HEMATOLOGICAL ONCOLOGY
Hematol Oncol 2003; **21**: 149–161.
Published online 15 December 2003 in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/hon.716

REVIEW ARTICLE

PATHOPHYSIOLOGY OF ACUTE GRAFT-VERSUS-HOST DISEASE

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SUMMARY

Graft-versus-host disease (GVHD) has been the primary limitation to the wider application of allogeneic bone marrow transplantation (BMT). The pathophysiology of acute GVHD is complex and can be conceptualized to be a three-step process based on murine studies. In step 1, the conditioning regimen leads to the damage and activation of host tissues and induces the secretion of inflammatory cytokines. As a consequence, the expression of MHC antigens and adhesion molecules is increased 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 lipopolysaccaride (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. The following review discusses the three-step process of the pathophysiology of experimental acute GVHD. Copyright © 2003 John Wiley & Sons, Ltd.

KEY WORDS: Th1; Th2; cytokines; APC; NK cells; CTL

INTRODUCTION

The ability of allogeneic stem cell transplantation (SCT) to cure certain hematologic malignancies is widely recognized. An important therapeutic aspect of SCT in eradicating malignant cells is the graft-versus-leukemia (GVL) effect. Forty years ago Barnes and colleagues noted that recipients of allogeneic grafts, although less likely to relapse, died of a 'wasting syndrome' now recognized as graft-versus-host disease (GVHD).[1,2] Thus, in addition to describing GVL, these experiments highlighted for the first time the intricate relationship between GVL and GVHD. Ten years after the work of Barnes and Loutit, Billingham formulated the 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 destroy the transplanted cells.[3] Clinical GVHD occurs secondary to mismatches between histocompatibility antigens between the donor and recipient.[4,5] Matching of the major histocompatibility complex (MHC) antigens speeds engraftment and reduces the severity of GVHD.[4] Despite HLA identity between a patient and donor, substantial numbers of patients still develop GVHD due to differences in minor histocompatibility antigens that lie outside the HLA loci. But the precise elucidation of many human minor antigens is yet to be accomplished.[6,7] Acute GVHD can occur within days or as late as 2 months after transplantation. The incidence ranges from less than 10 to more

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Received 3 April 2003
Revised 3 August 2003
Accepted 10 November 2003

than 80%, depending on the degree of histoincompatibility between donor and recipient, the number of T cells in the graft, the patient's age and the GVHD prophylactic regimen.[8] The principal target organs include the immune system, skin, liver and intestine. Pathologically, the *sine qua non* of acute GVHD is selective epithelial damage of target organs.[9,10] Standard grading systems generally include clinical changes in the skin, GI tract, liver and performance status.[11] While mild GVHD (grade I or II) is associated with little morbidity and almost no mortality, higher grades are associated with significantly decreased survival.[11] With grade IV GVHD, the mortality is greater than 90%.

Since the early experiments by Brent and Billingham, both GVHD and the GVL effect have been studied extensively, particularly in murine models of transplantation.[12] This review summarizes the current understanding of the pathophysiology of acute GVHD. Two important principles help to place the pathophysiology of GVHD in context. The first is that GVHD is not a disease *per se*; it reflects exaggerated but normal physiologic inflammatory mechanisms of the donor lymphocytes that have been infused into the recipient, given the foreign environment they encounter. The second principle is that donor lymphocytes are infused into a host that has been profoundly damaged by the underlying disease, prior infections and the intensity of the conditioning regimen. 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 by the increased expression of adhesion molecules, cytokines and co-stimulatory proteins. Thus the pathophysiology of acute GVHD is complex but can be conceptualized in three sequential phases: (1) effects of conditioning (2) donor T cell activation which constitutes the afferent phase and (3) efferent effector phase (Figure 1).[13]

This review will focus on the complex interactions of the donor T cells, NK cells, host antigen presenting cells and the cytokines and chemokines as they relate to the three phases of the pathophysiology of acute GVHD.

PHASE 1: EFFECTS OF CONDITIONING

The first step involves the conditioning regimen that may include total body irradiation (TBI) and/or chemotherapy (Figure 1). 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.[14] The presence of inflammatory cytokines during this phase increases the expression of adhesion molecules, co-stimulatory molecules and MHC antigens.[15,16] 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 the alloreaction.[17] This concept of altered host milieu promoting an alloreaction 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.[18,19] TBI is particularly important because it also induces endothelial apoptosis in the gastrointestinal tract followed by epithelial cell damage,[20] allowing immunostimulatory microbial products such as LPS to enter into systemic circulation, leading to further amplification of GVHD.[21] The relationship between conditioning intensity, inflammatory cytokine and GVHD severity was further supported by animal models and clinical observation.[22]

PHASE 2: DONOR T CELL ACTIVATION, PROLIFERATION AND DIFFERENTIATION

GVHD fundamentally depends on donor T cell 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 (Figure 1). The central role of host APC has recently been established by elegant murine studies which demonstrated that host APCs alone are sufficient to

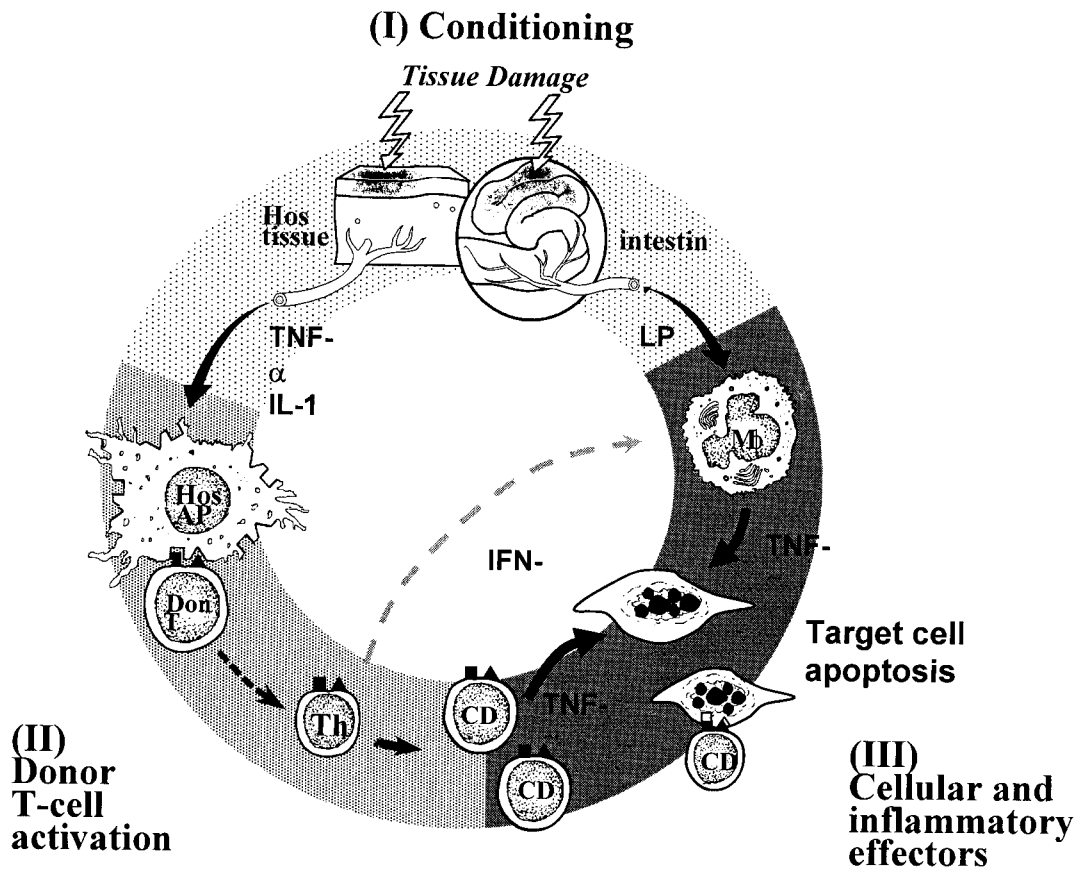

Figure 1. Pathophysiology of GVHD. During step 1, irradiation and chemotherapy both damage and activate host tissues, including intestinal mucosa, liver and the skin. Activated cell hosts then secrete inflammatory cytokines (e.g. TNF-α and IL-1), which can be measured in the systemic circulation. The cytokine release has important effects on antigen presenting cells (APCs) of the host, including increased expression of adhesion molecules (e.g. ICAM-1, VCAM-1) and of MHC class II antigens. These changes in the APCs enhance the recognition of host MHC and/or minor H antigens by mature donor T cells. During step 2, donor T-cell activation is characterized by proliferation of GVHD T cells and secretion of the Th1 cytokines IL-2 and IFN-γ. Both of these cytokines play central roles in clonal T-cell expansion. Induction of CTL and NK cell responses and the priming of mononuclear phagocytes: in step 3, mononuclear phagocytes primed by IFN-γ are triggered by a second signal such as endotoxin (LPS) to secrete cytopathic amounts of IL-I and TNF-α. LPS can leak through the intestinal mucosa damaged by the conditioning regimen, to stimulate gut-associated lymphoid tissue or Kupffer cells in the liver; LPS that penetrates the epidermis may stimulate keratinocytes, dermal fibroblasts and macrophages to produce similar cytokines in the skin. This mechanism results in the amplification of local tissue injury and further production of inflammatory effectors such as nitric oxide, which, together with CTL and NK effectors, leads to the observed target tissue destruction in the stem cell transplant host. CTL effectors use Fas/FasL, perforin/granzyme B and membrane-bound cytokines to lyse target cells

activate donor T cells.[23] Although alloantigen 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 MiHA and MHC mismatches.[23,24] 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.[25] APCs 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.[17,26] These effects are extremely important in producing the 'danger signals' that enhance the maturity of APCs and induce T cell activation.[17] But the relative *in vivo* contribution of the professinal APCs such as the dendritic cells and other semiprofessional APCs such as monocytes/macrophages and B cells in the induction of GVHD is yet to be determined. Donor T cells require the second signal from the co-stimulatory molecules provided by the APC.[27,28] Interruption of the second signal by blockade of the co-stimulatory molecules has been shown to reduce GVHD in some murine models.[29] 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.[30]

Proliferation and apoptosis

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.[31] 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.[30] Following proliferation some of the alloreactive donor T cells are eliminated by the process of deletion as an apparent mechanism to regulate the severity of the immune response. Deletional mechanisms of tolerance can be placed into two categories: (1) central (thymic) deletion and (2) peripheral deletion.[32] 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.[33,34] In this instance, donor cells seed the thymus and maturing donor-reactive T cell clones are deleted through intrathymic apoptosis.[33,34] 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.[35] 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.[35,36] 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).[35] Probably the most important mediator of AICD in T cells is the Fas receptor.[37] 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.[38–41]

PCD (or death by neglect), illustrates the exquisite dependence of activated T cells on growth factors (e.g. IL-2, IL-4, IL-7, and/or IL-15) for survival; apoptotic cell death in this instance is largely due to rapid downregulation of Bcl-2. Transplantation of Bcl-xL T cells into non-irradiated recipients significantly exacerbated GVHD, however no difference in GVHD mortality was observed in animals that had been lethally irradiated.[42]

Selective elimination of donor T cells *in vivo* after BMT using transgenic T cells in which a thymidine kinase (TK) suicide gene is targeted to the T cell, has also been shown to attenuate the severity of acute GVHD.[43] More recently selective depletion of alloantigen-specific donor T cells by a 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 prevent GVHD.[44]

Thus several deletional mechanisms which are not necessarily mutually exclusive have been shown to reduce the severity of acute GVHD. However the conditions under which one or the other of these deletional mechanisms plays a critical role, remain to be determined.

T-cell differentiation and cytokine secretion

The T-cell activation and proliferation is followed by their differentiation characterized by secretion of cytokines and chemokines.[45] 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.[45,46] The Th1 cytokines are preferentially produced and have been implicated in the pathophysiology of acute GVHD.[22,47] Th1 cells producing IL-2 have a pivotal role in controlling and amplifying the immune response against alloantigens, representing step 2 of the cytokine cascade which initiates acute GVHD.[47,48] Experimental data show that IL-2 is secreted primarily by donor CD4+ T cells and addition of low doses of IL-2 after allogeneic BMT enhanced the severity and mortality of GVHD.[49,50] The precursor frequency of host-specific IL-2-producing cells (pHTL) predicts the occurrence of GVHD after transplantation between HLA-identical siblings.[51] 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.[47] 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.[52] However, treatment with anti-IL-2 receptor mAb was only moderately successful in reducing the incidence of severe GVHD in clinical studies.[53,54] 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*.[55] Elevated serum levels of IL-15 are associated with acute GVHD in humans.[56] 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.[21] IFN-γ levels are significantly higher in mice with GVHD than those without it.[57] 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.[13] 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.[21,58,59] Experimental data suggest that IFN-γ is involved in several aspects of acute GVHD pathophysiology. First, IFN-γ upregulates numerous molecules such as adhesion molecules, chemokines, MHC and its associated machinery molecules, which are important for antigen presentation. Thus IFN-γ facilitates antigen presentation and effector recruitment.[47] Second, IFN-γ can mediate the development of pathologic processes in the gastro-intestinal tract and skin during GVHD.[60,61] Third, IFN-γ mediates GVHD-associated immuno-suppression in several experimental GVHD systems through the induction of nitric oxide (NO) and Fas.[62–64] 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.[65] 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.[40,66]

Th1/Th2 paradigm

Differential activation of Th1 or Th2 cells has been evoked in the pathogenesis of GVHD.[67] 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,[68,69] 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.[40,70–72] Furthermore the use of Th1 cytokine-deficient mice as BMT donors still results in GVHD;[73–75] and some studies failed to show a beneficial effect of Th2 polarization on acute GVHD.[73,76–78] Thus, a 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. However Th2 polarization of donor T cells by IL-4, both *in vivo* and *ex vivo*,[69] the use of G-CSF and IL-18 to mobilize donor cells,[69,79] administration of IL-11[80] and the secretion of IL-4 by NK1.1 + T cells,[81] all reduce acute GVHD. 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;[82] 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 and the donor–recipient strain combination may be critical to the eventual outcome of acute GVHD.

Regulatory T cells

Several recent studies have demonstrated a critical role for donor CD4 (+) CD25 (+) regulatory T (T (reg)) cells in the regulation 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.[83] The ability of CD4+ CD25+ regulatory T cells to suppress GVH reactivity after BMT depended partially on IL-10 production and or CD28 expression, the mechanisms for *in vivo* generation of these cells are not known.[83,84] *Ex vivo*-expanded CD4 (+) CD25 (+) regulatory T cells obtained after stimulation by allogeneic recipient-type antigen-presenting cells can also modulate GVHD.[85] Furthermore *ex vivo* blockade of the CD40: CD40L or LIGHTβ: CD40L co-stimulatory pathway in primary mixed lymphocyte reaction cultures generated regulatory T cells that protected from GVHD.[86–89]

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.[90] 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.[91,92] Several chemokines are over-expressed in GVHD target organs, such as MIP-1alpha, MIP-2 and MIG in liver and spleen. MIP-1alpha, MIP-2, MCP-1 and MCP-3 are predominantly expressed in other target organs such as the skin.[93] 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, 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 also in target organs.[94] Resident APCs in target tissues may play an important role in the selectivity of target organ damage.[95] Another recent study suggested that chemokine receptor CCR5 and integrin alpha(4)beta(7)-MAdCAM-1 (mucosal vascular addressin) interactions are critical for donor T-cell migration to APCs in Peyer's patches and the initiation of acute GVHD.[96] However the role of various chemokines in regulating donor T-cell migration to secondary lymphoid organs and/or GVHD target tissues remains unexplored.

PHASE 3: CELLULAR AND INFLAMMATORY EFFECTOR PHASE

The efferent phase of acute GVHD is a complex cascade of multiple effectors mediated by (a) cellular effectors such as CTLs and NK cells and (b) inflammatory effectors such as TNF-α, IL-1 and NO (Figure 1).[13]

Cellular effectors

The cellular effectors of acute GVHD are primarily CTLs and NK cells.[13] 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.[97,98]

Cytotoxic T lymphocytes

Transplantation of perforin-deficient T cells resulted in a marked delay in the onset of GVHD in transplants across MiHA disparities, but mortality and histological signs of GVHD were induced in the *absence* of perforin-dependent killing.[99] 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 the Fas/FasL pathway in CD4-mediated GVHD.[100,101] 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.[102]

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.[102] During GVHD, Fas expression on bile duct epithelial cells is upregulated[103] and administration of anti-FasL significantly blocked the hepatic damage occurring in murine models of GVHD.[104] Elevated serum levels of soluble FasL and Fas have been observed in at least some patients with acute GVHD.[105]

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.[106] However, two subsequent studies demonstrated that cytotoxic effector mechanisms of donor T cells are critical in preventing host resistance to GVHD[107,108] and with lethal irradiation, cdd T cells produced similar mortality to wild type T cells in a murine model of GVHD.[108] 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 GVL but resulted in similar severity of acute GVHD suggesting that donor T cells might utilize different effector mechanisms in mediating GVH and GVL responses.[109]

NK cells

Recent studies have generated a tremendous amount of interest in the role of NK cells in GVHD.[110] NK cells are negatively regulated by MHC class I-specific inhibitory receptors, thus HLA-mismatched transplants may trigger donor NK-mediated alloreactivity.[111] In murine models of BMT, infusion of donor NK cells can reduce GVHD, probably through the elimination of host APCs[112] or through the secretion of TGF-β.[113] 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.[110] 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.[114]

Inflammatory effectors

The inflammatory cytokines TNF-α 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 microbials which can leak through the intestinal mucosa or skin damaged by the conditioning regimen and GVHD.[21,115] Several experimental models strongly support the role of mononuclear phagocytes as sources of inflammatory cytokines during the effector phase of acute GVHD.[13,116] Murine studies demonstrate that TNF-α production by donor cells in response to LPS predicts the severity of GVHD and that direct antagonism of LPS reduces GVHD.[117] 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.[13]

TNF-α plays a critical role in the pathophysiology of intestinal GVHD in murine and human studies[104,118] and is also an important effector molecule in skin and lymphoid tissue.[104,119] Furthermore target organ damage could be inhibited by infusion of anti-TNF-α mAbs.[120] A role for TNF-α in clinical acute GVHD has been suggested by studies demonstrating elevated levels of TNF-α in the serum of patients with acute GVHD. Regardless of the source, donor or the host, TNF-α plays an important role in acute GVHD.[65,121] TNF-α may be involved in a multi-step process of GVHD pathophysiology. First, TNF-α activates APCs and enhances alloantigen presentation. Second, TNF-α recruits effector T cells and monocytes into target organs via the induction of inflammatory chemokines. Third, TNF-α causes direct tissue damage by inducing apoptosis and necrosis.[13,21,122]

The second major proinflammatory cytokine that appears to play an important role in the effector phase of acute GVHD is IL-1.[13] Secretion of IL-1 appears to occur predominantly during the effector phase of GVHD of the spleen and skin, two major GVHD target organs.[123] Mice receiving IL-1 after allogeneic BMT displayed a wasting syndrome and increased mortality that appeared to be an accelerated form of disease.[124] Although administration of an IL-1 receptor antagonist (IL-1ra) to recipients reduces GVHD mortality in animal models,[125,126] a recent randomized human study failed to demonstrate any benefit against acute GVHD.[127] These data would suggest that IL-1 might have a redundant and pleiotropic role in the disease and may be synergistic with TNF-α.

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.[128,129] NO also contributes to the deleterious effects on GVHD target tissues, particularly immunosuppression.[130,131] 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.[132]

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-α and IL-1 particularly for CD4-mediated acute GVHD but also in part, for CD8-mediated disease.[23] 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 pathogenesis 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 interaction occurs in step 2, where host APCs activate allogeneic donor T cells and the dysregulation of complex cytokine cascades occurs at various steps in the sequence and is eventually responsible for the manifestations of this disease.

Although some of these observations need to be validated in human studies further investigation 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 might make allogeneic BMT safer and ultimately more available to many patients who could benefit from this potent therapy.

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