Immunobiology of acute graft-versus-host disease

Pavan Reddy, James L.M. Ferrara

Departments of Internal Medicine and Pediatrics, University of Michigan Cancer Center, Ann Arbor, MI, USA

Abstract  Graft-versus-host disease (GVHD) has been the primary limitation to the wider application of allogeneic bone marrow transplantation (BMT). The immunobiology of acute GVHD is complex and can be conceptualized to be a three-step process. In step 1, the conditioning regimen (irradiation and/or chemotherapy) leads to the damage and activation of host tissues and induces the secretion of inflammatory cytokines TNF-α and IL-1. As a consequence expression of MHC antigens and adhesion molecules is increased, thus enhancing the recognition of host alloantigens by donor T cells. Donor T-cell activation in step 2 is characterized by donor T-cell interaction with host APCs and subsequent proliferation, differentiation, and secretion of cytokines. Cytokines such as IL-2 and IFN-γ enhance T-cell expansion, induce cytotoxic T cells (CTL) and natural killer (NK) cell responses, and prime additional mononuclear phagocytes to produce TNF-α and IL-1. These inflammatory cytokines in turn stimulate production of inflammatory chemokines, thus recruiting effector cells into target organs. In step 3, effector functions of mononuclear phagocytes are triggered via a secondary signal provided by lipopolysaccharide (LPS) that leaks through the intestinal mucosa damaged during step 1. This mechanism may result in the amplification of local tissue injury and further promotion of an inflammatory response, which, together with the CTL and NK components, leads to target tissue destruction in the transplant host.

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INTRODUCTION

Allogeneic bone marrow transplantation (BMT) is an important curative therapy for a number of hematologic diseases. Unfortunately its utility is limited by acute graft-versus-host disease (GVHD), the most major complication of allogeneic BMT.1 Acute GVHD occurs when donor T cells react to host antigens on antigen-presenting cells (APCs) with sequential activation of donor T cells and monocytes/macrophages causing target organ damage that we recognize as clinical acute GVHD. This report will focus on the complex immunobiology of T cells, antigen presenting cells, NK cells, and cytokines as they relate to the pathophysiology of acute GVHD. Acute GVHD pathophysiology can be conceptualized in three sequential phases: (1) effects of conditioning, (2) donor T-cell activation that constitute the afferent phase, and (3) effenter effector phase.2,5

PHASE 1: EFFECTS OF CONDITIONING

The first step involves the transplant conditioning regimen, which includes total body irradiation (TBI) and/or chemotherapy. Donor T cells are infused into a host that has been profoundly damaged by underlying disease, infection, and conditioning, all of which result in activation of host cells with secretion of proinflammatory cytokines, such as TNF-α and IL-1.1 The presence of inflammatory cytokines during this phase may increase expression of adhesion molecules, co-stimulatory molecules, and MHC antigens.5,6 Such “danger signals” expressed by injured host tissues are critical for the activation of host dendritic cells (DCs) and are necessary for the initiation of primary and secondary immune responses.7 This concept explains a number of clinical observations such as increased risks of GVHD associated with advanced stage leukemia, certain intensive conditioning regimens, and histories of viral infections.8,9 TBI is particularly important because it also induces endothelial apoptosis in gastrointestinal tract followed by epithelial cell damage,10 allowing immunostimulatory microbial products such as LPS to enter into systemic circulation, leading to further amplification of GVHD.11 The relationship between conditioning intensity, inflammatory cytokine, and GVHD severity was further supported by animal models and clinical observation.3

PHASE 2: T-CELL ACTIVATION

GVHD fundamentally depends on donor T cells interaction with host antigen-presenting-cells and their subsequent activation, proliferation, and differentiation. This process occurs during the second step of the afferent phase of acute GVHD. The central role of host APC has recently been established by elegant murine studies which demonstrated those hosts APCs alone are sufficient to activate donor T cells.12 Although allo-antigen can be presented directly by host-derived and indirectly by donor-derived APCs, host-derived APCs appear to be critical in inducing GVHD across both MHC mismatches.12,13 Furthermore, a recent murine study identified the enhanced allostimulatory activity of host APCs in aged mice as one of the important reasons for greater severity of GVHD in aged recipients.13 Dendritic cells (DCs) are the most potent APCs and are activated by (1) inflammatory cytokines such as TNF-α and IL-1, (2) microbial products such as LPS and CpG entering systemic circulation from intestinal mucosa damaged by conditioning, and (3) necrotic cells that are damaged by recipient conditioning.14 These effects are extremely important in producing the ‘danger signals’ that mature DCs and induce T-cell activation whereas immature DCs induce T-cell tolerance.7 However, the relative contribution of DCs and other semiprofessional APCs such as monocytes/macrophages and B cells in inducing GVHD remains to be elucidated.

Donor T cells require the second signal from the co-stimulatory molecules provided by the APC.16,17 Interruption of the second signal by blockade of the co-stimulatory molecules has been shown to reduce GVHD in some murine models.18 Furthermore, changes in phase 1 dramatically augment the signals delivered through CD28 that lower the threshold for T-cell activation and promote T-cell differentiation and survival.19

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Donor T cells proliferate following activation. The alloantigen composition of the host determines which T-cell subset proliferates and differentiates. In mouse models of GVHD, where genetic differences between multiple strain combinations can be controlled, CD4+ cells induce GVHD to MHC class II differences, and CD8+ cells induce GVHD to MHC class I differences. In the majority of HLA-identical HSC transplants, GVHD may be induced by either subset or by both subsets in response to MiHA, which are derived from the expression of polymorphic genes that distinguish host from donor. While the number of MiHA in humans is not yet defined, the actual numbers of so-called ‘major minor’ antigens that can potentially induce GVHD are likely to be limited. The T-cell activation and proliferation is followed by their differentiation and secretion of cytokines.

Cytokines

Alloantigen presentation induces the activation of individual T cells resulting in activation of several, rapidly occurring intracellular biochemical changes that activate transcription of genes for cytokines and their receptors. The Th1 cytokines are preferentially produced and have been implicated in the pathophysiology of acute GVHD. Th1 cells producing IL-2 have a pivotal role in controlling and amplifying the immune response against alloantigens, representing step two of the cytokine cascade that initiates acute GVHD. The importance of IL-2 is further underscored by donor CD4+ T cells and addition of low doses of IL-2 after allogeneic BMT enhanced the severity and mortality of GVHD. The precursor frequency of host-specific IL-2 producing cells (pHTL) predicts the occurrence of GVHD after transplantation between HLA-identical siblings. Due to their apparent importance in initiating acute GVHD, IL-2 producing donor T cells have been the target of many experimental approaches to control GVHD. Cyclosporine (CSP) and FK506, inhibitors of IL-2 production, are effective prophylactic agents against GVHD. The importance of IL-2 is further underscored by experiments showing that monoclonal antibodies (mAbs) against the IL-2 receptor are efficient in preventing GVHD in animal models. However, treatment with anti-IL-2 receptor mAb was only moderately successful in reducing the incidence of severe GVHD in clinical studies. Interestingly a recent study of the kinetics of T cell division and expression of IL-2 and IL-15 receptor subunits demonstrated that IL-15 is a critical cytokine in initiating allogeneic T cell division in vivo. Elevated serum levels of IL-15 are associated with acute GVHD in humans. IL-15 may therefore be a critical factor in initiating GVHD.

IFN-γ is another crucial cytokine that can be implicated in the second step of the pathophysiology of acute GVHD. IFN-γ levels are significantly higher in mice with GVHD than those without it. The release of IFN-γ is also an early event in the cascade leading to GVHD because IFN-γ production in animals with GVHD peaks at day 7 post transplant before clinical manifestations are apparent. In several models of experimental acute GVHD, T cells produce large amounts of IFN-γ and a large proportion of T-cell clones isolated from GVHD patients also produce IFN-γ. Experimental data suggest that IFN-γ is involved in several aspects of acute GVHD pathophysiology. First, IFN-γ up-regulates numerous molecules such as adhesion molecules, chemokines, MHC and its associated machinery molecules important for antigen presentation. Thus IFN-γ facilitates antigen presentation and effector recruitment. Second, IFN-γ can mediate the development of pathologic processes in the gastrointestinal tract and skin during GVHD. Third, IFN-γ mediates GVHD-associated immunosuppression in several experimental GVHD systems through the induction of nitric oxide (NO) and Fas. Fourth, exposure to IFN-γ results in a significant reduction in the amount of LPS needed to trigger macrophages to produce proinflammatory cytokines and NO. Lastly, IFN-γ also plays an important role in regulating the death of activated donor T cells by enhancing Fas-mediated apoptosis, thus regulating GVHD.

Th1/Th2 paradigm and GVHD

Differential activation of Th1 or Th2 cells has been evoked in the immunopathogenesis of GVHD. The role of Th1/Th2 polarization as it relates to acute GVHD is incompletely understood and controversial. Although the ‘cytokine storm’ amplified by the Th1 phenotype correlates with the development of acute GVHD, early Th1 polarization of donor T cells by administration of cytokines such as IFN-γ, IL-2, IL-12 and IL-18 to BMT recipients can attenuate acute GVHD. Furthermore the use of Th1 cytokine deficient mice as BMT donors still results in GVHD; and some studies failed to show a beneficial effect of Th2 polarization on acute GVHD. Thus, physiological and adequate amount of Th1 cytokine production is critical for GVHD induction, while inadequate production (extremely low or high) could modulate GVHD through a breakdown of negative feedback mechanisms for activated donor T cells.

Multiple studies have shown that Th2 secretion may reduce GVHD. These studies include polarization of donor T cells by IL-4, both in vivo and ex-vivo, the use of G-CSF and IL-18 to mobilize donor cells, administration of IL-11, and the secretion of IL-4 by NK1.1+ T cells. However, the causal mechanisms of these effects are yet to be completely determined. One study suggested that Th1 and Th2 subsets cause injury of distinct target tissues; Th2 (Stat 4−/−) cells were required for hepatic damage and Th1 cells (Stat6−/−) for GI tract damage after allogeneic BMT. Taken together, these data demonstrate that the timing of administration or the production of any given cytokine, the intensity of the conditioning regimen, the donor-recipient strain combination may be critical to the eventual outcome of acute GVHD.

T-cell apoptosis

Deletional mechanisms of tolerance can be placed into two categories: (1) central (thymic) deletion and (2) peripheral deletion. Central clonal deletion might be an effective way to deal with continued thymic production of alloreactive T cells. To this end, lymphoablative treatments have been used as a condition to create a mixed hematopoietic chimeric state in murine BMT models. In this instance, donor cells seed the thymus and maturing donor-reactive T cell clones are deleted through intrathymic apoptosis. However, a definitive role for central deletion in attenuating GVHD in humans is yet to be demonstrated.
The proportion of the peripheral T-cell repertoire that can respond to allogeneic MHC can play a critical role in the development of tolerance. In the case of MHC-mismatched transplantation, the frequency of alloreactive T cells is at least five orders of magnitude greater than the frequency of peptide-specific T cells responding to a nominal antigen. The pathways of T-cell apoptosis by which peripheral deletion occurs can be broadly categorized into activation-induced cell death (AICD) and passive cell death (PCD). Probably the most important mediator of AICD in T cells is the Fas receptor. Activated T cells induced to express the Fas molecule can undergo apoptotic cell death when brought into contact with cells expressing Fas ligand. A critical role for Fas-mediated AICD has been clearly demonstrated in attenuation of acute GVHD by the Th1 cytokines.

Photodynamic cell purging process (wherein donor T cells are treated with photoactive 4, 5-dibromorhodamine 123 and subsequently exposed to visible light) has been shown to attenuate GVHD. Moreover, the conditions under which one or the other of these deletional mechanisms dominate remains an area of active investigation.

Regulatory T cells

Several recent studies have demonstrated a critical role for donor CD4+CD25+ regulatory T (T(reg)) cells in the prevention of acute GVHD. The balance of donor-type CD4+CD25− T (reg) and conventional CD4+CD25− T cells can determine the outcome of acute GVHD. The ability of CD4+CD25+ regulatory T cells to suppress GVH reactivity after BMT depended partially on IL-10 production and/or CD28 expression, but the mechanisms for in vivo generation of these cells are not known. Ex vivo-expanded CD4+CD25+ regulatory T cells obtained after stimulation by allogeneic recipient-type antigen-presenting cells can also modulate GVHD. Furthermore, ex vivo blockade of the CD40:CD40L or LIGHT/CD40L costimulatory pathway in primary mixed lymphocyte reaction cultures generated regulatory T cells that protected from GVHD.

Chemokines and T-cell migration

Chemokines play a critical role in the migration of immune cells to secondary lymphoid organs and target tissues. Recruitment of CCR5+ T cells that usually secrete Th1 cytokines are associated with the development of hepatic GVHD. T-lymphocyte production of macrophage inflammatory protein-1alpha is critical to the recruitment of CD8+ T but not CD4+ cells to the liver, lung, and spleen during acute GVHD. Several chemokines are over-expressed in GVHD target organs, such as MIP-1α, MIP-2 and MIG in liver and spleen. MIP-1α, MIP-2, MCP-1 and MCP-3 are predominantly expressed in other target organs such as the skin. Recently, Choi et al used mouse models of GVHD to multiple minor H antigens in order to track donor T cells through several GVHD target organs (spleen, liver, and lung) in real time and demonstrated that the donor T cells expanded simultaneously in the liver, lung, and spleen, suggesting that donor T cells interact directly with antigen-presenting cells of the host not only in secondary lymphoid tissue but in target organs as well. However, the role of various chemokines and their regulation of donor T cells and host APC migration to secondary lymphoid organs and or target tissues in causing acute GVHD remain unexplored.

NK cells

Recent studies have generated a tremendous amount of interest on the role of NK cells in GVHD. NK cells are negatively regulated by MHC class I-specific inhibitory receptors; thus HLA mismatched transplants may trigger donor NK-mediated alloreactivity. In murine models of BMT, infusion of donor NK cells can reduce GVHD, probably through the elimination of host APC, or through the secretion of TGF-β. Interestingly, HLA class I disparity driving donor NK-mediated alloreactions in the GVH directions mediate strong GVL effects and produce higher engraftment rates without causing acute GVHD. A recent murine BMT study using mice lacking SH2-containing inositol phosphatase (SHIP), in which the NK compartment is dominated by cells that express two inhibitory receptors capable of binding either self or allogeneic MHC ligands, suggests that host NK cells may play a role in the initiation of GVHD.

Phase 3: Cellular and Inflammatory Effector Phase

The effector phase of acute GVHD is a complex cascade of multiple effectors mediated by cellular effectors such as CTLs and NK cells, and inflammatory effectors such as TNF-α, IL-1 and NO.

Cellular effectors

The cellular effectors of acute GVHD are primarily CTLs and NK cells. The Fas/Fas ligand (FasL) and the perforin/granzyme (or granule exocytosis) pathways are the classic effector mechanisms that CTLs and NK cells utilize to lyse target cells. A number of other ligands have also been identified on T cells that can induce CTL. Transplantation of perforin deficient T cells resulted in a marked delay in the onset of GVHD in transplants across MHC disparities, but mortality and histological signs of GVHD were induced in the absence of perforin dependent killing. The importance of the perforin/granzyme pathway for GVHD induction has been evident in studies employing
donor T cell subsets. Perforin or granzyme B deficient CD8+ T cells induced significantly less mortality compared to wild type T cells in experimental transplants across a single MHC class I mismatch, while this pathway seems to be less important compared to Fas/FasL pathway in CD4+ mediated GVHD.92,93 Thus, it has been thought that CD4+ CTLs preferentially use the Fas/FasL pathway, while CD8+ CTLs mostly use the perforin/granzyme pathways. Most studies failed to detect a role for the perforin/granzyme pathway in target organ pathology.94

In contrast, FasL-mediated cytotoxicity may be a particularly important effector pathway in target organ GVHD. FasL defective T cells markedly diminish GVHD in liver, skin and lymphoid organs.94 During GVHD, Fas expression on bile duct epithelial cells is upregulated94 and administration of anti-asL significantly blocked the hepatic damage occurring in murine models of GVHD.96 Elevated serum levels of soluble FasL and Fas have been observed in at least some patients with acute GVHD.97

The utilization of mutant mice provides the opportunity to address whether other effector pathways are capable of inducing GVHD target organ pathology. An initial study demonstrated that perforin/granzyme and FasL cytotoxic double deficient (cdd) T cells were unable to induce GVHD lethality in recipients after sublethal irradiation.98 However, two subsequent studies demonstrated that cytotoxic effector mechanisms of donor T cells are critical in preventing host resistance to GVHD99,100 and with lethal irradiation, cdd T cells produced similar mortality to wild type T cells in a murine model of GVHD.100 These results demonstrate that other effector molecules are sufficient to induce GVHD mediated by T cells in the absence of perforin/granzyme and FasL dependent functions. A recent study demonstrated that the absence of TRAIL on donor cells significantly reduced GVH but resulted in similar severity of acute GVHD suggesting that donor T cells might utilize different effector mechanisms in mediating GVH and GVL responses.101

**Inflammatory effectors**

The inflammatory cytokines TNF-z and IL-1 are produced by monocytes and macrophages after stimulation. This stimulus may be provided through Toll-like receptors (TLRs) by microbial products such as LPS and other components of microorganisms, which can leak through the intestinal mucosa or skin damaged by the conditioning regimen and GVHD.11,102 The role of LPS in acute GVHD has been elucidated in several experimental models.103 These experiments strongly supported the role of mononuclear phagocytes as sources of inflammatory cytokines during the effector phase of acute GVHD.11,103 Subsequent murine studies further demonstrated that TNF-z production by donor cells in response to LPS predicts the severity of GVHD and that direct antagonism of LPS reduces GVHD.104 Thus, the gastrointestinal tract plays a major role in the amplification of systemic GVHD and is critical in the propagation of the “cytokine storm” characteristics of acute GVHD.33

TNF-z plays a critical role in the pathophysiology of intestinal GVHD in murine and human studies96,105 and is also an important effector molecule in skin and lymphoid tissue.96,106 Furthermore target organ damage could be inhibited by infusion of anti-TNF-z mAbs.107 A role for TNF-z in clinical acute GVHD has been suggested by studies demonstrating elevated levels of TNF-z in the serum of patients with acute GVHD. Regardless of the source, donor or the host, TNF-z plays an important role in acute GVHD.41,108 TNF-z may be involved in a multistep process of GVHD pathophysiology. First, TNF-z activates DCs and enhances alloantigen presentation. Second, TNF-z recruits effector T cells, neutrophils, and monocytes into target organs via the induction of inflammatory chemokines. Third, TNF-z causes direct tissue damage by inducing apoptosis and necrosis.11,109

The second major proinflammatory cytokine that appears to play an important role in the effector phase of acute GVHD is IL-1.33 Secretion of IL-1 appears to occur predominantly during the effector phase of GVHD of the spleen and skin, two major GVHD target organs.110 Mice receiving IL-1 after llogeneic BMT displayed a wasting syndrome and increased mortality that appeared to be an accelerated form of disease.111 Although administration of an IL-1 receptor antagonist (IL-1ra) to recipients reduces GVHD mortality in animal models112,113 a recent randomized human study failed to demonstrate any benefit against acute GVHD.114 These data would suggest that IL-1 might have a redundant and pleiotropic role in the disease and may be synergistic with TNF-z.

Nitric oxide (NO) is another inflammatory effector molecule that plays an important role in GVHD. Development of GVHD is preceded by an increase in serum levels of oxidation products.115,116 NO also contributes to the deleterious effects on GVHD target tissues, particularly immunosuppression.117,118 As a result of activation during GVHD, macrophages produce NO and induce the release of iron from target cells, resulting in an inhibition of the recovery of injured target tissues by inhibiting proliferation of epithelial stem cells in the gut and skin.119

More recently, a central role of inflammatory cytokines in acute GVHD was confirmed in a murine study by using bone marrow chimeras wherein mortality and target organ injury was prevented by the neutralization of TNF-z and IL-1. This neutralization was particularly effective for CD4-mediated acute GVHD but also partially for CD8-mediated disease.12 These inflammatory effectors may synergize with the lytic component provided by CTLs in a complex milieu of chemotactic signals and cytokine cascades resulting in the amplification of local tissue injury and further promotion of an inflammatory response, which ultimately leads to the observed target tissue destruction in the transplant recipient.

In conclusion the immunobiology of acute GVHD involves a number of complex interactions between several cell types of both donor and host. Although the disease process can be schematized in three overall steps, it should be noted that each of the three steps does not carry equal weight in its pathogenesis. The pivotal interactions occur in step 2, where host APCs activate allogeneic donor T cells and the dysregulation of complex cytokine cascades occur at various steps in the sequence and eventually is responsible for the manifestations of this disease. Further insights into the mechanisms of these cascades should provide insight into the unique target organ distribution of acute GVHD as well as...
provide new strategies to prevent and treat this complicated disorder. Such approaches should make allogeneic BMT safer and ultimately more available to many patients who could benefit from this potent therapy.

Research agenda

- The relative contribution of host professional (dendritic cells) and semiprofessional (monocyte/macrophages and B cells) APCs in the induction of acute GVHD and GVL.
- The relevance of the timing, induction and the impact of conditioning on Th1/Th2 secretion by donor T cells on the outcome of acute GVHD.
- The mechanisms of regulatory T cells in the separation of GVHD from GVL responses.
- The role of resident APCs and chemokines in defining the target organ distribution of acute GVHD.
- The role of alloreactive NK cells and KIR variability on the occurrence of acute GVHD and GVL.
- The specific role of different cellular and inflammatory effectors in separating acute GVHD from GVL.

References


