC-haploidentical or MHC-matched GVHD models improved immune reconstitution without aggravating GVHD [25]. They found that activated and memory alloreactive donor-derived T cells from recipients of allogeneic HSCT expressed little IL-7R and thought that this might explain the failure of IL-7 administration to exacerbate GVHD. Different doses, timing and routes of IL-7 administration might explain the differences of these results. Further studies need to be performed in order to resolve that IL-7 ameliorates or exacerbates GVHD.

3. Protection of epithelial cells in GVHD target organs

Keratinocyte growth factor (KGF) can stimulate epithelial cell proliferation and then increase the thickness of the epithelial cell layers. Thus, KGF may contribute to epithelial cell rich target organs (*i.e.* the gastrointestinal tract) to better withstand both conditioning and GVHD-mediated destruction. In murine GVHD models, KGF was found to alleviate the tissue injury from GVHD, particularly gastrointestinal damage, and also modulate T cell responses [26, 27]. KGF was shown to also promote thymic recovery due to the effects on thymic epithelial cells which were damaged by conditioning regimens and GVHD [28]. Hepatocyte growth factor has also been shown to reduce GVHD, probably by similar mechanisms to KGF [29, 30]. Likewise, IL-11 has been found to be able to decrease GVHD morbidity and mortality by protection of the small bowel, besides by polarization of donor T cells to Th2 and suppression of inflammatory cytokines such as TNF-α [31].

4. Blockade of costimulatory pathways of T cell activation

Given the indispensable role of costimulatory signals in T cell activation, blocking these signals has been a subject of intense investigation for GVHD control. The blockade of B7/CD28 interaction by anti-CD28 mAbs or anti-B7 mAbs inhibited donor T cell expansion and alleviated GVHD lethality [32–34]. The blockade of CD154 (CD40 ligand)-CD40 pathway by anti-CD154 mAbs alleviated GVHD mediated by CD4⁺ T cells, but not GVHD mediated by only CD8⁺ T cells [35]. Similarly, the blockade of CD134 (OX40)-CD134L (CD134 ligand) interaction by anti-CD134L mAbs ameliorated GVHD-mediated lethality in mice [36]. The administration of anti-CD137L mAbs to block CD137 (4-1BB)-CD137L interaction was found to significantly reduce GVHD-mediated morbidity and mortality by impairing donor CD8⁺ T cell expansion and IFNγ production without significant effects on CD4⁺ T cell expansion [37].

Other costimulatory signals have also been examined for roles in GVHD pathophysiology recently. Inducible costimulator (ICOS) is expressed on activated CD4⁺ and CD8⁺ T cells and the ligand for ICOS (ICOS-L) is constitutively expressed on B cells, macrophages, and dendritic cells and is up-regulated on some nonlymphoid tissues by TNF-α or LPS. The blockade of ICOS/ICOS-L interaction by using ICOS^{-/-} mice or anti-ICOS mAbs has been shown to reduce CD4⁺ and CD8⁺ T cell-mediated GVHD under lethal irradiation conditions [38]. Delayed ICOS blockade until day 5 post-HSCT, a time in which donor T cell activation has already occurred, significantly prolonged GVHD survival, similar to the increased survival

derived from the blockade of ICOS starting the day prior to transplant [38]. Thus, ICOS/ICOS-L blockade might be an especially attractive target for downregulating T cell responses in GVHD once it has been initiated.

LIGHT (Lymphotoxin-like, exhibits Inducible expression, and competes with HSV Glycoprotein D for HVEM, a receptor expressed by T lymphocytes) is a TNF superfamily member that is expressed on activated T cells and immature dendritic cells. It has three receptors, including the herpes virus entry mediator (HVEM), the lymphotoxin β receptor (LT β R) and the soluble decoy receptor 3 (DcR3). LIGHT-HVEM interaction is thought to be a costimulatory signal for T cell activation [39]. The blockade of LIGHT by administration of soluble receptors or antibodies was found to be capable of ameliorating GVHD [39].

5. Blockade of the migration of donor alloreactive T cells to GVHD target organs

T cell migration is a complex process involving chemokines and adhesion molecules. It may be possible that by selectively targeting certain chemokines/adhesion molecules involved in T cell trafficking to particular GVHD organs, GVHD can be alleviated without significant impairment of GVT effects. In a murine GVHD model in which no cytoreductive conditioning was applied to evaluate the role of CCR5 in GVHD, a blocking antibody to CCR5 resulted in significant alleviation of liver GVHD [40]. In contrast, in models in which lethal conditioning was applied, donor T cells from CCR5^{-/-} mice resulted in more severe GVHD than wild type donor T cells [41,42]. Intensive conditioning resulted in CXCL10 and CXCL11 most highly expressed in tissues of irradiated recipients during the first week post-transplant and CCR5^{-/-} T cells had enhanced migration to CXCL10, and blocking this ligand *in vivo* improved survival in irradiated recipients receiving CCR5^{-/-} T cells [42]. Thus, cytoreductive conditioning appears to dramatically affect GVHD induction and progression. Other chemokines have also been evaluated for roles in GVHD. Allogeneic donor CXCR3^{-/-} T cells infused into lethally irradiated recipients resulted in fewer donor T cells in the host gut and liver, although more in the spleen, resulting in less overall GVHD lethality [43]. CCL5^{-/-} donor T cells infused into lethally irradiated MHC-haploidentical recipients resulted in less severe GVHD-induced lung injury than wild type donor T cells [44]. CCL3^{-/-} donor T cells infused into the mice conditioned with both CsA and lethal irradiation resulted in accelerated lung lesion [45]. In lethally irradiated parent to F1 models, CCR2^{-/-} donor cells resulted in reduced lung injury as compared to wild type donor cells and the neutralization of MCP-1 significantly decreased lung injury compared with control-treated host mice [46]. In another lethally irradiated parent to F1 model, CCR2^{-/-} donor CD8⁺ T cells resulted in a reduction in overall GVHD morbidity and mortality due to less migration in host gut and liver than wild type CD8⁺ T cells [47]. Interestingly, the GVT effect mediated by CCR2^{-/-} CD8⁺ T cells was maintained, which suggests that interference with CD8⁺ T-cell trafficking by the blockade of CCR2 pathway can separate GVHD from GVT activity [47]. Similar to CCR2^{-/-} T cells, in a lethally irradiated parent to F1 model, CCR1^{-/-} donor cells significantly reduced systemic and target organ GVHD severity as compared to wild type donor cells, and GVT activity was preserved, but the survival advantage decreased with increasing tumor burden. This was because although cytolytic function was maintained on a per-cell basis, T cell proliferation and IFN- γ secretion of CCR1^{-/-} T cells were significantly reduced [48]. Similar to CCR1 and CCR2^{-/-} T cells, the infusion of CCR6^{-/-} donor T cells also resulted in less morbidity and mortality of GVHD as compared to wild type T cells [49].

Adhesion molecules which play a role in T cell trafficking have also been studied as a potential target in GVHD prevention and treatment. *In vivo* administration of anti- α 4 integrin mAbs resulted in protection from gastrointestinal GVHD in a murine parent to F1 GVHD model [50]. MHC-mismatched splenocytes incubated with both mAbs to L-selectin and α 4-integrin resulted in delayed GVHD induction by reducing donor T cell homing to lymph nodes in SCID

mice without conditioning [51]. LPAM ($\alpha_4\beta_7$ integrin) is responsible for T cell homing to gut-associated lymphoid tissues by binding the mucosal addressin cellular adhesion molecule (MAdCAM). LPAM^{-/-} donor T cells resulted in significantly less severe gut GVHD as compared to wild type T cells, and importantly, GVT responses were preserved [52].

FTY720 is a sphingosine-1-phosphate receptor modulator capable of trapping T cells in secondary lymphoid organs [53]. It has been shown that FTY720 administration can inhibit GVHD without compromising GVT in a murine HSCT model [53]. This is further confirmed in a recent study in which FTY720 was found to be capable of reducing splenic CD11c⁺ cells by 50% besides trapping T cells in secondary lymphoid organs [54]. In another recent study, FTY720 was shown to facilitate a rapid contraction of the donor T cell pool in association with an increased degree of apoptosis of donor T cells by the sequestration of donor T cells in lymph nodes, and thereby resulted in a reduction in GVHD [55].

6. Cellular therapies to prevent GVHD

6.1 T regulatory cells (Tregs)—CD4⁺CD25⁺ Treg cells have potent suppressor activity *in vitro* and *in vivo*. Donor or host Treg cell depletion accelerates GVHD and Treg cell infusion inhibits acute GVHD lethality [56–58]. *In vitro* expanded and activated Tregs using polyclonal stimulators (anti-CD3 mAbs) or recipient APCs are more effective on GVHD inhibition than freshly isolated Tregs [57]. Another advantage of *in vitro* expansion prior to infusion is to increase the number of Tregs that are available for infusion. And importantly, in most instances, Treg cell infusion has preserved GVL effects [58,59]. The impact of Tregs on T-cell development and immunity following allogeneic HSCT has also been explored [58,60]. Tregs have been found to enhance immune reconstitution by preventing GVHD-induced damage of the thymic and secondary lymphoid microenvironment [60].

6.2 NK and NKT cells—Adoptive transfer of activated donor-type NK cells have been demonstrated in preclinical models to inhibit GVHD and promote GVT [61••]. TGF- β , an immunosuppressive cytokine, was at least partially responsible for the suppression of alloreactivity in this model [61••]. Recent studies have also suggested that donor NK cells can attack host dendritic cells which have been shown to play a pivotal role in initiating GVHD [6,62••].

NKT cells are a heterogeneous group of lymphocytes with markers present on both NK and T cells. The majority of murine NKT cells bear TCRs that are far more limited in diversity than those on conventional T cells and recognize glycolipid antigens presented by CD1d expressed on APCs. NKT cells have been found to inhibit GVHD through the ability to polarize donor T cells toward Th2 cytokine production after allogeneic HSCT [63]. The researchers activated host NKT cells by the injection of α -galactoceramide (α -GalCer) after allogeneic HSCT. As a result, significant skewing of donor T cells to Th2-type cytokine production (IL-4) occurred and the inhibition of GVHD ensued, suggesting that adoptive transfer of NKT cells or simply their activation may suppress GVHD [63]. In the study by Haraguchi et al, the adoptive transfer of invariant NKT cells from donor or host mice significantly prolonged the survival of GVHD mice [64].

6.3 Mesenchymal stem cells (MSCs)—Mesenchymal stem cells (MSCs) are an adherent, CD34⁺ and CD45⁺ cell population that can differentiate into a wide-spectrum of cells. MSCs can inhibit T cell proliferation induced either in a MLR or by nonspecific mitogens [65]. MSCs have been shown to be capable of alleviating acute GVHD in murine GVHD models [66,67]. However, there are also reports showing that MSCs had no effects on GVHD prevention in murine models [68]. This may be due to different doses and timing of MSC infusion, or different MSC populations and protocols used.

Clinical prevention of acute GVHD

The choice of the donor is one of the most important variables for the prevention of GVHD. The use of a young related HLA-matched, sex-matched donor can minimize GVHD severity. With limitations of the donor pool it is often necessary to utilize HLA-matched unrelated donors.

Post-transplant immunosuppressive therapy is the most common form of GVHD prevention [69]. In general, the regimen is based on the combination of cyclosporine A (CsA; 1mg/kg) and a short course of methotrexate (MTX) [69]. Higher doses of CsA are more effective at controlling GVHD, but an increased frequency of leukemia relapse ensues due to strong immunosuppression.

The acrolide tacrolimus (FK506) is an alternative to CsA in GVHD prevention. FK506 can inhibit IL-2 production and T cell activation by forming a complex with FK binding protein-12 to block the serine-threonine phosphatase activity of calcineurin [70]. In a phase III clinical trial involving HLA-identical sibling HSCT, the efficacy of FK506 and MTX for prophylaxis of acute GVHD was compared with that of CsA and MTX [71]. The incidence of grade II–IV acute GVHD was significantly lower in patients who received FK506 than patients in the CsA group (31.9% versus 44.4%, $P=0.01$). But the incidence of grade III–IV acute GVHD was similar in both groups (17.1% versus 13.3%). There was no difference in the incidence of chronic GVHD in both groups, but the development of severe chronic GVHD was more common with CsA. The tumor relapse rates of the two groups were similar. In another phase III clinical study involving HSCT from unrelated donors, the incidence of grade III–V acute GVHD in the FK506 group was also significantly lower than in the CsA group (56% versus 74%, $P<0.01$) [72]. The incidence of chronic GVHD was similar in both groups. Also, adverse events and the incidence of leukemia relapse were similar in both groups.

Mycophenolate mofetil (MMF) is a novel immunosuppressive drug that shows promise in the prevention and treatment of GVHD. Orally administered MMF is metabolized to release mycophenolic acid (MPA), which is a potent, selective, noncompetitive inhibitor of the type 2 isoform of inosine monophosphate dehydroxygenase (IMPDH) expressed in activated T and B lymphocytes. By inhibiting IMPDH, MPA depletes the pool of dGTP required for DNA synthesis [73]. A randomized trial compared CsA + MMF with CsA + MTX on GVHD prophylaxis in HLA-matched sibling myeloablative HSCT [74]. The group receiving MMF ($n=21$) showed significantly less severe mucositis than the group receiving MTX ($n=19$) (21% vs. 65%), and the median time for neutrophil engraftment was shorter in the MMF group (11 vs. 18 days). There were no significant differences in the incidence of acute GVHD and 100 day survival between the groups. A phase I/II trial performed in a different transplant center confirmed that CsA + MMF regimen had similar efficacy to CsA + MTX for GVHD prophylaxis [75].

Preclinical animal studies have demonstrated that repeated total lymphoid irradiation can increase the frequency of NKT cells in mice [76]. This population is associated with regulatory functions and is a primary source of host T cell derived IL-4 [76]. In a clinical trial reported in 2005, Lowsky et al applied a nonmyeloablative conditioning regimen consisting of 10 doses of total lymphoid irradiation (80 cGy each) plus antithymocyte globulin (ATG) to 37 patients with hematopoietic malignancies prior to a HLA-matched transplant from either related or unrelated donor [77]. A lower incidence of acute GVHD was seen in this study compared to historical controls. In addition, the GVT effects of an allogeneic transplant appeared to be retained with this regimen. While peripheral blood NKT cells were not assessed in most patients post-transplant, the investigators did observe an increase in interleukin-4 production in donor CD4⁺ T cells after in vitro stimulation and a reduction in the allogeneic proliferation in vitro,

as compared with normal control subjects and control subjects who underwent conditioning with a single dose of 200 cGy total-body irradiation.

Clinical treatment of acute GVHD

1. First-line treatment

Corticosteroids are the current standard of care for first-line therapy of acute GVHD [1]. In a study of 443 patients with acute GVHD who received prednisone (60mg/m² for 14 days) as first-line therapy, then followed by an 8-week taper, the overall response was observed in 55% of the patients [78]. Generally, primary treatment of acute GVHD is prednisone or methylprednisone (2mg/kg/d, IV) for 5 or 7 days [69]. The dose is then tapered in responding patients starting on day 7 [69]. A 5-day course of corticosteroids is sufficient to identify steroid-refractory acute GVHD [69]. Nonresponders should receive second-line therapy [1,69].

2. Second-line treatment

The patients who do not respond to first-line steroid therapy are generally treated with higher-dose steroids [69]. If a response is observed in 3 to 5 days, the dose is decreased to 2mg/kg/day and the patients are treated in a way same as individuals who response to low-dose steroid therapy [1]. However, less than 60% of steroid-refractory patients responded well to second-line treatment [79].

2.1 Anti-thymocyte globulin (ATG)—In a clinical study evaluating the responses of 79 HSCT patients treated with equine ATG in addition to prednisone (or methylprednisolone), plus CSA (75%) or tacrolimus (4%), for steroid-resistant acute GVHD, ATG was found to improve survival when giving early after the diagnosis of steroid-refractory acute GVHD [78]. Khoury reviewed the results of treatment with ATG of 58 patients with steroid-resistant acute GVHD at two institutions [80]. Skin GVHD improved well with ATG (79%), while progression of gut and liver GVHD was observed in 40% and 66% of patients, respectively. Despite initial improvement, 52 patients (90%) died in a median of 40 days after ATG therapy from progressive GVHD and/or infection (74%), adult respiratory distress syndrome (15%), or relapse (11%). Only six patients (10%), three of whom had GVHD limited to the skin at the time when ATG was administered, were long-term survivors. They concluded that this treatment did not significantly improve the survival and was associated with a high rate of major complications. A prospective randomized multicenter trial analyzed the efficacy of 6-methyl-prednisone (5mg/kg/d for 10 days) alone or in combination with rabbit ATG (6.25mg/kg/d in 10 days) for control of steroid-refractory acute GVHD [81]. There was no significant difference in response, transplant related mortality, and survival between the non-ATG and ATG groups.

2.2 Interleukin 2 receptor antibodies—Daclizumab (Zenapax) is a humanized IgG1 mAb that binds specifically to the alpha subunit (CD25) of the human interleukin-2 (IL-2) receptor, which is expressed on activated lymphocytes, and inhibits IL-2 binding. Daclizumab has been shown to have efficacy in steroid-refractory acute GVHD, but appears to be associated with an increased incidence of infectious complications [82,83]. The effect of Daclizumab when combined with steroids for the treatment of acute GVHD has been studied in a multicenter, double-blind, randomized study [84]. The combination therapy did not show any benefit over a steroid only regimen for acute GVHD. In fact, long term survival was worse.

Denileukin diftitox (Ontak®), a recombinant protein composed of human IL-2 fused to diphtheria toxin, has selective cytotoxicity against activated lymphocytes expressing CD25, the high-affinity IL-2 receptor [85]. Denileukin diftitox has been approved by the US Food

and Drug Administration for the treatment of relapsed or refractory cutaneous T-cell lymphoma [86]. It has been showed to have activity on steroid-refractory acute GVHD [85,87].

Inolimomab is a murine anti-human mAb directed against CD25. A retrospective study about the safety and efficacy of inolimomab on GVHD patients showed that it had activity on steroid-refractory acute GVHD [88]. Another retrospective study of 40 steroid-refractory acute GVHD patients also showed that inolimomab was an effective salvage therapy for patients with steroid-refractory GVHD, particularly for those without gastrointestinal involvement [89]. The median overall survival time was 294 days for the antibody responders vs. 14 days for the nonresponders, with one year probability of 59% vs. 0% for overall survival.

Basiliximab is a chimeric mAb that binds to CD25 with different pharmacokinetic features from daclizumab. Massenkeil et al evaluated the safety and efficacy of basiliximab in 17 patients with steroid-refractory acute GVHD after HSCT [90]. None of the patients had infusion-associated or cytokine-related side-effects after basiliximab. Twelve of 17 patients (71%) responded to basiliximab, 9/17 (53%) had a complete response (CR) of acute GVHD and 3/17 (18%) had a partial response (PR). In a prospective phase II trial, basiliximab has also been shown to have efficacy on steroid-refractory acute GVHD [91]. In another study involving 34 patients with refractory acute GVHD (grade III–IV), complete responses to basiliximab were seen in 27/32 patients (84%) with skin, 12/25 (48%) with gut and 6/23 (26%) with liver GVHD [92].

Unfortunately, the effects of targeting IL-2 may also have effects on Tregs as well as the activated T cells so caution must be exercised when applying in GVHD [93].

2.3 TNF- α antibodies—Infliximab is a chimeric anti-TNF- α mAb that binds the soluble subunit and the membrane-bound precursor of TNF- α . In a study involving four patients with severe steroid-refractory acute GVHD (grade III–IV), infliximab appeared to have activity on acute GVHD, in that three of four patients had a complete resolution of diarrhea and significant improvement of skin and liver disease [94]. In a retrospective analysis evaluating the activity of infliximab in 32 patients with severe steroid-refractory acute GVHD, infliximab was found active, particularly when the intestine was involved, but it increased the risk of infections [95]. Couriel et al arrived at the same conclusion in their retrospective study on infliximab use in 21 patients with steroid-refractory acute GVHD [96]. The efficacy and safety of infliximab in children with steroid-resistant GVHD was also retrospectively evaluated [97]. The overall response rate in 22 evaluable children was 82%. However, long-term outcome was poor, and GVHD recurred commonly upon discontinuation of infliximab. Within 100 days of the final infliximab dose, 77% of patients had bacterial infections, 32% had viral infections and 13.6% had probable or proven non-candidal invasive fungal infections.

Etanercept is a recombinant human soluble TNF- α receptor fusion protein that inhibits TNF- α . The safety and efficacy of etanercept therapy in 21 patients with steroid-refractory acute GVHD were evaluated, and etanercept was found well tolerated and can induce a high response rate in patients with steroid-refractory GVHD, particularly in the setting of gastrointestinal involvement [98]. However, CMV reactivation occurred in 48% of patients, bacterial infections in 14% of patients, and fungal infections in 19% of patients.

2.4 Anti-CD147 mAb—Human CD147 is a member of the immunoglobulin superfamily and expresses weakly on human leukocytes, granulocytes, red blood cells, and several other cell types [99]. Upon activation, CD147 is up-regulated on T and B lymphocytes. ABX-CBL is a murine IgM mAb that recognizes CD147 on the cell surface and initiates cell killing through complement-mediated lysis [99]. Deeg et al reported a pilot study of the use of ABX-CBL on steroid-refractory acute GVHD [99]. Among 51 patients evaluable for efficacy, 26(51%)

responded to anti-CD147 treatment, including 13 with complete responses (CR) and 13 with partial responses (PR). At 6 months after the initiation of ABX-CBL therapy, 26 (44%) patients survived [99]. Recently, a prospective, multicenter, open-label, randomized clinical trial comparing ABX-CBL with ATG for treatment of steroid-resistant acute GVHD was conducted in 95 patients at 21 centers [100]. Forty-eight patients received ABX-CBL daily for 14 consecutive days followed by up to 6 weeks of ABX-CBL twice weekly, and Forty-seven patients received equine ATG, 30 mg/kg every other day for a total of 6 doses with additional courses as needed. By day 180, overall improvement was similar in both groups (56% vs. 57%). Patient survival at 18 months was less favorable on ABX-CBL than on ATG (35% vs. 45%). The results from this trial suggested that ABX-CBL is not superior to ATG in the treatment of acute steroid-resistant GVHD.

2.5 Extracorporeal photopheresis/photochemotherapy (ECP)—ECP is a leukapheresis-based immunomodulatory method. The lymphocytes are mixed with 8-methoxypsoralen which intercalates into the DNA of the lymphocytes. This makes them susceptible to apoptosis upon exposure to ultraviolet radiation. The modified cells are then infused back to the patients [1]. Greinix et al reported a prospective pilot study on the use of ECP on 21 patients with steroid-refractory acute GVHD after HSCT from siblings or unrelated donors [101]. Three months after initiation of treatment, 60% of patients achieved a complete response. Complete responses were obtained in 100%, 67% and 12% of patients with grade II, grade III and grade IV acute GVHD, respectively. Three months after start of treatment, complete responses were obtained in 60%, 67% and 0% of patients with cutaneous, liver and gut involvement, respectively. Fifty-five percent of patients were alive at a median observation time of 25 months after HSCT. Recently, Greinix et al have reported their Phase II study results on 59 patients: Complete responses of GVHD were obtained in 82% of patients with cutaneous involvement, 61% with liver involvement, and 61% with gut involvement. The probability of long-term survival in complete responders was 59% as compared to 11% in patients not responding completely [102].

2.6 Mesenchymal stem cells (MSCs)—Le Blanc et al reported a case in which GVHD symptoms of a 9 year-old boy with severe steroid-refractory GVHD were significantly improved and survival was prolonged after treatment with MSCs from his mother [103]. Recently, they reported their further study on the use of MSCs in eight patients with steroid-refractory grades III–IV GVHD [104]. Acute GVHD disappeared completely in six of eight patients. Complete resolution was seen in intestinal GVHD in all 6 responding patients as well as resolution of liver and skin symptoms in 1 patient each. The survival rate of patients with gastrointestinal GVHD treated with MSCs was significantly better than that of 16 patients with steroid-resistant gastrointestinal GVHD, not treated with MSCs during the same period.

Conclusions

There has been much progress in our understanding of GVHD and development of means to control this complication. However, although these approaches hold promise, so far none of them has been able to be standard methods that we may use routinely to decrease the incidence of GVHD in the future. The more we know about basic GVHD processes and T cell biology, the more possibility we can find good approaches to control GVHD.

Expert opinions

Donor-type NK cells have been demonstrated in murine models to inhibit GVHD and promote GVT [61••]. In human transplants, alloreactive donor NK cells have also been found to be able to eliminate leukemia relapse and protect patients against GVHD [62••]. In a clinical study evaluating adoptive transfer of donor NK cells with killing immunoglobulin-like receptor (KIR)

mismatches from hosts after allogeneic HSCT, anti-tumor effects increased without increased GVHD [105]. These studies suggest that NK cells may be of use to prevent GVHD and promote GVT responses in tumor-bearing HSCT patients. NK cells have inhibitory and activating receptors specific for MHC class I or other molecules (KIR in humans, Ly49 in mice, and NKG2D in both), which affect their responses. Subsets of NK cells are characterized by the expression of these inhibitory and/or stimulatory receptors. Further characterization and exploitation of NK cell subsets may contribute to augmentation of these protective effects.

Recently, a subset of CD4⁺ T cells (Th17) noted for their production of IL-17 has been characterized. Th17 T cells have been implicated as pathogenic in mouse models of autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE) and collagen-induced arthritis [106–108]. IL-17 knockout mice were resistant to both EAE and collagen-induced arthritis [106,107]. Also, the mice with EAE had increased numbers of Th17 cells but were resistant to disease if given neutralizing IL-17 antibodies [108]. IL-17 has been shown to induce production of TNF- α and other proinflammatory cytokines and result in an inflammatory pathology in target tissues [109,110]. Proinflammatory cytokines play a pivotal role in the occurrence of acute GVHD. Does IL-17 play a role in the pathophysiology of acute GVHD? So far, the roles of IL-17 and Th17 cells have not been well characterized in acute GVHD, although Chen et al showed that donor-derived Th17 cells emerged during acute GVHD [111]. If IL-17 and Th17 cells are demonstrated to play an important role in the pathophysiology of GVHD, they will become new potential therapeutic targets for GVHD prevention and treatment.

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References

- = of interest,
 - = of considerable interest
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Abbreviations

(GVHD), Graft-versus-host disease; (HSCT), Hematopoietic stem cell transplantation; (GVT), Graft-versus-tumor; (TBI), Total body irradiation; (APCs), Antigen-presenting cells; (SCID), Severe combined immunodeficiency.