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Pathophysiology of Acute Graft-vs-Host Disease: Recent Advances

Yaping Sun¹, Isao Tawara¹, Tomomi Toubai¹, and Pavan Reddy^{1,*}

¹ Department of Internal Medicine, University of Michigan Comprehensive Cancer Center, Ann Arbor, MI.

Abstract

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative therapy for many malignant and non-malignant hematological diseases. Donor T cells from the allografts are critical for the success of this effective therapy. Unfortunately these T cells not only recognize and attack the disease cells/tissues but also the other normal tissues of the recipient as ‘foreign’ or ‘nonself’ and cause severe immune mediated toxicity, Graft-versus-Host Disease (GVHD). Several insights into the complex pathophysiology of GVHD have been gained from recent experimental observations, which show that acute graft-vs.-host disease (GVHD) is a consequence of interactions between both the donor and the host innate and adaptive immune systems. These insights have identified a role for a variety of cytokines, chemokines, novel T cell subsets (naïve, memory, regulatory and NKT cells) and also for non-T cells of both the donor and host (antigen presenting cells, $\gamma\delta$ T cells, B cells and and NK cells) in modulating the induction, severity and maintenance of acute GVHD. This review will focus on the immunobiology of experimental acute GVHD with an emphasis on the recent observations.

Keywords

allogeneic; BMT; T cells; dendritic cells; antigens; cytokines

I. INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) represents an important therapy for many hematological, some epithelial malignancies and also for a spectrum of non-malignant diseases (1). The development of novel strategies such as donor leukocyte infusions (DLI), nonmyeloablative HCT and cord blood transplantation (CBT) have helped expand the indications for allogeneic HCT over the last several years, especially among older patients (2). However, the major toxicity of allogeneic HCT, graft-versus-host disease (GVHD), remains a lethal complication that limits its wider application(3). Depending on the time at which it occurs after HCT, GVHD can be either acute or chronic(4–7). Acute GVHD is responsible for 15% to 40% of mortality and is the major cause of morbidity after allogeneic HCT, while chronic GVHD occurs up to 50% of patients who survive three months after HCT

* To whom correspondence should be addressed: 6310 CCGC, University of Michigan Cancer Center, 1500 East Medical Center Drive, Ann Arbor, MI 48109-0942, Phone; (734) 647-5954, Fax; (734) 647-9271, Email; redypr@umich.edu, Email: yapings@umich.edu (Y.S.), itawara@umich.edu (I.T.), tomomit@umich.edu (T.T.), redypr@umich.edu (P.R.).

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(1,7). Research efforts over years have provided increasing insight into the biology of this complex disease process.

The GVH reaction was first noted when irradiated mice were infused with allogeneic marrow and spleen cells(8). Although mice recovered from radiation injury and marrow aplasia, they subsequently died with 'secondary disease(8),' a syndrome consisting of diarrhea weight loss, skin changes, and liver abnormalities. This phenomenon was subsequently recognized as GVH disease (GVHD). Three requirements for the development of GVHD were formulated by Billingham(9). First, the graft must contain immunologically competent cells, now recognized as mature T cells. In both experimental and clinical allogeneic BMT, the severity of GVHD correlates with the number of donor T cells transfused(10,11). The precise nature of these cells and the mechanisms they use are now understood in greater detail (discussed below). Second, the recipient must be incapable of rejecting the transplanted cells i.e. immuno-compromised. A patient with a normal immune system will usually reject cells from a foreign donor. In allogeneic BMT, the recipients are usually immuno-suppressed with chemotherapy and/or radiation before stem cell infusion(2). Third, the recipient must express tissue antigens that are not present in the transplant donor. This area has been the focus of intense research that has led to the discovery of the major histocompatibility complex (MHC)(12). Human Leukocyte Antigens (HLA) are proteins that are the gene products of the MHC and that are expressed on the cell surfaces of all nucleated cells in the human body. HLA proteins are essential to the activation of allogeneic T cells (12,13) (discussed below).

This review on the pathophysiology of acute GVHD will place the genetic basis and the immuno-biological mechanisms of Billingham's postulates in perspective.

II. GENETIC BASIS OF GVHD

Billingham's third postulate stipulates that GVH reaction occurs when donor immune cells recognize disparate host antigens(9). These differences are governed by the genetic polymorphisms of the HLA system and the non-HLA systems such as the killer immunoglobulin receptors (KIR) family of NK receptors, nucleotide-binding oligomerization domain (NOD) 2 and cytokine gene polymorphisms(2,13).

A. HLA matching

Alloreactive T-cell-antigen recognition can be divided based on whether the presenting MHC molecule is matched or mismatched. For reasons as yet unclear, the precursor frequency of T cells that can recognize a mismatched MHC is very high (14–16). In humans, MHC is governed by the the HLA antigens that are encoded by the MHC gene complex on the short arm of chromosome 6 and can be categorized as Class I, II and III. Class I antigens (HLA A, B, and C) are expressed on almost all cells of the body at varying densities (12). Class II antigens include DR, DQ, and DP antigens and are primarily expressed on hematopoietic cells (B cells, DCs, monocytes) although their expression can also be induced on many other cell types following inflammation or injury(12). The incidence of acute GVHD is directly related to the degree of MHC mismatch (17–20). The role of HLA mismatching in cord blood transplant (CBT) is more difficult to analyze than in unrelated HSCT, because allele level typing of CB units for HLA-A, B, C, DRB1, and DQB1 is not routinely performed(21). Nonetheless, the total number of HLA disparities between recipient and the CB unit has been shown to correlate with risk of acute GVHD and the frequency of severe acute GVHD was lower in patients transplanted with matched (6/6) CB units(21–23).

B. Minor histocompatibility antigens (MiHAs)

In MHC matched BMT context, as is the case with most clinical allo-BMT, donor T cells recognize MHC-bound peptides derived from the protein products of polymorphic genes (minor histocompatibility antigens or MiHAs) that are present in the host but not in the donor (24–28). Recent data suggest that genes not expressed in the donor but in the host can also generate relevant MiHAs(29,30).

Thus, despite HLA identity between a patient and donor, substantial numbers (40%) of patients receiving HLA-identical grafts and optimal post grafting immune suppression, develop acute GVHD due to differences in MiHAs that lie outside the HLA loci(27,31). MiHAs are widely expressed, but can differ in their tissue expression(25,31). This might be one of the reasons for the unique target organ involvement in GVHD. For example, most described human MiHAs although show wide but variable tissue expression pattern, but all are expressed in hematopoietic cells(25). This preponderance of MiHA expression on hematopoietic cells might account for making the host immune system a primary target for GVH response and further help explain the critical role of direct presentation by professional recipient antigen presenting cells (APCs) in causing anti-tumor and GVHD responses (by at least the donor CD8+ T cells) after allogeneic BMT. Experimental murine models have shown that different MiHAs dictate the phenotype, target organ involvement and the kinetics of GVHD(32). Recent efforts have identified some MiHAs such as, HA-1 and HA-2 that are primarily found on hematopoietic cells(33). These proteins may therefore induce GVHD. By contrast, other MiHAs, such as H-Y and HA-3, are expressed ubiquitously (see Table 1) (31). Experimental data have demonstrated that despite the presence of numerous potential MiHAs, all of the MiHAs are not equal in their ability to induce lethal GVHD and that they show hierarchical immunodominance(34,35). Furthermore, difference in single immuno-dominant MiHAs alone was shown to be insufficient for causing GVHD in murine models although T cells targeting single MiHA can induce tissue damage in a skin explant model (36,37). However, the role of specific and immuno-dominant MiHAs that are relevant in clinical GVHD has not been systematically evaluated in large groups of patients(38).

C. Other Non-HLA genes

Genetic polymorphisms in several non-HLA genes such as in KIRs, cytokines and NOD2 genes have recently been shown to modulate the severity and incidence of GVHD.

1. KIR polymorphisms—KIR receptors on NK cells that bind to the HLA class I gene products are encoded on chromosome 19. Polymorphisms in the trans-membrane and cytoplasmic domains of KIR receptors governs whether the receptor has inhibitory potential (such as KIR2DL1, -2DL2, -2DL3 and 3DL1 and their HLA class I ligands (HLA-C and HLA-Bw4) or activating potential; at this time, there is limited information on the clinical significance of activating KIR genes. Two competing models have been proposed for HLA-KIR allo-recognition by donor NK cells following HSCT: “mismatched ligand” and the “missing ligand” models(39–43). The former posits that NK alloreactivity occurs when donor NK cells recognize recipient target cells that lack the class I allele of the donor (HLA mismatching between the donor and recipient in the GVH direction); the latter hypothesizes that donor NK alloreactivity occurs when the host lacks the correct HLA class I ligand(s) to provide the inhibitory signal for donor KIR(42,43). Both models are supported by some clinical observations, albeit in patients receiving very different transplant and immunosuppressive regimens(40,44–46). Clearly, further validation is warranted and it is likely that the immunobiology of the interface between HLA and KIR genetics will be an area of intense future investigation.

2. Cytokine gene polymorphisms—Pro-inflammatory cytokines, involved in the classical ‘cytokine storm’ of GVHD (discussed below), cause pathological damage of target organs such as skin, gut and liver(47). Several cytokine gene polymorphisms, both in hosts and donors, have been implicated. Specifically, TNF polymorphisms (TNFd3/d3 in the recipient, TNF-863 and -857 in donors and/or recipients and TNFd4, TNF- α -1031C and TNFR11-196R- in the donors) have been associated with an increased risk of acute GVHD and transplant-related mortality(48,49). The three common haplotypes of the IL-10 gene promoter region in recipients representing high, intermediate and low production of IL-10 have been associated with the severity of acute GVHD following allo-BMT after HLA-matched sibling donors(50). By contrast, smaller studies have found neither IL-10 nor TNF- α polymorphisms to be associated with GVHD after HLA-mismatched cord blood transplants(49,51). Furthermore, it is important to note that not all of the polymorphism analyses so far support the paradigm that excess of pro-inflammatory and Th1 cytokines are always associated with greater GVHD or mortality. For example IFN- γ polymorphisms of the 2/2 genotype (high IFN- γ production) and 3/3 genotype (low IFN- γ) have been associated with decreased or increased acute GVHD, respectively(49,52). This is consistent with accelerated acute GVHD in IFN- γ knockout mouse models suggesting therefore that IFN- γ may be involved in the down-regulation of GVHD via a negative feed-back loop (discussed below)(53,54). Similarly, possession of *IL-6-174* polymorphism in the recipients (high production of IL-6) associated with both acute and chronic GVHD after MRD sibling HCT(52), which is in contrast to the results of IL-6 genetics in other Th1 mediated disease such as juvenile onset chronic arthritis (55).

By contrast, genotype (high production) of another Th1 cytokine, IL-2 (-330 allele G) was noted to increase the risk of acute GVHD in MUD transplants (56) while a different study showed that Th2 cytokine, IL-13, production by donor T cells is also predictive of acute GVHD in unrelated donor stem cell cohorts (57). Thus data from cytokine polymorphism studies so far do not seem to suggest a simple paradigm for GVHD. Nonetheless, the fact that most of the studies are small, not properly stratified must be considered while interpreting these data.

A small study in pediatric recipients of unrelated HSCT suggested that the presence of the IL-1 α -889 allele in either donor or recipient decreased transplant related mortality but did not decrease GVHD(49). NOD2/CARD15 gene polymorphisms in both the donors and recipients were recently shown to have a striking association with GI GVHD and overall mortality after both related and unrelated allogeneic HSCT(58). It is likely non-HLA gene polymorphisms might play differing roles depending on the donor source (related vs. unrelated), HLA disparity (matched vs. mismatched), source of the graft (CB vs. PBSC vs. BM), and the intensity of the conditioning.

III. IMMUNOBIOLOGY

It is helpful to remember two important principles when considering the pathophysiology of acute GVHD. First, acute GVHD represents exaggerated but normal inflammatory responses against foreign antigens (allo-antigens) that are ubiquitously expressed in a setting where they are undesirable. The donor lymphocytes that have been infused into the recipient function appropriately, given the foreign environment they encounter. Second, donor lymphocytes encounter tissues in the recipient that have been often profoundly damaged. The effects of the underlying disease, prior infections, and the intensity of conditioning regimen all result in substantial changes not only in the immune cells but also in the endothelial and epithelial cells. Thus the allogeneic donor cells rapidly encounter not only a foreign environment, but one that has been altered to promote the activation and proliferation of inflammatory cells. Thus, the pathophysiology of acute GVHD may be considered a distortion of the normal inflammatory cellular responses that in addition to the absolute requirement of donor T cells, involves

multiple other innate and adaptive cells and mediators(59). The development and evolution of acute GVHD can be conceptualized in three sequential phases (Figure 1) to provide a unified perspective on the complex cellular interactions and inflammatory cascades that lead to acute GVHD: (1) activation of the antigen presenting cells (APCs) (2) donor T cell activation, differentiation and migration and (3) effector phase(59).

It is important to note that the three phase description as discussed below allows for a unified perspective in understanding the biology. It is, however, not meant to suggest that all three phases are of equal importance or that GVHD occurs in a step-wise and sequential manner. The spatio-temporal relationships between the biological processes described below, depending on the context, are more likely to be chaotic and of varying intensity and relevance in the induction, severity and maintenance of GVHD.

A. Phase 1: Activation of antigen presenting cells (APCs)

The earliest phase of acute GVHD is set into motion by the profound damage caused by the underlying disease and infections and further exacerbated by the BMT conditioning regimens (which include total body irradiation (TBI) and/or chemotherapy) that are administered even before the infusion of donor cells(60–64). This first step results in activation of the APCs. Specifically, damaged host tissues respond with multiple changes, including the secretion of proinflammatory cytokines, such as TNF- α and IL-1, described as the ‘cytokine storm’(62, 63,65). Such changes increase expression of adhesion molecules, costimulatory molecules, MHC antigens and chemokines gradients that alert the residual host and the infused donor immune cells(63). These “danger signals” activate host APCs(66,67). Damage to the gastrointestinal (GI) tract from the conditioning is particularly important in this process because it allows for systemic translocation of immuno-stimulatory microbial products such as lipopolysaccharide (LPS) that further enhance the activation of host APCs and the secondary lymphoid tissue in the GI tract is likely the initial site of interaction between activated APCs and donor T cells (63) (68,69). This scenario accords with the observation that an increased risk of GVHD is associated with intensive conditioning regimens that cause extensive injury to epithelial and endothelial surfaces with a subsequent release of inflammatory cytokines and increases in expression of cell surface adhesion molecules(63,64). The relationship among conditioning intensity, inflammatory cytokine, and GVHD severity has been supported by elegant murine studies(65). Furthermore, the observations from these experimental studies have led to two recent clinical innovations to reduce clinical acute GVHD: (a) reduced intensity conditioning to decrease the damage to host tissues and thus limit activation of host APC and (b) KIR mismatches between donor and recipients to eliminate the host APCs by the alloreactive NK cells(41,70).

Host type APCs that are present and have been primed by conditioning, are critical for the induction of this phase; recent evidence suggests that donor type APCs exacerbate GVHD, but, in certain experimental models, donor type APC chimeras also induce GVHD(67,71–73). In clinical situations, if donor type APCs are present in sufficient quantity and have been appropriately primed, they too might play a role in the initiation and exacerbation of GVHD (74–76). Amongst the cells with antigen presenting capability, DCs are the most potent and play an important role in the induction of GVHD(77). Experimental data suggest that GVHD can be regulated by qualitatively or quantitatively modulating distinct DC subsets(78–83). Langerhans cells were also shown to be sufficient for the induction of GVHD when all other APCs were unable to prime donor T cells, although the role for Langerhans cells when all APCs are intact is unknown(84). Studies have yet to define roles for other DC subsets. In one clinical study persistence of host DC after day 100 correlated with the severity of acute GVHD while elimination of host DCs was associated with reduced severity of acute GVHD(75). The allo-stimulatory capacity of mature monocyte derived DCs (mDCs) after reduced intensity

transplants was lower for up to six months compared to the mDCs from myeloablative transplant recipients, thus suggesting a role for host DCs and the reduction in ‘danger signals’ secondary to less intense conditioning in acute GVHD(85). Nonetheless this concept of enhanced host APC activation explains a number of clinical observations such as increased risks of acute GVHD associated with advanced stage malignancy, conditioning intensity and histories of viral infections.

Other professional APCs such as monocytes/macrophages or semi-professional APCs might also play a role in this phase. For example, recent data suggests that host type B cells might play a regulatory role under certain contexts(86). Also host or donor type non-hematopoietic stem cells, such as mesenchymal stem cells or stromal cells when acting as APCs have been shown to reduce T cell allogeneic responses, although the mechanism for such inhibition remains unclear. The relative contributions of various APCs, professional or otherwise, remain to be elucidated.

B. Phase 2: Donor-T-Cell Activation, differentiation and migration

The infused donor T cells interact with the primed APCs leading to the initiation of the second phase of acute GVHD. This phase includes antigen presentation by primed APCs, the subsequent activation, proliferation, differentiation and migration of alloreactive donor T cells.

After allogeneic HSC transplants, both host- and donor-derived APCs are present in secondary lymphoid organs(87,88). The T-cell receptor (TCR) of the donor T cells can recognize alloantigens either on host APCs (direct presentation) or donor APCs (indirect presentation) (89,90). In direct presentation, donor T cells recognize either the peptide bound to allogeneic MHC molecules or allogeneic MHC molecules without peptide (90,91). During indirect presentation, T cells respond to the peptide generated by degradation of the allogeneic MHC molecules presented on self-MHC (91). Experimental study demonstrated that APCs derived from the host, rather than from the donor, are critical in inducing GVHD across MiHA mismatch (89). Recent data suggest that presentation of distinct target antigens by the host and donor type APCs might play a differential role in mediating target organ damage(32,92). In humans, most cases of acute GVHD developed when both host DCs and donor dendritic cells (DCs) are present in peripheral blood after BMT (75).

1. Co-stimulation—The interaction of donor lymphocyte TCR with the host allo-peptide presented on the MHC of APCs alone is insufficient to induce T cell activation(93). Both TCR ligation and co-stimulation via a ‘second’ signal through interaction between the T cell co-stimulatory molecules and their ligands on APCs are required to achieve T proliferation, differentiation and survival(94). The danger signals generated in phase 1 augment these interactions and significant progress has been made on the nature and impact of these ‘second’ signals(95,96). Costimulatory pathways are now known to deliver both positive and negative signals and molecules from two major families, the B7 family and the TNF receptor (TNFR) family play pivotal roles in GVHD(97). Interruption of the second signal by blockade of various positive co-stimulatory molecules (CD28, ICOS, CD40, CD30, 4-1BB and OX40) reduces acute GVHD in several murine models while antagonism of the inhibitory signals (PD-1 and CTLA-4) exacerbates the severity of acute GVHD(2,98–103). The various T cell and APC co-stimulatory molecules and the impact on acute GVHD are summarized in Table 2. The specific context and the hierarchy in which each of these signals play a dominant role in the modulation of GVHD remain to be determined.

2. T cell subsets—T cells consist of several subsets whose responses differ based on antigenic stimuli, activation thresholds and effector functions. The alloantigen composition of the host determines which donor T-cell subsets proliferate and differentiate.

CD4+ and CD8+ cells: CD4 and CD8 proteins are co-receptors for constant portions of MHC class II and class I molecules, respectively(104). Therefore MHC class I (HLA-A, -B, -C) differences stimulate CD8+Tcells and MHC class II (HLA-DR -DP, -DQ) differences stimulate CD4+T cells(104–107). But clinical trials of CD4⁺ or CD8⁺ depletion have been inconclusive(108). This perhaps is not surprising, because GVHD is induced by MiHAs in the majority of HLA-identical BMT, which are peptides derived from polymorphic cellular proteins that are presented by MHC molecules(28). Because the manner of protein processing depends on genes of the MHC, two siblings will have many different peptides in the MHC groove(28). Thus in the majority of HLA-identical BMT, acute GVHD may be induced by either or both CD4⁺ and CD8⁺ subsets in response to minor histocompatibility antigens(108). The peptide repertoire for class I or class II MHC remains unknown and might even be different in different individuals(109). But it is plausible that only a few of the many of these peptides might behave as immuno-dominant “major minor” antigens that can potentially induce GVHD. In any event, such antigens remain to be identified and validated in large patient population.

Central deletion by establishment of stable mixed hematopoietic chimeric state is an effective way to eliminate continued thymic production of both CD4⁺ and CD8⁺ alloreactive T cells and thus reduce GVHD(110–112). In contrast peripheral mechanisms to induce tolerance of CD4⁺ and CD8⁺ T cells appears to be distinct(113,114). 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)(115). Experimental data suggests that deletional tolerance by AICD is operative via the Fas (for CD4⁺) or TNFR (CD8⁺) pathways in Th1 cells and when the frequency of alloreactive T cells is at much greater(116–121). PCD or ‘death by neglect’ is due to rapid down regulation of Bcl-2 and appears to be critical in non-irradiated but not after irradiated BMT(122). Thus distinct mechanisms of tolerance induced by apoptosis have a dominant role depending on the T cell subsets, the conditioning regimens and the histocompatibility differences. Nonetheless strategies aimed at selective elimination of donor T cells in vivo after HCT either by targeting a suicide gene to the allo-T cells or by photodynamic cell purging appears to be promising in reducing experimental acute GVHD (123–129).

Naïve and Memory subsets: Several independent groups have intriguingly found that the naïve (CD62L⁺) T cells were alloreactive and caused acute GVHD but not the memory (CD62L⁻) T cells across different donor/recipient strain combinations(130–133). Furthermore, expression of naïve T cell marker CD62L was also found to be critical for regulation of GVHD by donor natural regulatory T cells(134,135). By contrast, another recent study demonstrated that alloreactive memory T cells and their precursor cells (memory stem cells) caused robust GVHD(136,137). It remains as yet unknown whether the reduced GVHD potential of memory type T cells from a naïve murine donor, in contrast to their ability to cause greater solid organ allo-rejection(138), is due to a consequence of the intense conditioning regimen and/or altered trafficking or from a restricted repertoire and/or from T cell intrinsic defect.

Regulatory T cells: Recent advances indicate that distinct subsets of regulatory CD4⁺CD25⁺, CD4⁺CD25⁻IL10⁺ Tr cells, $\gamma\delta$ T cells, DN⁻ T cells, NK T cells and regulatory DCs control immune responses by induction of anergy or active suppression of alloreactive T cells(79,80,139–147). Several studies have demonstrated a critical role for the natural donor CD4⁺CD25⁺ Foxp3⁺ regulatory T (Treg) cells, obtained from naïve animals or generated ex-vivo, in the outcome of acute GVHD. Donor CD4⁺CD25⁺ T cells suppressed the early expansion of alloreactive donor T cells and their capacity to induce acute GVHD without abrogating GVL effector function against these tumors(148,149). CD4⁺CD25⁺ T cells induced/generated by of immature or regulatory host type DCs and by regulatory donor type myeloid APCs were also able to suppress acute GVHD(79). One of the clinical studies that evaluated the relationship between donor CD4⁺CD25⁺ cells and acute GVHD in humans after matched

sibling donor grafts and found that in contrast to the murine studies, donor grafts containing larger numbers of CD4⁺ CD25⁺T cells developed more severe acute GVHD(150). These data suggest that co-expression of CD4⁺ and CD25⁺ is insufficient because an increase in CD25⁺ T cells in donor grafts is associated with greater risks of acute GVHD after clinical HCT. Another recent study found that Foxp3 mRNA expression (considered a specific marker for naturally occurring CD4⁺CD25⁺Tregs) was significantly decreased in peripheral blood mononuclear cells from patients with acute GVHD(151,152). But Foxp3 expression in humans, unlike mice, may not be specific for T cells with a regulatory phenotype(153). It is likely that the precise role of regulatory T cells in clinical acute GVHD will therefore not only depend upon identification of specific molecular markers in addition to Foxp3 but also on the ability for ex vivo expansion of these cells in sufficient numbers. Several clinical trials are underway in the US and Europe with attempts to substantially expand these cells ex-vivo and use for prevention of GVHD.

Host NK1.1⁺ T cells are another T cell subsets with suppressive functions have also been shown to suppress acute GVHD in an IL-4 dependent manner(146,147,154). By contrast, donor NKT cells were found to reduce GVHD and enhance perforin mediated GVL in an IFN- γ dependent manner(155,156). Recent clinical data suggests that enhancing recipient NKT cells by repeated TLI conditioning promoted Th2 polarization and dramatically reduced GVHD(147). Experimental data also show that activated donor NK cells can reduce GVHD through the elimination of host APCs or by secretion of transforming growth factor- β (TGF- β) secretion (156). A 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(157).

3. Cytokines and T cell differentiation—APC and T cell activation result in rapid intracellular biochemical cascades that induce transcription of many genes including cytokines and their receptors. The Th1 cytokines (IFN- γ , IL-2 and TNF- α) have been implicated in the pathophysiology of acute GVHD(158–160). IL-2 production by donor T cells remains the main target of many current clinical therapeutic and prophylactic approaches, such as cyclosporine, tacrolimus and monoclonal antibodies (mAbs) against the IL-2 and its receptor to control acute GVHD(161,162). But emerging data indicate an important role for IL-2 in the generation and maintenance of CD4⁺CD25⁺ Foxp3⁺ T regs, suggesting that prolonged interference with IL-2 may have an unintended consequence in the prevention of the development of long term tolerance after allogeneic HCT(163–166).

Studies by Sykes and colleagues and others have demonstrated that the role of Th1 cytokines is complex. For example exogenous administration of IFN- γ or T cells from IFN- γ deficient donors have demonstrated a reduction and enhancement of GVHD respectively(53,54,167, 168). These data are consistent with the clinical IFN- γ polymorphism data (discussed above). A recent study suggests that IFN- γ might play a differential role in the severity of distinct GVHD target organs(169). Likewise, early injection of IL-2 has also been shown to reduce GVHD(170,171). Thus whether the Th1 cytokines are the regulators or inducers of GVHD severity depends on the degree of allo-mismatch, the intensity of conditioning and the T cell subsets that are involved after BMT(172–174). Thus although the “cytokine storm” initiated in phase I and amplified by the Th1 cytokines correlates with the development of acute GVHD, early Th1 polarization of donor T cells to HCT recipients can attenuate acute GVHD suggesting that physiological and adequate amounts of Th1 cytokines are critical for GVHD induction, while inadequate production (extremely low or high) could modulate acute GVHD through a breakdown of negative feedback mechanisms for activated donor T cells(160,174–177). Several different cytokines that polarize donor T cells to Th2 such as IL-4, G-CSF, IL-18, IL-11, rapamycin and the secretion of IL-4 by NK1.1⁺ T cells can reduce acute GVHD(178–

185). But Th1 and Th2 subsets cause injury of distinct acute GVHD target tissues and some studies failed to show a beneficial effect of Th2 polarization on acute GVHD(186). Thus the Th1/Th2 paradigm of donor T cells in the immuno-pathogenesis of acute GVHD has evolved over the last few years and its causal role in acute GVHD is complex and incompletely understood.

IL-10 plays a key role in suppression of immune responses and its role in regulating experimental acute GVHD is unclear(187). Recent clinical data demonstrate an unequivocal association of IL-10 polymorphisms with the severity of acute GVHD(50). TGF- β , another suppressive cytokine was shown to suppress acute GVHD but to exacerbate chronic GVHD (188). The roles of some other cytokines, such as IL-7 (that promotes immune reconstitution) and IL-13 remain unclear(189–192). The role for Th17 cells, a recently described novel T cell differentiation in many immunological processes, is not yet known(193). In any case, all of the experimental data so far collectively suggest that the timing of administration, the production of any given cytokine, the intensity of the conditioning regimen and the donor-recipient combination may all be critical to the eventual outcome of acute GVHD.

4. Leukocyte migration—Donor T cells migrate to lymphoid tissues, recognize alloantigens on either host or donor APCs and become activated. They then exit the lymphoid tissues and traffic to the target organs and cause tissue damage(194). The molecular interactions necessary for T cell migration and the role of lymphoid organs during acute GVHD have recently become the focus of a growing body of research. Chemokines play a critical role in the migration of immune cells to secondary lymphoid organs and target tissues(195). T-lymphocyte production of macrophage inflammatory protein-1 α (MIP-1 α) is critical to the recruitment of CD8⁺ but not CD4⁺ T cells to the liver, lung, and spleen during acute GVHD(196). Several chemokines such as CCL2-5, CXCL2, CXCL9-11, CCL17 and CCL27 are over-expressed and might play a critical role in the migration of leukocyte subsets to target organs liver, spleen, skin and lungs during acute GVHD(194,197). CXCR3⁺ T and CCR5⁺ T cells cause acute GVHD in the liver and intestine(194,198–200). CCR5 expression has also been found to be critical for Treg migration in GVHD(201). In addition to chemokines and their receptors, expression of selectins and integrins and their ligands also regulate the migration of inflammatory cells to target organs(195). For example, interaction between α 4 β 7 integrin and its ligand MadCAM-1 are important for homing of donor T cells to Peyer's patches and in the initiation of intestinal GVHD(68,202). α L β 2/ICAM1, 2, 3 and α 4 β 1/VCAM-2 interactions are important for homing to the lung and liver after experimental HCT(194). The expression of CD62L on donor Tregs is critical for their regulation of acute GVHD suggesting that their migration in secondary tissues is critical for their regulatory effects(88). The migratory requirement of donor T cells to specific lymph nodes (e.g. Peyer's patches) for the induction of GVHD might depend on other factors such as the conditioning regimen, inflammatory milieu etc(68,203). Furthermore, FTY720, a pharmacologic sphingosine-1-phosphate receptor agonist, inhibited GVHD in murine but not in canine models of HCT(204,205). Thus, there might also be significant species differences in the ability of these molecules to regulate GVHD.

C. Phase 3: Effector Phase

The effector phase that leads to the GVHD target organ damage is a complex cascade of multiple cellular and inflammatory effectors that further modulate each others responses either simultaneously or successively. Effector mechanisms of acute GVHD can be grouped into cellular effectors (e.g., CTLs) and inflammatory effectors such as cytokines. Inflammatory chemokines expressed in inflamed tissues upon stimulation by proinflammatory effectors such as cytokines are specialized for the recruitment of effector cells, such as CTLs(206). Furthermore the spatio-temporal expression of the cyto-chemokine gradients might determine

not only the severity but also the unusual cluster of GVHD target organs (skin, gut, and liver) (194,207).

1. Cellular Effectors—Cytotoxic T cells (CTLs) are the major cellular effectors of GVHD (208,209). The Fas-Fas ligand (FasL), the perforin-granzyme (or granule exocytosis) and TNFR-like death receptors (DR), such as TNF-related apoptosis-inducing ligand (TRAIL: DR4, 5 ligand) and TNF-like weak inducers of apoptosis (TWEAK: DR3 ligand) are the principle CTL effector pathways that have been evaluated after allogeneic BMT(209–214). The involvement of each of these molecules in GVHD has been testing by utilizing donor cells that are unable to mediate each pathway. Perforin is stored in cytotoxic granules of CTLs and NK cells, together with granzymes and other proteins. Although the exact mechanisms remain unclear, following the recognition of a target cell through the TCR-MHC interaction, perforin is secreted and inserted into the cell-membrane, forming “perforin pores” that allow granzymes to enter the target cells and induce apoptosis through various downstream effector pathways such as caspases(215). Ligation of Fas results in the formation of the death-inducing signaling complex (DISC) and also activates caspases(216,217).

Transplantation of perforin deficient T cells results in a marked delay in the onset of GVHD in transplants across MiHA disparities only, both MHC and MiHA disparities (126), and across isolated MHC I or II disparities(209,218–222). However, mortality and clinical and histological signs of GVHD were still induced even in the absence of perforin-dependent killing in these studies, demonstrating that the perforin-granzyme pathways plays little role in target organ damage. A role for the perforin-granzyme pathway for GVHD induction is also evident in studies employing donor-T-cell subsets. Perforin- or granzyme B-deficient CD8+ T cells caused less mortality than wild-type T cells in experimental transplants across a single MHC class I mismatch. This pathway, however, seems to be less important compared to Fas/FasL pathway in CD4-mediated GVHD(221–223). Thus, it seems that CD4+ CTLs preferentially use the Fas-FasL pathway, whereas CD8+CTLs primarily use the perforin-granzyme pathway.

Fas, a TNF-receptor family member, is expressed by many tissues, including GVHD target organs(224). Its expression can be upregulated by inflammatory cytokines such as IFN- γ and TNF- α during GVHD, and the expression of FasL is also increased on donor T cells, indicating that FasL-mediated cytotoxicity may be a particularly important effector pathway in GVHD (209,225). FasL-defective T cells cause less GVHD in the liver, skin and lymphoid organs (220,223,225). The Fas-FasL pathway is particularly important in hepatic GVHD, consistent with the keen sensitivity of hepatocytes to Fas-mediated cytotoxicity in experimental models of murine hepatitis(209). Fas-deficient recipients are protected from hepatic GVHD, but not from other organ GVHD, and administration of anti-FasL (but not anti-TNF) MAbs significantly blocked hepatic GVHD damage occurring in murine models(209,226,227). Although the use of FasL-deficient donor T cells or the administration of neutralizing FasL MAbs had no effect on the development of intestinal GVHD in several studies, the Fas-FasL pathway may play a role in this target organ, because intestinal epithelial lymphocytes exhibit increased FasL-mediated killing potential(228). Elevated serum levels of soluble FasL and Fas have also been observed in at least some patients with acute GVHD(229,230).

The utilization of a perforin-granzyme and FasL cytotoxic double-deficient (*cdd*) mouse provides an opportunity to address whether other effector pathways are capable of inducing GVHD target organ pathology. An initial study demonstrated that *cdd* T cells were unable to induce lethal GVHD across MHC class I and class II disparities after sublethal irradiation (219). However, subsequent studies demonstrated that cytotoxic effector mechanisms of donor T cells are critical in preventing host resistance to GVHD(213,231). Thus when recipients were conditioned with lethal dose of irradiation, *cdd* CD4+ T cells produced similar mortality to wild type CD4+ T cells(213). These results were confirmed by a recent study demonstrating

that GVHD target damage can occur in mice that lack alloantigen expression on the epithelium, preventing direct interaction between CTLs and target cells(214).

The participation of another death ligand receptor signaling pathway, TNF/TNFRs, has also been evaluated. Experimental data suggests that this pathway is crucial for GI GVHD (discussed more below). Recently, several additional TNF family apoptosis-inducing receptors/ligands have been identified, including TWEAK, TRAIL and LT β /LIGHT have all been proposed to play a role in GVHD and GVL responses(2,232–238). However whether these distinct pathways play a more specific role for GVHD mediated by distinct T cell subsets in certain situations remains unknown. Intriguingly, recent data suggest that none of these pathways might be critical for mediating the rejection of donor grafts(232,239). Thus it is likely that their role in GVHD might be modulated by the intensity of conditioning and by the recipient T cell subsets. Existing experimental data suggest that perforin and TRAIL cytotoxic pathways are associated with CD8+ T cell-mediated GVL(209). The available experimental data are strongly skewed toward CD8+ T cell-mediated GVL based on the dominant role of this effector population in most murine GVT models; however, CD4+ T cells can mediate GVL and might be crucial in clinical BMT depending on the type of malignancy and the expression of immuno-dominant antigens.

Taken together, although experimental data suggest that might be some distinction between the use of different lytic pathways for the specific GVHD target organs and GVL, but the clinical applicability of these observations is as yet largely unknown

2. Inflammatory Effectors—Inflammatory cytokines synergize with CTLs resulting in the amplification of local tissue injury and further promotion of an inflammation, which ultimately leads to the observed target tissue destruction in the transplant recipient(47). Macrophages, which had been primed with IFN- γ during step 2, produce inflammatory cytokines TNF- α and IL-1 when stimulated by a secondary triggering signal(240). This stimulus may be provided through Toll-like receptors (TLRs) by microbial products such as LPS and other microbial particles, which can leak through the intestinal mucosa damaged by the conditioning regimen and gut GVHD(241,242). It is now apparent that immune recognition through both TLR and non-TLRs (such as NOD) by the innate immune system also controls activation of adaptive immune responses(241,243). Recent clinical studies of GVHD suggested the possible association with TLR/NOD polymorphisms and severity of GVHD(58,244,245). LPS and other innate stimuli may stimulate gut-associated lymphocytes, keratinocytes, dermal fibroblasts, and macrophages to produce pro-inflammatory effectors that play a direct role in causing target organ damage. Indeed experimental data with MHC mismatched BMT suggest that under certain circumstances these inflammatory mediators are sufficient in causing GVHD damage even in the absence of direct CTL induced damage(71). The severity of GVHD appears to be directly related to the level of innate and adaptive immune cell priming and release of pro-inflammatory cytokines such as TNF- α , IL-1 and nitric oxide (NO)(71,242,246–248).

The cytokines TNF- α and IL-1 are produced by an abundance of cell types during processes of both innate and adaptive immunity; they often have synergistic, pleiotrophic, and redundant effects on both activation and effector phases of GVHD(160). A critical role for TNF- α in the pathophysiology of acute GVHD was first suggested over 20 years ago because mice transplanted with mixtures of allogeneic BM and T cells developed severe skin, gut, and lung lesions that were associated with high levels of TNF- α mRNA in these tissues(249). Target organ damage could be inhibited by infusion of anti-TNF- α MABs, and mortality could be reduced from 100% to 50% by the administration of the soluble form of the TNF- α receptor (sTNFR), an antagonist of TNF- α (62,65,247). Accumulating experimental data further suggest that TNF- α is involved in a multistep process of GVHD pathophysiology. TNF- α can (1) cause cachexia, a characteristic feature of GVHD, (2) induce maturation of DCs, thus enhancing

alloantigen presentation, (3) recruit effector T cells, neutrophils, and monocytes into target organs through the induction of inflammatory chemokines, and (4) cause direct tissue damage by inducing apoptosis and necrosis. TNF- α also involves in donor-T-cell activation directly through its signaling via TNFR1 and TNFR2 on T cells. TNF-TNFR1 interactions on donor T cells promote alloreactive T-cell responses and TNF-TNFR2 interactions are critical for intestinal GVHD(235,250). TNF- α also seems to be important effector molecules in GVHD in skin and lymphoid tissue(249,251). Additionally, TNF- α might also be involved in hepatic GVHD, probably by enhancing effector cell migration to the liver via the induction of inflammatory chemokines(252). An important role for TNF- α in clinical acute GVHD has been suggested by studies demonstrating elevated serum levels of TNF- α or elevated TNF- α mRNA expression in peripheral blood mononuclear cells in patients with acute GVHD and other endothelial complications, such as hepatic veno-occlusive disease (VOD)(252–255). Phase I–II trials using TNF- α antagonists reduced the severity of GVHD suggesting that it is a relevant effector in causing target organ damage(256,257).

The second major pro-inflammatory cytokine that appears to play an important role in the effector phase of acute GVHD is IL-1(258). Secretion of IL-1 appears to occur predominantly during the effector phase of GVHD of the spleen and skin, two major GVHD target organs (259). A similar increase in mononuclear cell IL-1 mRNA has been shown during clinical acute GVHD. Indirect evidence of a role for IL-1 in GVHD was obtained with administration of this cytokine to recipients in an allogeneic murine BMT model. Mice receiving IL-1 displayed a wasting syndrome and increased mortality that appeared to be an accelerated form of disease. By contrast, intra-peritoneal administration of IL-1ra starting on d 10 post-transplant was able to reverse the development of GVHD in the majority of animals, providing a significant survival advantage to treated animals(260). However, the attempt to use IL-1ra to prevent acute GVHD in a randomized trial was not successful(261).

As a result of activation during GVHD, macrophages also produce NO, which contributes to the deleterious effects on GVHD target tissues, particularly immunosuppression(248,262). NO also inhibits the repair mechanisms of target tissue destruction by inhibiting proliferation of epithelial stem cells in the gut and skin(263). In humans and rats, the development of GVHD is preceded by an increase in serum levels of NO oxidation products(264–267).

Existing data demonstrate important role for various inflammatory effectors in GVHD. The relevance of currently studied or as yet unknown specific effectors might however be determined by other factors, including the intensity of preparatory regimens, the type of allograft, the T cell subsets and the duration of BMT. In any event, both experimental and clinical data suggest an important role for both the cellular and inflammatory mediators in GVHD induced target organ damage.

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Figure 1.
Three phases of GVHD immuno-biology

Table 1

Minor histocompatibility Antigens	Tissue Distribution
HA-1	Hematopoietic
HA-2	Hematopoietic
HB-1	Hematopoietic
BCL2A1	Hematopoietic
HA-3	Ubiquitous
HA-8	Ubiquitous
UGT2B17	Ubiquitous
HY (A, B, DR, DQ) antigens	Ubiquitous

Table 2

T Cell co-stimulation

	<i>T cell</i>	<i>APC</i>
Adhesion	ICAMs LEA-1 CD2 (LEA-2)	LEA-1 ICAM~ LFA-3
Recognition	TCR/CD4 TCR/CD8	NIIC hi Mi-Icc I
Costimulation	CD28 CD152 (CTLA-4) ICOS PD-1 Unknown CD 154 (CD4OL) CD134 (OX40) CD137 (4-1BB) HVEM	CD80/86 CD80/86 B7H/B7RP-1 PD-L1, PD-L2 B7-H3 CD40 CD134L (OX4OL) CD137L (4-1BBL) LIGHT

Abbreviations: HVEM HSV glycoprotein D for herpesvirus entry mediator; LIGHT, homologous to lymphotoxins, shows inducible expression, and competes with herpes simplex virus glycoprotein D for herpes virus entry mediator (HVEM), a receptor expressed by T lymphocytes.