EXPERT REVIEWS

Current and future approaches for control of graft-versus-host disease

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Graft-versus-host disease (GVHD), both acute and chronic, remains one of the major barriers to improving outcomes after allogeneic stem cell transplantation. The pathophysiology of GVHD is complex and incompletely understood. GVHD is believed to arise from the interaction of: tissue damage and proinflammatory cytokines causing activation of antigen-presenting cells (APCs, donor T-cell activation by APCs and cytokines and host tissue injury by effector T lymphocytes and proinflammatory cytokines. There is also a role for additional lymphocyte subtypes (naive and memory T cells, regulatory T cells, natural killer T cells and B cells) in GVHD pathogenesis. Strategies to improve donor–recipient HLA match, and to minimize conditioning toxicity, cytokine release and APC and effector T-lymphocyte activation, will likely improve prophylaxis of acute (and possibly chronic) GVHD. Therapy of established acute and chronic GVHD is still heavily dependent on corticosteroids, despite their limited efficacy and considerable toxicity. Novel agents (and/or combinations of agents) comprising pharmacologic, biologic and cellular therapies targeting specific steps or subsets involved in immune activation will likely comprise future advances in GVHD control. This article reviews the current state of knowledge regarding the prevention and treatment of acute and chronic GVHD. Novel approaches currently undergoing evaluation are also highlighted.

KEYWORDS: allogeneic stem cell transplantation • graft-versus-host disease

Allogeneic stem cell transplantation (alloSCT) is often the only curative option for patients with various hematologic and/or immune disorders, particularly those with aggressive or advanced hematologic malignancies. However, the toxicity of alloSCT remains a significant barrier to its wider utilization. Graft-versus-host disease (GVHD) remains the most frequent complication after alloSCT.

Clinically, GVHD was categorized as 'acute' and 'chronic' based on time of presentation. Any GVHD before day 100 was known as 'acute', and after day 100 it was known as 'chronic'. The severity of GVHD was graded: acute GVHD was categorized as grade I–IV by modified Glucksberg criteria (A–D by the International Bone Marrow Transplant Registry index) (TABLE 1) [1,2]; chronic GVHD was commonly categorized as limited or extensive [3]. Based on these criteria, grade II–IV acute GVHD is thought to occur in approximately 35% of recipients of matched, related donor transplants, and in up to 50% of unrelated or alternative donor transplant recipients. Chronic GVHD can affect up to 60% of recipients who survive beyond 100 days after matched donor alloSCT.

While the simplicity of the day 100 definition is appealing, it is irrelevant biologically and clinically. For instance, in patients receiving reduced intensity conditioning (RIC) alloSCT, or after donor lymphocyte infusion (DLI), clinical acute GVHD may develop months after the procedure [4]. Hence, there is a current attempt by the National Institutes of Health chronic GVHD consensus project working group to reclassify acute GVHD into classic acute and late-onset acute; and chronic GHVD into classic chronic and overlap syndrome [5]. Classic acute GVHD is characterized by a maculopapular erythematous skin rash, gastrointestinal symptoms or cholestatic hepatic abnormalities occurring within 100 days of alloSCT or DLI, while late acute GVHD presents similarly beyond 100 days after alloSCT or DLI. Classic chronic GVHD consists solely of manifestations ascribable to chronic GVHD (without acute GVHD features) (TABLE 2), while overlap syndrome has clinical features of both acute and chronic GVHD occurring together.

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Table 1. Modified Glucksberg criteria for acute graft-versus-host disease grading.

Organ stage	Skin*	Liver	Gut
1	Rash < 25%	Bilirubin 2-2.9 mg/dl	Diarrhea 500-1000cc/d or biopsy-proven upper GI involvement
2	Rash 25-50%	Bilirubin 3-6 mg/dl	Diarrhea 1000-1500cc/d
3	Rash > 50%	Bilirubin 6.1-15 mg/dl	Diarrhea 1500-2000cc/d
4	Generalized erythroderma with bullae	Bilirubin > 15 mg/dl	Diarrhea > 2000 cc/d or severe abdominal pain with or without ileus
Overall grade			
I	Stage 1 or 2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	-	Stage 2 or 3 or	Stage 2–4
IV	Stage 4 or	Stage 4	

*Use 'rule of nines' to determine body surface area.
Data from [1].

Risk factors for GVHD

The risk factors for GVHD include:

- Donor–recipient match at the major histocompatibility complex (MHC) loci, for instance, HLA class I (HLA-A, -B and -C) and class II (HLA-DR, -DP and -DQ). Mismatches at HLA-A, -B, -C or -DRB1 (and possibly also -DQ and -DP) increase the risk of GVHD (nonpermissive donor–recipient HLA mismatches may particularly influence GVHD severity) and negatively impact survival [6–10];
- Donor stem cell source: compared with bone marrow stem cells, peripheral blood stem cells (PBSCs) have a higher GVHD risk, while umbilical cord blood cells appear to have a lower risk [11–14];
- T-cell dose: compared with T-cell replete grafts, 2–3 log depletion of $CD3^+$ T lymphocytes of the graft can effectively reduce acute GVHD incidence (although the effect on chronic GVHD is less clear), while less-intensive log reductions of T cells have no significant impact [15,16]. However, the benefit of T-cell depletion is counteracted by increased risks of graft failure, opportunistic infection and disease relapse such that pan-T-cell depletion strategies are not currently favored [17];
- Additional risk factors include donor and recipient age, donor–recipient sex mismatch (female donor to male recipient), donor parity and allosensitization, disease stage and intensity of conditioning (for acute GVHD). Acute GVHD is a powerful predictor of chronic GVHD risk [18].

Measures to reduce GVHD risk would therefore include improvements in donor selection, improved HLA matching, as well as reduced intensity conditioning where possible. However, other trends, such as the increased use of donor PBSCs as a source of stem cells, extending alloSCT to older/sicker patients and the use of alternative donors (haploidentical and HLA-mismatched donors), suggest that GVHD control will remain a significant issue for the foreseeable future.

Etiopathogenesis of GVHD

The etiology of GVHD is complex, but Billingham's criteria still apply [19]. First, the graft must contain immunologically competent cells (T lymphocytes and possibly B lymphocytes). Second, the recipient must be incapable of rejecting the transplanted cells (achieved by conditioning chemotherapy or radiation). Third, the recipient must express tissue antigens that are not present in the donor (major or minor histocompatibility mismatch).

Our current understanding of acute GVHD, although incomplete, is better than that of chronic GVHD. In part, this is due to the better availability of mouse models of acute GVHD. Broadly however, both forms of GVHD are believed to be caused by similar alloimmune responses that also underlie the beneficial graft-versus-leukemia (GVL) effect. Maintaining control of GVHD, while enabling the curative GVL response remains the holy grail of allotransplantation.

The development of acute GVHD is frequently divided into three phases (Figure 1):

- Tissue damage, owing to underlying disease, infections and conditioning regimen toxicity, resulting in leakage of bacterial lipopolysaccharides across the damaged gut epithelium and a 'cytokine storm' with the production of inflammatory cytokines, such as TNF-α, and IL-1 by injured cells, resulting in secondary changes in expression of adhesion molecules, MHC antigens and chemokines, which can act as danger signals and activate residual host and donor antigen-presenting cells (APCs) [20–24]. APC activation can occur via both Toll-like receptor (TLR) and non-TLR (e.g., nucleotide-binding oligomerization domain [NOD]) pathways [25,26];
- Donor T-cell activation, cytokine release, proliferation and tissue localization occurs in the context of the proinflammatory post-transplant milieu and after alloantigen presentation and costimulation by APCs (donor or host) [27–30];

Table 2. Definite and probable manifestations of chronic graft-versus-host disease.

Organ system	Definite manifestations of chronic GVHD	Possible manifestations of chronic GVHD
Skin	Scleroderma (superficial or fasciitis), lichen planus, vitiligo, scarring alopecia, hyperkeratosis pilaris, contractures from skin immobility, nail bed dysplasia	Eczematoid rash, dry skin, maculopapular rash, hair loss, hyperpigmentation
Mucous membranes	Lichen planus, noninfectious ulcers, corneal erosions/ noninfectious conjunctivitis	Xerostomia, keratoconjunctivitis sicca
GI tract	Esophageal strictures, steatorrhea	Anorexia, malabsorption, weight loss, diarrhea, abdominal pain
Liver	None	Elevation of alkaline phosphatase, transaminitis, cholangitis, hyperbilirubinemia
GU tract	Vaginal stricture, lichen planus	Noninfectious vaginitis, vaginal atrophy
Musculoskeletal/serosa	Nonseptic arthritis, myositis, myasthenia, polyserositis, contractures from joint immobilization	Arthralgia
Hematologic	None	Thrombocytopenia, eosinophilia, autoimmune cytopenias
Lung	Bronchiolitis obliterans	Bronchiolitis obliterans with organizing pneumonia, interstitial pneumonitis

GI: Gastrointestinal; GU: Genitourinary; GVHD: Graft-versus-host disease.

- The effector phase of GVHD target organ damage involves a complex interaction of cytokine and cellular effectors. Cytotoxic T lymphocytes (CTLs), both $CD4^+$ and $CD8^+$, are the major cellular effectors of GVHD and cause cell death by a variety of pathways, such as Fas–Fas ligand (FasL), TNF receptor (TNFR)-like death receptors (e.g., TRAIL and TWEAK) and perforin–granzyme [31–36]. Inflammatory cytokines, such as TNF-α and IL-1, synergize with CTLs and can also act directly to promote tissue injury and inflammation in GVHD target organs [37–40].

Based on their cytokine expression pattern, there are at least two types of T helper (Th) effector cells involved in GVHD: Th1 and Th2 cells. Th1 cells generate IL-2, TNF-α and IFN-γ, while Th2 cells produce IL-4 and IL-10. While the 'cytokine storm' phase of GVHD, which is amplified by Th1 cytokines, correlates with the development of acute GVHD, cytokines that polarize donor T cells to Th2 (e.g., granulocyte colony-stimulating factor [G-CSF], IL-4 and IL-18) can reduce acute GVHD [41–44]. However, this model may be an oversimplification, as Th1 and Th2 subsets can each cause injury to distinct GVHD target organs in some mouse models of acute GVHD [45]. Additional complexities involve possible roles for newly identified Th17 cells in GVHD and the interaction between Th17 effector cells and peripheral regulatory T cells (Tregs), given their alternate developmental fates from common naive precursor T cells [46–48].

Additionally, genetic polymorphisms that lead to altered cytokine expression levels (e.g., IL-6, IL-10 and TNF-α) have also been linked to differences in acute and chronic GVHD incidence [49–58]. Furthermore, polymorphisms involving natural killer (NK) cell receptor/ligand complex, collectively termed the killer immunoglobulin-like receptor family (KIR), have been linked to differences in both GVHD and relapse rates after alloSCT [59–61]. Similarly, polymorphisms in the non-TLR (NOD) pathway of adaptive immune activation can impact GVHD risk [62]. Genes involved in drug metabolism have also been linked to toxicity and GVHD after alloSCT [63,64]. Finally, genes with only indirect associations with immune activity have also been linked to GVHD [65–67]. Both donor and recipient polymorphisms are often relevant with regards to GVHD risk, as in the case of IL-10 [68].

There is increasing awareness of the role of additional cellular subsets in GVHD:

- Naive and memory T cells: naive ($CD62L^+$) T cells, but not memory ($CD62L^-$) T cells, are often considered to have alloreactive potential that can result in acute GVHD [69,70]. However, contrasting recent data also suggest a role for alloreactive memory T cells and their precursor stem cells in the development of GVHD [71,72];

- Tregs: $CD4^+CD25^+$ $FoxP3^+$ Tregs from the donor have been shown to suppress the expansion of alloreactive donor T cells and the development of GVHD, without abrogating GVL in this MHC-mismatched murine model [73]. IL-2, initially identified as a lymphocyte growth factor and thought primarily to promote effector T-cell responses *in vivo*, is now identified as a cytokine critical for the development, expansion and activity of Tregs [74,75]. In humans, FoxP3 mRNA levels (considered a relatively specific marker for Tregs) was significantly decreased in patients with GVHD [76,77]. The expression of the cell surface marker CD62L was also found to be critical for the ability of donor Tregs to control GVHD [78,79];

- NK T cells: host NKT cells also have immune suppressive effects that can control GVHD in an IL-4-dependent fashion [80,81]. Human clinical data suggest that enhancing recipient

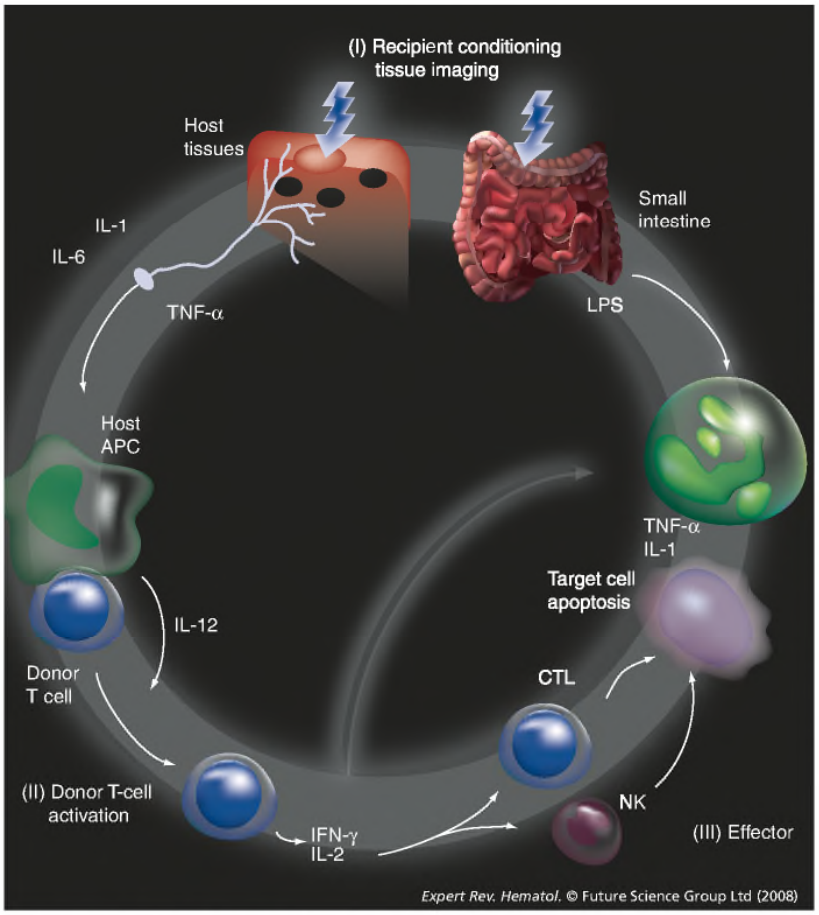

Figure 1. Etiopathogenesis of acute graft-versus-host disease.
Modified with permission from [245].

NKT cells by total lymphoid irradiation (TLI) in conjunction with anti-thymocyte globulin-based conditioning similarly promoted Th2 polarization and significantly reduced GVHD [82]. However, it is important to note that NKT cells are heterogeneous and their roles in GVHD are incompletely understood;

- B cells: traditionally, a major role for B cells and humoral immunity in the development of GVHD has not been considered. However, recent work suggests that, in the context of matched sibling PBSC allotransplantation, the concentration of $CD20^+$ B cells in the apheresis product may predict the development of acute GVHD [83]. Additionally, auto- and alloantibodies have been described in chronic GVHD, some of which may play a direct role in disease progression (e.g., activating PDGF receptor antibodies) [84–87]. High circulating levels of B-cell activation factor at 6-months post-transplant were a predictor of subsequent chronic GVHD, further supporting a role for B-cell dysfunction in chronic GVHD [88]. The role of humoral immunity in GVHD remains an area of controversy and further investigation.

Prophylaxis of GVHD

Pilot studies omitting GVHD prophylaxis indicated an acute GVHD incidence of nearly 100% [89]. Studies using methotrexate as a single agent for GVHD prophylaxis via inhibition of rapidly dividing alloreactive T cells, indicated an acute grade II–IV GVHD rate of over 50%, even in the setting of HLA-matched sibling donors [90]. The introduction of a calcineurin inhibitor, cyclosporine (and subsequently tacrolimus), represented the next advance in the prevention of GVHD, with improved efficacy in GVHD control compared with methotrexate [91–93]. Cyclosporine and tacrolimus bound to cyclophilin or FK-binding protein 12 (FKBP12), respectively, inhibit calcineurin (a protein phosphatase that is calcium- and calmodulin-dependent) and prevent the dephosphorylation and nuclear translocation of the transcription factor nuclear factor of activated T cells (NFAT). By blocking NFAT, one of the most important regulators of cytokine gene transcription following T-cell activation, calcineurin inhibitors block T-cell activation and proliferation [94,95]. The combination of calcineurin inhibitor (cyclosporine) and methotrexate was more effective than either agent alone, with grade II–IV acute GVHD rates of 20–56% after HLA-matched sibling alloSCT [96,97]. Compared with cyclosporine, tacrolimus has an improved toxicity profile and, more importantly, randomized data indicate improved acute GVHD prophylaxis in both HLA-matched siblings and unrelated donor allotransplants [98,99]. The length of immunosuppressive therapy appears to have no role in improving control of chronic GVHD. Patients with acute GVHD or biopsy evidence of subclinical acute GVHD were randomly assigned to 6 versus 24 months of cyclosporine therapy. The rates of clinical extensive chronic GVHD were 39 and 51%, respectively, a nonsignificant difference [100]. Similarly, the presence or absence of day 11 methotrexate does not likely impact chronic GVHD rates [101,102].

Corticosteroids, the mainstay of therapy for established acute GVHD, do not have a significant role in GVHD prophylaxis. Various trials compared prednisone and cyclosporine to the three-drug combination of methotrexate, cyclosporine and prednisone. In one large trial, the acute GVHD rate in the cyclosporine and prednisone control arm was 23%, compared with only 9% in the three-drug arm of methotrexate, cyclosporine and prednisone [103]. However, subsequent trials could not demonstrate similarly improved GVHD control, or improved long-term outcomes with the three-drug combination, and, currently, steroids are not routinely used in GVHD prophylaxis [104].

Cyclophosphamide has been used post-transplant since the 1980s for GVHD prevention and act via inhibition of rapidly dividing T cells (in a manner similar to methotrexate) [105]. Stem cells contain high levels of aldehyde dehydrogenase that converts the active metabolite 4-hydroxycyclophosphamide to an inactive nonalkylating metabolite, thus protecting the stem cell from the antiproliferative activity of the agent. Similarly, the gut epithelium has high levels of aldehyde dehydrogenase that is protective against excess mucosal toxicity despite prior intensive conditioning. Used as a single agent after myeloablative conditioning in related and unrelated allotransplants, the grade II–IV acute GVHD rate was 41%, with few late infections, attributed to the brief duration of immune suppressive therapy [106]. It is also currently being evaluated for alternative donor transplants (haploidentical donor) [107].

Mycophenolate mofetil (MMF) is a potent, selective, noncompetitive reversible inhibitor of inosine monophosphate dehydrogenase that inhibits the *de novo* pathway of guanosine nucleotide synthesis. It has potent cytostatic effects on lymphocytes (both T and B) whose proliferation is dependent on *de novo* purine synthesis. With good oral bioavailability, the optimal dosing interval remains uncertain, usually two- to three-times daily. It has been used for GVHD prophylaxis in various combinations (usually with a calcineurin inhibitor ± methotrexate). The incidence of grade II–IV acute GVHD has ranged between 38 and 62% [108,109]. In a single-center randomized study, the combination of cyclosporine plus MMF was associated with faster engraftment and reduced mucositis incidence, but with similar incidence of acute and chronic GVHD and survival comparable to cyclosporine plus methotrexate, possibly affected by limited sample size and follow-up duration for these secondary end points [110]. Longer-term use of cyclosporine in combination with MMF after RIC alloSCT with matched related donors did not impact the rates of acute grade II–IV or chronic GVHD [111].

Sirolimus (also called rapamycin) binds uniquely to FKBP12 and forms a complex with mammalian target of rapamycin (mTOR) that interacts with various upstream pathways including PTEN/PI3 kinase/Akt pathway and the Janus kinase pathway [112,113]. The sirolimus–mTOR complex inhibits several biochemical pathways, resulting in reduction of DNA transcription/translation, protein synthesis and cell cycle progression, which results in T-cell immunosuppression [114,115]. Interestingly, there is apparent differential inhibition of T-cell subsets, possibly involving selective inhibition of Th1 cell responses, and sparing of Th2 and Treg activity [116–120]. Despite theoretical concerns for competition for FKBP binding with calcineurin inhibitors, these agents appear to work synergistically, and sirolimus does not interact with calcineurin or its downstream effectors [112]. In contrast to calcineurin inhibitors, sirolimus may also exert its immunosuppressive effects through suppression of APC activity via a reduction in antigen uptake, cellular processing, intracellular signaling and induction of apoptosis [121–123]. The combination of sirolimus and tacrolimus appears more effective than sirolimus plus cyclosporine in reducing alloreactive memory T-cell production, abrogation of effector CTL induction and apoptosis induction [124]. Single-institution clinical studies of sirolimus and tacrolimus with and without low-dose methotrexate for GVHD prophylaxis after myeloablative conditioning with cyclophosphamide/total-body irradiation (TBI) indicate excellent efficacy and acceptable toxicity in the matched related and unrelated donor context, with grade II–IV acute GVHD rates of 19 and 23%, respectively [125]. The rates of chronic GVHD, however, were not significantly impacted. Similar efficacy in acute GVHD control was noted despite omitting low-dose methotrexate, and toxicity was further reduced [126]. Similar low-acute GVHD rates were also noted in the context of RIC. Other recent single-institution reports indicate concordant as well as variant estimates of sirolimus efficacy for GVHD prophylaxis in the myeloablative alloSCT context [127,128]. Sirolimus plus tacrolimus is currently being evaluated in a Phase III multi-institution context in comparison to methotrexate plus tacrolimus.

Biologic agents have also been evaluated for GVHD prophylaxis. *In vivo* T-cell depletion with horse- or rabbit-derived polyclonal antithymocyte globulin (ATG) has been evaluated for prevention of GVHD, as initially proposed by Ramsey *et al.* [129]. Such agents administered pre- and peritransplant can simultaneously target host and donor T cells to control both graft rejection and GVHD [130–132]. However, additional cellular components, such as B cells, NK cells and APCs, can also be affected by polyspecific antibodies. Their use does appear to reduce the incidence of chronic GVHD and chronic lung dysfunction, with improved late transplant-related mortality [133]. Whether the reduction in chronic GVHD is also associated with increased disease relapse remains to be determined. Higher doses of rabbit ATG (thymoglobulin) are associated with increased infections that can abrogate its positive impact on GVHD [134]. TLI in conjunction with ATG-based conditioning also significantly reduced GVHD [82].

Monoclonal antibodies, such as alemtuzumab (Campath-1H; anti-CD52), are widely used for *in vivo* GVHD prophylaxis. It has been found to reduce GVHD and nonrelapse mortality after related and unrelated transplants, and can also facilitate engraftment [135]. Monoclonal antibodies targeting the IL-2 receptor (CD25) may also show benefit [136]. However, IL-2 is also critical for Treg development, expansion and activity, hence IL-2 targeting in GVHD may have the unintended consequence of impairing Tregs that are important to control GVHD [74,75]. Low-dose IL-2 is currently being evaluated for GVHD prophylaxis. Some biologic agents that may have activity in established active GVHD, such as IL-1 antagonists and ricin-conjugated CD5 antibody, do not show benefit in the prophylactic setting [137–141]. Interestingly, rituximab, a monoclonal CD20 antibody that depletes B cells, may independently decrease acute GVHD risk [142]. It is also being evaluated for the prophylaxis of chronic GVHD.

In vitro T-cell depletion (TCD) has also been attempted to control GVHD, with some success in controlling acute (and possibly chronic) GVHD. However, in a randomized study comparing GVHD prophylaxis with approximately 1-log TCD (with monoclonal antibody T10B9 targeting the T-cell receptor) plus

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