

Acute Graft Versus Host Disease: Pathophysiology, Risk Factors, and Prevention Strategies

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Abstract: Acute graft versus host disease (GVHD) remains the greatest complication of allogeneic bone marrow transplantation and a major cause of morbidity and mortality. This article summarizes the risk factors and prevention strategies for acute GVHD by considering them within the context of disease pathophysiology. Acute GVHD can be considered a 3-step process: 1) damage from chemotherapy/radiotherapy; 2) host antigen-presenting cell activation and amplification of donor T cells; and 3) target cell apoptosis via cellular and inflammatory mediators. This conceptual framework helps to explain the effectiveness of current prevention strategies and points to areas where new drugs and approaches may be of clinical benefit.

Pathophysiology

In order to appreciate the strategies to prevent acute graft versus host disease (GVHD), it helps to understand the pathophysiology of the disease, which can be considered as a 3-step process (Figure 1). These 3 steps are tissue damage to the recipient by the radiation/chemotherapy pretransplant conditioning regimen, donor T-cell activation and clonal expansion, and cellular and inflammatory factors. In the first step, the conditioning regimen (irradiation and/or chemotherapy) leads to damage and activation of host antigen presenting cells (APCs) by inflammatory cytokines. In step 2, host APCs present alloantigens to the resting T cells and activate them. Donor T-cell activation is characterized by cellular proliferation and the secretion of cytokines, including interleukin (IL)-2 and interferon- γ (INF- γ). In step 3, mononuclear phagocytes and neutrophils cause inflammation and are triggered by mediators such as lipopolysaccharides (LPS) that leak through the intestinal mucosa damaged during step 1. The inflammation recruits effector cells into target organs, amplifying local tissue injury with further secretion of an inflammatory cytokines response that, together with cytotoxic T lymphocytes (CTLs), leads to target tissue destruction.^{1,2}

Step 1: Effects of Hematopoietic Cell Transplantation Conditioning

The first step of acute GVHD starts before donor cells are infused. Prior to hematopoietic cell transplantation (HCT), a patient's tissues

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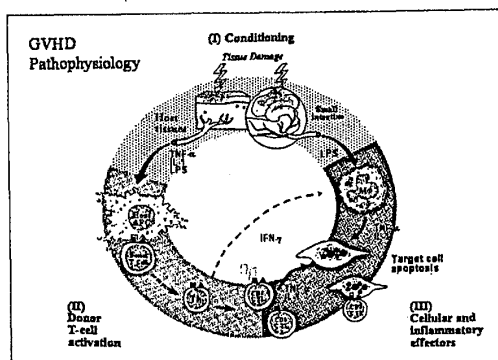


Figure 1. Graft-versus-host disease (GVHD) pathophysiology.

LPS = lipopolysaccharide; TNF = tumor necrosis factor.

have been damaged by a number of factors, including the underlying disease and its treatment, infection, and transplant conditioning. High-intensity chemoradiotherapy, characteristic of many HCT conditioning regimens, activates host APCs that are critical to the stimulation of donor T cells infused in the stem cell inoculum. Total body irradiation (TBI) is particularly important in this process because it activates host tissues to secrete inflammatory cytokines, such as tumor necrosis factor (TNF)- α and IL-1, and it induces endothelial apoptosis that leads to epithelial cell damage in the gastrointestinal (GI) tract.^{3,4} GVHD damage to the GI tract amplifies GVHD by allowing the translocation of microbial products such as LPS into systemic circulation. This scenario helps to explain the increased risk of GVHD associated with intensive conditioning regimens.⁵⁻⁷

Step 2: Donor T-Cell Activation and Cytokine Secretion

Murine studies have demonstrated that host APCs alone are both necessary and sufficient to stimulate donor T cells to proliferate as early as day 3 after HCT, preceding the engraftment of donor stem cells.⁸⁻¹⁰ Inflammatory cytokines and microbial products such as LPS may all be considered "danger signals"¹¹ that help to activate T cells and may make the difference between an immune response and tolerance.¹² When T cells are exposed to antigens in the presence of adjuvants such as LPS, the migration and survival of T cells are dramatically enhanced *in vivo*.¹³ The effect of advanced age in enhancing allostimulatory activity of host APCs may also help explain the increased incidence of acute GVHD in older recipients.¹⁴ The elimination of host APCs by activated natural killer

(NK) cells can prevent GVHD in experimental models.¹⁵ This suppressive effect of NK cells on GVHD has been confirmed in humans: human leukocyte antigen (HLA) class I differences driving donor NK-mediated alloreactions in the graft-versus-host direction mediate potent graft-versus-leukemia/lymphoma (GVL) effects and produce higher engraftment rates without causing severe, acute GVHD.^{15,16} Cytokines secreted by activated T cells are generally classified as Th1 (secreting IL-2 and INF- γ) or Th2 (secreting IL-4, IL-5, IL-10, and IL-13).¹⁷

Monoclonal antibodies (mAbs) against IL-2 or its receptor can prevent GVHD when administered shortly after the infusion of T cells,¹⁸⁻²⁰ but this strategy was only moderately successful in reducing established GVHD.^{21,22} Cyclosporine (CSP) and tacrolimus dramatically reduce IL-2 production and effectively prevent GVHD. IL-15 is another critical cytokine in initiating allogeneic T-cell division *in vivo*,²³ and elevated serum levels of IL-15 are associated with acute GVHD in humans.²⁴ INF- γ increases the expression of numerous molecules involved in GVHD, including adhesion molecules, chemokines, major histocompatibility complex antigens, and Fas, resulting in enhanced antigen presentation and the recruitment of effector cells into target organs.²⁵⁻²⁷ INF- γ also alters target cells in the GI tract and skin so that they are more vulnerable to damage during GVHD; the administration of anti-INF- γ mAbs prevents GI GVHD²⁸ and high levels of both INF- γ and TNF- α correlate with the most intense cellular damage in skin.²⁹ Paradoxically, at early time points after HCT, INF- γ can reduce GVHD by enhancing Fas-mediated apoptosis of activated donor T cells.^{8,9,30}

Subpopulations of regulatory donor T cells can prevent experimental GVHD. Repeated *in vitro* stimulation of donor CD4(+) T cells with alloantigens results in the emergence of a population of regulatory T cell clones that secrete high amounts of IL-10 and tissue growth factor- β .³¹ The immunosuppressive properties of these cytokines are explained by their ability to inhibit APC function and to suppress proliferation of responding T cells directly.³²⁻³⁴ Natural suppressor cells and NK1.1(+) T cells can also prevent GVHD in experimental models.³⁵⁻³⁷

Step 3: Cellular and Inflammatory Effectors

Significant experimental and clinical data suggest that soluble inflammatory mediators act in conjunction with direct cell-mediated cytotoxicity by CTLs and NK cells to cause the full spectrum of deleterious effects seen during acute GVHD. As such, the effector phase of GVHD involves aspects of both the innate and adaptive immune response and the synergistic interactions of components generated during steps 1 and 2.

The Fas/Fas ligand (FasL) and the perforin/granzyme (or granule exocytosis) pathways are the principal effector mechanisms used by CTLs and NK cells to lyse their target cells.^{38,39} A number of T-cell surface proteins also possess the capability to trimerize TNF receptor-like death receptors that also induce apoptosis in their targets.⁴⁰⁻⁴² CD4(+) CTLs preferentially use the Fas/FasL pathway during acute GVHD, while CD8+ CTLs primarily use the perforin/granzyme pathway, consistent with other conditions involving cell-mediated cytotoxicity. FasL defective donor T cells cause markedly reduced experimental GVHD in liver, skin, and lymphoid organs. The Fas/FasL pathway is particularly important in hepatic GVHD, consistent with the marked sensitivity of hepatocytes to Fas-mediated cytotoxicity in models of murine hepatitis.⁴³

A central role for inflammatory cytokines in acute GVHD was confirmed by a recent murine study using bone marrow chimeras where GVHD-target organ injury was induced, even in the absence of epithelial alloantigens, and mortality and target organ injury were prevented by the neutralization of TNF- α and IL-1.² TNF- α plays a central role in intestinal GVHD in murine and human studies.^{32,44,45} Two recent studies demonstrated that neutralization of TNF- α alone or in combination with IL-1 resulted in a significant reduction of GVHD.^{2,33} Although neutralization of IL-1 with an IL-1 receptor antagonist was able to prevent GVHD in mice, its use in a randomized clinical trial did not prevent GVHD.^{34,42}

Macrophages secrete cytokines after ligation of Toll-like receptors by LPS and other microbial products that have leaked through a damaged intestinal mucosa. Since the GI tract is known to be particularly sensitive to the injurious effects of cytokines,^{44,46} damage to the GI tract incurred during the effector phase can lead to a positive feedback loop wherein increased translocation of LPS results in further cytokine production and progressive intestinal injury. Thus, the GI tract may be critical to propagating the "cytokine storm" characteristic of acute GVHD.⁴⁷ Elevated serum levels of LPS have been shown to correlate directly with the degree of intestinal histopathology occurring after allogeneic HCT,^{46,48,49} and gram-negative gut decontamination during HCT has been shown to reduce GVHD.⁵⁰⁻⁵³

Risk Factors and Prevention

GVHD pathophysiology can thus be considered an exaggerated and dysregulated response of a normal immune system (that of the donor) to tissue damage that is intrinsic to transplantation. Risk factors for acute GVHD can be considered according to this 3-phase model,

as can the prophylactic strategies designed to reduce its morbidity.

Reduced Intensity Conditioning Regimens

One common thread among GVHD target organs is their exposure to the environment. Skin and gut have very obvious barrier functions and a well developed reticuloendothelial system. Similarly, the liver is the first line of defense downstream of the gut. The lung's less intense exposure to organisms, particularly Gram-negative rods, reduces the frequency of its involvement. All of these organs are subject to injury from conditioning and breaches of a protective barrier that allows organisms or endotoxins into the circulation. The 3-phase model predicts that less intense conditioning regimens will be associated with less severe GVHD. Available data also suggest that the severity of GVHD after reduced intensity regimens is, indeed, less than that seen after conventional-dose conditioning despite the fact that these patients were at risk for a much more severe form of GVHD.⁵⁴⁻⁵⁷

Modulation of Donor T Cells

Histocompatibility differences between donor and recipient are major determinants of donor T-cell activation and, thus, increased HLA disparity is one of the most important risk factors for acute GVHD. Female donors, particularly those with multiple pregnancies, cause greater GVHD in male recipients because proteins encoded on the Y chromosome can serve as minor histocompatibility antigens in male recipients.

The number of T cells in the donor marrow is directly associated with the severity of acute GVHD, and T-cell depletion is one of the most effective forms of prophylaxis; a T-cell dose less than 10^5 /kg was associated with complete control of GVHD if an HLA-identical sibling served as the donor.⁵⁸ The combination of very high stem cell numbers and CD3 T-cell numbers less than 3×10^4 /kg allowed haploidentical transplantation without GVHD.⁵⁹ Unfortunately, the nonspecific removal or clearance of T cells results in increased fatal opportunistic infections, resulting in equivalent overall survival.⁶⁰⁻⁶²

Administration of intermittent low-dose methotrexate immediately after bone marrow transplantation induces proliferation of T cells that have started to divide after exposure to allogeneic antigens.³⁴ CSP inhibits signaling through the T-cell receptor and is about as effective as methotrexate alone. The combination of methotrexate and CSP significantly reduces GVHD and is widely used.^{63,64} More recently, the immunosuppressive agent tacrolimus, which is similar to colony-stimulating factor, has shown similar control of GVHD. Both drugs inhibit

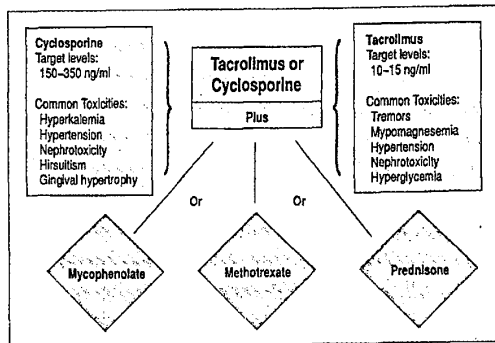


Figure 2. Two-drug regimens for GVHD prophylaxis.

T-lymphocyte activation by binding to immunophilins; CSP binds cyclophilin and tacrolimus binds FKBP-12. The net result is the inhibition of T lymphocyte activation.⁶⁵ Subsequent comparisons of tacrolimus versus CSP in combination with methotrexate showed no advantage for either combination.^{66,67}

More recently, mycophenolate mofetil (MMF), an inhibitor of the de novo pathway of guanosine nucleotide synthesis, has been studied. MMF does not inhibit the activation of T cells as such, but blocks the coupling of activation to DNA synthesis and proliferation.⁶⁸ Recent limited trials of the combination of MMF with CSP or tacrolimus are promising.^{57,69-71} The most common approaches to chemical control of donor T cells as prophylaxis for GVHD are schematized in Figure 2.

Blockade of Inflammatory Stimuli and Effectors

Elimination of intestinal colonization with bacteria reduces GVHD by minimizing the triggering signal for monocytes and macrophages, as well as minimizing the actuation of APCs. Elimination of exposure to microorganisms prevents GVHD in germ-free mice, where GVHD was not observed until the mice were colonized with Gram-negative organisms.⁷² Additionally, gut decontamination and use of a laminar air flow environment was associated with less GVHD and better survival in patients with severe aplastic anemia.⁵⁰

An important role for TNF- α in clinical acute GVHD was suggested by studies demonstrating elevated levels of TNF- α in the serum of patients with acute GVHD and other endothelial complications, such as veno-occlusive disease.⁷³⁻⁷⁵ Recently, therapy of GVHD with humanized anti-TNF- α (infliximab [Remicade, Centocor])^{76,77} or a dimeric TNF receptor fusion protein (etanercept [Enbrel, Wyeth/Amgen]) have shown some promise.⁷⁸ More

studies are required to understand the pharmacokinetics and proper use of these agents after allogeneic transplantation, since TNF inhibition may increase the risk of opportunistic infections.

Two phase I/II trials showed promising data suggesting that specific inhibition of IL-1 (with soluble IL-1 receptor or IL-1 receptor antagonist), could result in remissions in 50-60% of patients with steroid-resistant GVHD.^{79,80} However, a randomized trial of the addition of IL-1 receptor antagonist or placebo to CSP and methotrexate did not show any protective effect of the drug, despite attaining very high plasma levels.³⁴ IL-11 was able to protect the GI tract and prevent GVHD in animal models,^{81,82} but it did not prevent clinical GVHD.⁸³ Therefore, not all preclinical data successfully translate to new therapies.

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(continued on page 428)

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