Pathogenesis and Management of Graft-versus-Host Disease

Sung W. Choi, MD[a,*], John E. Levine, MD[b], James L.M. Ferrara, MD, DSc[c]

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- Acute graft-versus-host disease
- Chronic graft-versus-host disease
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Allogeneic hematopoietic cell transplantation (HCT) is an important therapeutic option for various malignant and nonmalignant conditions.[1] The indication for its use has expanded, especially among older patients, in recent years through novel strategies using donor leukocyte infusions, nonmyeloablative conditioning and umbilical cord blood (UCB) transplantation.[2] As allogeneic HCT continues to increase, with more than 20,000 allogeneic transplantations performed annually worldwide, greater attention is given to improvements in supportive care, infectious prophylaxis, immunosuppressive medications, and DNA-based tissue typing. Despite advances, graft-versus-host disease (GVHD) remains the most frequent and serious complication following allogeneic HCT and limits the broader application of this important therapy.[3] GVHD can be considered an exaggerated manifestation of a normal inflammatory mechanism in which donor lymphocytes encounter foreign antigens in a milieu that fosters inflammation. In the context of hematological malignancies, a delicate balance exists between the harmful consequences of GVHD and the beneficial effects incurred

Dr Choi is a St Baldrick's Career Development Scholar.
Dr Ferrara is an American Cancer Society Clinical Research Professor.
[a] Department of Pediatrics, Blood and Marrow Transplant Program, University of Michigan Medical School, 1500 E. Medical Center Drive, 6303 Comprehensive Cancer Center, Ann Arbor, MI 48109-5942, USA
[b] Department of Internal Medicine and Pediatrics, Blood and Marrow Transplant Program, University of Michigan Medical School, 1500 E. Medical Center Drive, 5303 Comprehensive Cancer Center, Ann Arbor, MI 48109-5941, USA
[c] Department of Internal Medicine and Pediatrics, Blood and Marrow Transplant Program, University of Michigan Medical School, 1500 E. Medical Center Drive, 6303 Comprehensive Cancer Center, Ann Arbor, MI 48109-5942, USA
* Corresponding author.
E-mail address: sungchoi@med.umich.edu (S.W. Choi).

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when donor lymphocytes attack recipient malignant cells, a process referred to as the graft-versus-leukemia/tumor (GVL) effect. Given the increasing number of transplant recipients, there will be an increasing population of patients with GVHD. Recent advances in the understanding of the pathogenesis of GVHD have led to new approaches to its management, including using it to preserve the GVL effect following allogeneic transplant. This article reviews the important elements in the complex immunologic interactions involving cytokine networks, chemokine gradients, and the direct mediators of cellular cytotoxicity that cause clinical GVHD, and discusses the risk factors and strategies for management of GVHD.

ACUTE GVHD

Epidemiology and Risk Factors

In 1966, Billingham[4] formulated three requirements for the development of GVHD: the graft must contain immunologically competent cells; the recipient must express tissue antigens that are not present in the transplant donor; and the recipient must be incapable of mounting an effective response to eliminate the transplanted cells. It is now known that T cells are the immunologically competent cells, and when tissues containing T cells (blood products, bone marrow [BM], and solid organs) are transferred from one person to another who is unable to eliminate those cells, GVHD can develop.[5,6]

Allogeneic HCT is the most common setting for the development of GVHD, in which recipients receive immunoablative chemotherapy or radiation before hematopoietic cell infusion containing donor T cells. GVHD ultimately develops when donor T cells respond to recipient tissue antigens secondary to mismatches between major or minor histocompatibility antigens between the donor and recipient. The major histocompatibility complex (MHC) contains the genes that encode tissue antigens. In humans, the MHC region lies on the short arm of chromosome 6 and is called the human leukocyte antigen (HLA) region.[7] Class I HLA (A, B, and C) proteins are expressed on almost all nucleated cells of the body at varying densities. Class II (DR, DQ, and DP) proteins are primarily expressed on hematopoietic cells (B cells, dendritic cells, monocytes, and activated T cells), but their expression can be induced on many other cell types following inflammation or injury. High-resolution DNA typing of HLA genes with polymerase chain reaction (PCR)-based techniques has now largely replaced earlier methods. The incidence of GVHD is directly related to HLA disparity[8,9] and with more HLA mismatches, the likelihood of developing GVHD increases.[10,11] Recent data from the National Marrow Donor Program (NMDP) suggest that high-resolution matching for HLA-A, -B, -C, and -DRBI (8/8 match) maximizes post transplant survival.[12,13]

Despite HLA identity between a patient and donor, the incidence of acute GVHD ranges from 26% to 32% in recipients of sibling donor grafts, and 42% to 52% in recipients of unrelated donor grafts (Center for International Blood and Marrow Transplant Research [CIBMTR] Progress Report January–December 2008). The incidence is likely related to genetic differences that lie outside the HLA loci, or ‘‘minor’’ histocompatibility antigens (HA), which are immunogenic peptides derived from polymorphic proteins presented on the cell surface by MHC molecules.[14] Some minor HAs, such as HY and HA-3, are expressed on all tissues and are targets for GVHD and GVL, whereas other minor HAs, such as HA-1 and HA-2, are expressed abundantly on hematopoietic cells (including leukemic cells) and may induce a greater GVL effect with less GVHD.[14,15] However, the precise elucidation of most human minor antigens remains to be accomplished.[14,16]

The impact of donor and recipient polymorphisms in cytokine genes with critical roles in the classic ''cytokine storm'' of GVHD has been examined as a risk factor for GVHD.[17] Various polymorphic genes, including tumor necrosis factor α (TNF-α), interleukin 10 (IL-10), and interferon-γ (IFN-γ) variants, have been associated with GVHD, although not always.[18–20] There is no unequivocal evidence that polymorphic genes for cytokines or other proteins involved in innate immunity [21–24] sufficiently influence GVHD and transplant outcome to change clinical practice. Nonetheless, future strategies to identify the best possible transplant donor will likely incorporate HLA and non-HLA genetic factors.

In addition to genetic factors, other risk factors which have been associated with the development of GVHD include older donor and recipient age,[25–28] multiparous female donor,[28,29] advanced malignant condition at transplantation,[9,29] donor type,[28] and donor hematopoeitic cell source.[30–32] In the last decade, there has been a shift in clinical practice from the use of intraoperative harvested BM to granulocyte colony-stimulating factor mobilized peripheral blood stem cells (PBSC) as the donor hematopoietic cell source. However, definitive data demonstrating long-term advantages of PBSC rather than BM are lacking. One meta-analysis found that acute and chronic GVHD are more common following peripheral blood stem cell transplant (PBSCT) compared with bone marrow transplant (BMT) and indicated a trend toward decreased relapse rate following PBSCT.[31] The relative risk (RR) for acute GVHD after PBSCT was 1.16 (95% confidence interval [CI], 1.04–1.28) compared with BMT; the RR for chronic GVHD after PBSCT was 1.53 (95% CI, 1.25–2.05); and the RR for relapse after PBSCT was 0.81 (95% CI, 0.62–1.05). Thus, the survival benefit of PBSC versus BM remains in question. A large prospective, randomized, multicenter clinical trial of PBSC versus BM in unrelated donor transplantation conducted through the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) has recently finished accrual.

For individuals without a suitable HLA-matched donor, UCB has become an alternative to BM or PBSC.[33–36] The incidence and severity of acute GVHD seem to be lower following UCB transplant than after HLA-matched marrow unrelated donor transplant, despite HLA disparities between the donor and recipient.[37,38] In an effort to meet the minimum cell dose required to ensure reliable engraftment, the simultaneous transplantation of 2 partially HLA-matched UCB units has been studied.[39] A recent report comparing transplantation with 2 partially HLA-matched UCB units versus a single unit demonstrated an increased incidence and earlier presentation of acute GVHD associated with the double UCB graft.[40]

Prevention

Prevention of acute GVHD is an integral component to the management of patients undergoing allogeneic HCT. The primary strategy employed is in the use of pharmacologic GVHD prophylaxis. The most widely used GVHD prophylaxis following full intensity conditioning includes a combination of a calcineurin inhibitor (eg, cyclosporine, tacrolimus) with methotrexate (MTX). This standard regimen was initially described in 1986[41] and since then several clinical trials have shown the superiority of this combination in reducing the incidence of GVHD and improving survival compared with either agent alone.[42–45] The calcineurin inhibitors cyclosporine and tacrolimus impede the function of the cytoplasmic enzyme calcineurin, which is critical to the activation of T cells. The most common side effects include hypomagnesemia, hyperkalemia, hypertension, and nephrotoxicity.[46] Large randomized studies comparing tacrolimus-MTX with cyclosporine-MTX have demonstrated a reduced incidence of grade II to IV acute GVHD with tacrolimus, but no overall survival advantage.[43,46]

Recently, sirolimus, a widely used immunosuppressant in solid organ transplantation,[47] has become attractive as a GVHD prophylactic agent because of the nonoverlapping toxicities with calcineurin inhibitors and the different mechanism of action. Sirolimus binds uniquely to FK binding protein 12 (FKBP12) and then complexes with mammalian Target of Rapamycin (mTOR).[48] Several studies have shown that the combination of sirolimus and tacrolimus has resulted in rapid engraftment, low incidence of acute GVHD, reduced transplant-related toxicity, and improved survival.[49] A prospective, randomized, multicenter trial is being conducted through the BMT CTN (protocol 0402) comparing sirolimus-tacrolimus versus tacrolimus-MTX following HLA-matched, related donor PBSCT.

A commonly used GVHD prophylaxis following reduced-intensity conditioning includes a combination of a calcineurin inhibitor (eg, cyclosporine, tacrolimus) with mycophenolate mofetil (MMF) instead of MTX. MMF, the prodrug of mycophenolic acid, selectively inhibits inosine monophosphate dehydrogenase, an enzyme critical to the de novo synthesis of guanosine nucleotide, which is needed for proliferation of T cells. In a prospective randomized trial, patients who received MMF as part of GVHD prophylaxis experienced significantly less severe mucositis and more rapid neutrophil engraftment than those who received MTX.[50] Although the optimal prophylaxis regimen following reduced-intensity HCT is not well established, MMF has been shown to be safe in this context.[51–55] MMF is also often preferred to MTX in UCB transplants because of its advantageous toxicity profile with respect to neutropenia and mucositis.

Many centers have previously attempted to decrease the risk of GVHD by ex vivo T cell depletion. Despite significant reductions in the incidence and severity of GVHD, T cell depletion has not achieved wide acceptance because of high rates of graft rejection, life-threatening infections, and leukemia relapse.[56–58] In vivo T cell depletion has also been widely studied using alemtuzumab, a monoclonal antibody specific for CD52 antigen expressed abundantly on the surface of normal and malignant lymphocytes,[59,60] or antithymocyte globulin (ATG), a polyclonal antibody mixture of either horse or rabbit origin directed against multiple epitopes of human T cells.[61] These approaches are associated with significant reduction in acute GVHD, but at the cost of impaired immune reconstitution and increased risk of leukemia relapse. Thus, the focus of most prevention strategies remains pharmacologic manipulation of T cells following transplant.

Pathophysiology

Acute GVHD is mediated by donor lymphocytes infused into the recipient, in whom they encounter profoundly damaged tissues from the effects of the underlying disease, prior infections, and the transplant conditioning regimen. The allogeneic donor cells encounter a foreign environment that has been altered to promote the activation and proliferation of inflammatory cells. Thus, acute GVHD reflects an exaggerated response of the normal inflammatory mechanisms that involves donor T cells and multiple innate and adaptive cells and mediators. Three sequential phases can be conceptualized to illustrate the complex cellular interactions and inflammatory cascades that ultimately evolve to acute GVHD: (1) activation of antigen-presenting cells (APCs); (2) donor T cell activation, proliferation, differentiation and migration; and (3) target tissue destruction.[62]

Phase 1: activation of APCs

In the first phase, APCs are activated by the underlying disease and the HCT conditioning regimen.[63] Animal models [63,64] and clinical HCT [65] have supported the

observation that increased risk of GVHD is associated with intensive conditioning regimens that contribute to extensive tissue injury and subsequent release of inflammatory cytokines. Damage to host tissues leads to the secretion of proinflammatory cytokines, such as TNF-α and IL-1, and chemokines, such as CCL2-5 and CXCL9-11, thereby producing increased expression of adhesion molecules, MHC antigens and costimulatory molecules on host APCs. For example, increase of plasma TNF-α receptor 1 levels, a surrogate marker for TNF-α, at 1 week after HCT strongly correlates with the later development of GVHD.[66] Systemic translocation of immunostimulatory microbial products, such as lipopolysaccharide (LPS), as a result of damage to the gastrointestinal (GI) tract induced by the conditioning regimen, enhances the activation of host APCs.[67,68] The initial site of interaction between activated APCs and donor T cells is likely the secondary lymphoid tissue in the GI tract.[69] Different distinct subsets of APCs, including host and donor type APCs,[68,70,71] dendritic cells,[72,73] Langerhans cells,[74] and monocytes/macrophages,[75] have been implicated in this phase. However, the relative contributions of these various APCs remain to be elucidated. The intensity of the conditioning regimen and the degree of tissue injury seem to be associated with the risk of GVHD. Reduced intensity conditioning regimens have thus become more widely employed in an effort to reduce acute GVHD by decreasing the damage to host tissues.[65,76]

Phase 2: donor T cell activation

Donor T cell activation, proliferation, differentiation, and migration in response to primed APCs occur during the second phase of acute GVHD. The T cell receptors (TCR) of donor T cells recognize alloantigens on host and donor type APCs that are present in secondary lymphoid organs.[77,78] During direct presentation, donor T cells recognize either the peptide bound to host MHC molecules, or the foreign MHC molecules themselves.[79] During indirect presentation, donor T cells respond to the peptides generated by degradation of the host MHC molecules that are presented on donor-derived MHC.[80]

Following antigen recognition, signaling through the TCR induces a conformational change in adhesion molecules, resulting in high affinity binding to the APC.[81] The complex interaction between T cell costimulatory molecules and their ligands on APCs facilitates full T cell activation. Many T cell costimulatory molecules display unique and overlapping functions.[82] Receptors of the B7 family and the TNF family play especially critical roles in GVHD, and are known to deliver positive and negative signals to T cells.[83] Blockade of costimulatory and inhibitory pathways can reduce acute GVHD in murine models, but this approach has not yet been tested in clinical trials.[2]

Murine studies have shown that control of alloreactive responses responsible for GVHD depends at least in part on CD4+ CD25+ regulatory T cells. Studies in mice suggest that regulatory T cells added to donor grafts can prevent or delay GVHD.[84] However, the role of regulatory T cells in clinical allogeneic HCT has not been well established, in part because of the lack of clear identification of human regulatory T cell phenotype. In contrast to murine studies, more severe acute GVHD developed clinically when donor grafts contained larger numbers of donor CD4+ CD25+ T cells.[85] One recent study found that HCT recipients with higher absolute numbers of FOXP3+ CD4+ T cells were associated with a reduced risk of developing GVHD.[86] However, FOXP3 expression in humans is not specific for regulatory T cell phenotype,[87] and improved techniques to identify and expand regulatory T cells are required for its wider application in clinical BMT.

Several intracellular biochemical pathways are rapidly amplified following T cell activation. Activated T cells secrete cytokines that are generally classified as Th1 (IFN-γ,

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