Mouse models of graft-versus-host disease*

Pavan Reddy[1] and James L.M. Ferrara[1,§], [1]University of Michigan Cancer Center, Ann Arbor, MI 48109-5942, USA

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1. Introduction

Allogeneic hematopoietic cell transplantation (HCT) represents an important therapy for many hematological and some epithelial malignancies and for a spectrum of nonmalignant diseases (Appelbaum, 2001). 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 (Welniak et al., 2007). However, the major toxicity of allogeneic HCT, Graft-Versus-Host disease (GVHD), remains a lethal complication that limits its wider application (Ferrara and Reddy, 2006). Depending on when it occurs after HCT, GVHD can be either acute or chronic (Deeg, 2007; Weiden et al., 1979; Weiden et al., 1981; Lee, 2005). Acute GVHD is responsible for 15% to 40% of mortality and is the major cause of morbidity after allogeneic HCT, while chronic GVHD occurs in up to 50% of patients who survive three months after HCT. Mouse models have provided the majority of insights into the biology of this complex disease process.

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§To whom correspondence should be addressed. E-mail: ferrara@umich.edu

The GVHD reaction was first noted when irradiated mice were infused with allogeneic marrow and spleen cells (van Bekkum and De Vries, 1967). Although mice recovered from radiation injury and marrow aplasia, they subsequently died with "secondary disease" (van Bekkum and De Vries, 1967), a syndrome that causes diarrhea, weight loss, skin changes, and liver abnormalities. This phenomenon was subsequently recognized as GVHD disease (GVHD). Three requirements for the developing of GVHD were formulated by Billingham (Billingham, 1966–1967). First, the graft must contain immunologically competent, now recognized as mature T cells. In both experimental and clinical allogeneic BMT, the severity of GVHD correlates with the number of transfused donor T cells (Kernan et al., 1986; Korngold et al., 1987). 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., immunocompromised). A patient with a normal immune system will usually reject cells from a foreign donor. In allogeneic BMT, the recipients are usually immunosuppressed with chemotherapy and/or radiation before stem cell infusion (Welniak et al., 2007). 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; Petersdorf and Malkki, 2006). 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 (Petersdorf and Malkki, 2006; Krensky et al., 1990) discussed below. This chapter on mouse models of acute GVHD will place the immuno-biological mechanisms of Billingham's postulates in perspective.

In addition to these seminal postulates on GVH reaction, the critical requirement of immune cells from the donor graft for optimal leukemia/tumor elimination: a process called graft-versus-leukemia (GVL) effect, and its tight link with GVHD were initially made from mouse models (43). Other models such as the canine, nonhuman primate, and rat models also played important roles, particularly in the development of clinically used immuno-suppressants. Nonetheless, the presence of well-characterized in-bred strains, availability of knock-out and transgenic animals, easy availability of reagents, and the relative low cost have made mouse models the most utilized systems for investigating the mechanisms of GVH responses.

2. Mouse models

Mouse models of GVHD can be grouped into those in which GVHD is directed to MHC (class I, class II, or usually both) or to isolated multiple minor HA alone. Although multiple minor HA mismatches also exist in the former, their impact is usually limited relative to that induced by full MHC disparities (Reddy et al., 2008). The GVHD that develops in response to a full (class I and II) MHC disparity is dependent on CD4 T cells and CD8 T cells provide additive pathology. These systems result in an inflammatory "cytokine storm," capable of inducing GVHD in target tissues without the requirement for cognate T cell interaction with MHC on tissue (Teshima et al., 2002). In contrast to CD4-dependent GVHD, CD8 T cells induce GVHD primarily by their cytolytic machinery, which requires the TCR to engage MHC on target tissue (Reddy et al., 2008) The induction of GVHD to multiple minor HA results in a process where either CD8 T cells, CD4 T cells, or both, depending on the strain combination (see Table 1) may play a role in disease. These different models have helped dissect and refine the various other complex aspects of GVHD (see below). It is critical from the outset to understand that although most clinical BMT recipients are MHC matched but minor HA disparate with the donor, there is no one single most appropriate mouse model of clinical BMT. Experimentally both the MHC disparate and minor HA disparate systems can also induce the full or certain specific aspects of the spectrum of clinically relevant GVHD while permitting the dissection of immunologic mechanisms.

Most mouse models employ radiation for conditioning the recipient animals. Inbred mouse strains demonstrate variable sensitivity to radiation, so maximal tolerated total body irradiation (TBI) doses differ from strain to strain. For example, B6 are more resistant that BALB/C mice, and F1 hybrids are usually either more resistant than parental strain. Generally, the higher the TBI dose, the earlier and greater the intensity of the inflammatory arm of GVHD(see below) and BMT models utilizing low TBI doses and high donor T cell doses will result in GVHD dominated by later onset T cell-dependent pathology (Reddy et al., 2008). Chemotherapeutic conditioning with cyclophosphamide, fludarabine, and busulfan can also be delivered in mouse systems (Ferrara et al., 2005).

Available mouse models (see Table 1) nicely mimic the spectrum of acute GVHD but the induction of clinically relevant chronic GVHD in mouse models using nonmutated inbred strains is challenging. Amongst the commonly utilized models, they either mimic only a few and not all of the manifestations or the kinetics of chronic GVHD. As such, this paucity of appropriate mouse models for chronic GVHD has resulted in a lack of significant understanding of the immunobiology of chronic GVHD when compared with acute GVHD. Below we briefly discuss the current understanding of immuno-biological mechanisms of acute GVHD derived from utilizing mouse models.

Donor	Host	GVHD targets	T cell dependence
Acute GVHD Models			
B6	(B6 × DBA/2)F1	I, II, mHAs	CD4 +/or CD8
B6	BALB/c	I, II, mHAs	CD4 +/or CD8
BALB/c	B6	I, II, mHAs	CD4 +/or CD8
B6	bm I	I	CD8
B6	bm 12	II	CD4
C3H.SW	B6	mHAs	CD8
B6	BALB/b	mHAs	CD4
B10.D2	DBA/2	mHAs	CD4
DBA/2	B10.D2	mHAs	CD8
B10.BR	CBA	mHAs	CD8
Chronic GVHD Models			
B10.D2	BALB/c	mHAs	CD4
LP/J	B6	mHAs	CD4
DBA/2	B6D2F1	I, II, mHAs	CD4
B6	(B6 × DBA/c)F1	I, II, mHAs	CD4
BALB/c	BALB/c × A)F1	I, II, mHAs	CD4

Table 1. Mouse models of BMT.
Donor and host strains used in common BMT models, usual total body irradiation (TBI) doses (delivered) in two split doses on a single day at <150 cGy/min), target GVHD antigens-MHC class I (I), or minor HA (mHA), T cell dependence of subsequent GVHD (CD4 and/or CD8). Source: Biol Blood Marrow Transplantation 14:129–135(2008) *PMID S1083–8791(07)00551–4*.

3. Immunobiology

It is helpful to remember two important principles when considering the pathophysiology of acute GVHD. First, acute GVHD reflects exaggerated, but normal inflammatory mechanisms that occur 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 often been 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 (Reddy and Ferrara 2003). The development and evolution of acute GVHD can be conceptualized in three sequential phases (see 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 (Reddy and Ferrara 2003).

3.1. 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 its treatment or infections that might be further exacerbated by the BMT conditioning regimens of variable intensity which include total body irradiation (TBI and/or chemotherapy) that are administered even before the infusion of donor cells (Clift et al., 1990; Gale et al., 1987; Hill and Ferrara, 2000; Paris et al., 2001; Xun et al., 1994). This first step results in activating 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" (Hill and Ferrara, 2000; Xun et al., 1994; Hill et al., 1997). 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 (Hill and Ferrara, 2000). These "danger signals" activate host APCs (Matzinger, 2002; Shlomchik et al., 1999). 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 lipopolysaccaride (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

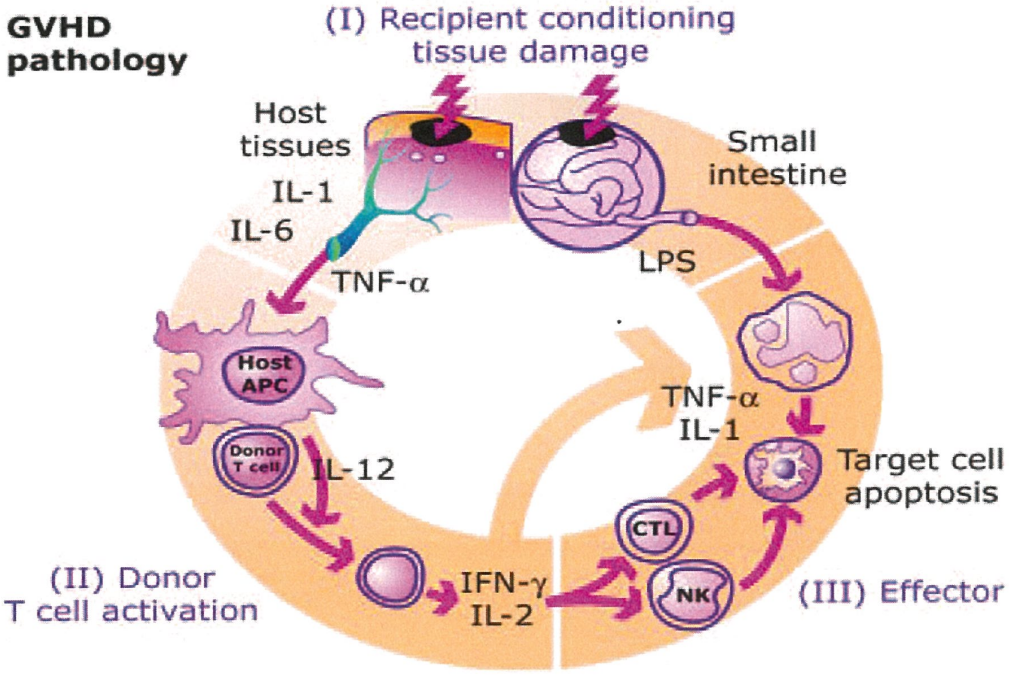

Figure 1. Three phases of GVHD immuno-biology.

between activated APCs and donor T cells (Hill and Ferrara, 2000; Paris et al., 2001; Cooke et al., 1998; Murai et al., 2003). 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 the expression of cell surface adhesion molecules (Hill and Ferrara, 2000; Paris et al., 2001). The relationship among conditioning intensity, inflammatory cytokine, and GVHD severity has been supported by elegant murine studies (Paris et al., 2001; Hill et al., 1997). 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 (Slavin, 2000; Velardi et al., 2002). However, reduced intensity conditioning also causes substantial GVHD. This suggests that in out-bred species that are exposed to infectious agents and in some parent into F1 mouse models, tissue stress and inflammation not caused by conditioning regimen are also sufficient to prime and induce a GVH response.

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 (Teshima et al., 2002; Shlomchik et al., 1999; Jones et al., 2003; Reddy et al., 2005). 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 (Arpinati et al., 2000; Auffermann-Gretzinger et al., 2002; MacDonald et al., 2005). Amongst the cells with antigen-presenting capability, DCs are the most potent and play an important role in the induction of GVHD (Banchereau and Steinman, 1998). Experimental data suggest that GVHD can be regulated by qualitatively or quantitatively modulating distinct DC subsets (Chorny et al., 2006; Duffner et al., 2004; Macdonald et al., 2007; Paraiso et al., 2007; Sato et al., 2003). 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 (Auffermann-Gretzinger et al., 2002). 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 (Nachbaur et al., 2003). 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. This has been further suggested by recent NOD2, MBL and TLR4 polymorphism studies in humans (Holler, 2006; Rocha, 2002; Lorenz et al., 2001).

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 (Rowe, 2006). Also host or donor type nonhematopoietic 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.

The other aspects of the innate immune system such as complement activation, PMNs, and defensins remain poorly understood and they too might play a role in enhancing or regulating the induction and propagation of GVHD. In this regard, a recent study suggests that target tissue inflammation might account for the unique organ specificity of acute GVHD (Chakraverty, 2006).

3.2. Phase 2: Donor T cell activation, differentiation and migration

The infused donor T cells interact with the primed APCs and initiate 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 (Beilhack et al., 2005; Korngold and Sprent, 1980). The T cell receptor (TCR) of the donor T cells can recognize alloantigens either on host APCs (direct presentation) or donor APCs (indirect presentation; Lechler et al., 2001; Shlomchik, 2003). In direct presentation, donor T cells recognize either the peptide bound to allogeneic MHC molecules or allogeneic MHC molecules without peptide (Lechler et al., 2001; Sayegh and Carpenter, 1996). During indirect presentation, T cells respond to the peptide generated by degradation of the allogeneic MHC molecules presented on self-MHC (Sayegh and Carpenter, 1996). An experimental study demonstrated that APCs derived from the host, rather than from the donor, are critical in inducing GVHD across MiHA mismatch (Shlomchik, 2003). Recent data suggest that presenting distinct target antigens by the host and donor type APCs might play a differential role in mediating target organ damage (Shlomchik, 2003; Anderson et al., 2005; Kaplan et al., 2004). In humans, most cases of acute GVHD developed when both host DCs and donor dendritic cells (DCs) were present in peripheral blood after BMT (Auffermann-Gretzinger et al., 2002).

3.2.1. Costimulation

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 (Appleman and Boussiotis, 2003). Both TCR ligation and costimulation via a "second" signal through interaction between the T cell costimulatory molecules and their ligands on APCs are required to achieve T proliferation, differentiation and survival (Sharpe and Freeman, 2002). 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 (Bromley et al., 2001; Dustin, 2001). 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 (Greenwald et al., 2005). Interrupting the second signal by blockade of various positive costimulatory 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 (Welniak et al., 2007; Blazar et al., 1994; Blazar et al., 1995; Blazar et al., 1997; Blazar et al., 2001; Blazar et al., 2003; Blazar et al., 2003). The various T cell and APC costimulatory 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.

3.2.2. 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.

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