Mouse models of graft-versus-host disease*  

Pavan Reddy¹ and James L.M. Ferrara¹,§, ¹University of Michigan Cancer Center, Ann Arbor, MI 48109-5942, USA

Table of Contents

1. Introduction .............................................................................1
2. Mouse models .............................................................................2
3. Immunobiology .........................................................................3
   3.1. Phase 1: Activation of Antigen Presenting Cells (APCs) ........3
   3.2. Phase 2: Donor T cell activation, differentiation and migration .5
      3.2.1. Costimulation .................................................................5
      3.2.2. T cell subsets ...............................................................5
      3.2.3. Cytokines and T cell differentiation .................................7
      3.2.4. Leukocyte migration .......................................................8
   3.3. Phase 3: Effector phase ........................................................8
      3.3.1. Cellular effectors .........................................................9
      3.3.2. Inflammatory effectors .................................................10
4. Conclusion .............................................................................11
5. References ............................................................................11

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.


Copyright: © 2009 Pavan Reddy and James L.M. Ferrara. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

§To whom correspondence should be addressed. E-mail: ferrara@umich.edu
Mouse models of graft-versus-host disease

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.
### Mouse models of graft-versus-host disease

<table>
<thead>
<tr>
<th>Donor</th>
<th>Host</th>
<th>GVHD targets</th>
<th>T cell dependence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute GVHD Models</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B6</td>
<td>(B6 × DBA/2)F1</td>
<td>I, II, mHAs</td>
<td>CD4 +/or CD8</td>
</tr>
<tr>
<td>B6</td>
<td>BALB/c</td>
<td>I, II, mHAs</td>
<td>CD4 +/or CD8</td>
</tr>
<tr>
<td>BALB/c</td>
<td>B6</td>
<td>I, II, mHAs</td>
<td>CD4 +/or CD8</td>
</tr>
<tr>
<td>B6</td>
<td>bm I</td>
<td>I</td>
<td>CD8</td>
</tr>
<tr>
<td>B6</td>
<td>bm 12</td>
<td>II</td>
<td>CD4</td>
</tr>
<tr>
<td>C5H.SW</td>
<td>B6</td>
<td>mHAs</td>
<td>CD8</td>
</tr>
<tr>
<td>B6</td>
<td>BALB/b</td>
<td>mHAs</td>
<td>CD4</td>
</tr>
<tr>
<td>B10.D2</td>
<td>DBA/2</td>
<td>mHAs</td>
<td>CD4</td>
</tr>
<tr>
<td>B10.D2</td>
<td>B6</td>
<td>mHAs</td>
<td>CD8</td>
</tr>
<tr>
<td>B10.BR</td>
<td>CBA</td>
<td>mHAs</td>
<td>CD8</td>
</tr>
<tr>
<td><strong>Chronic GVHD Models</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B10.D2</td>
<td>BALB/c</td>
<td>mHAs</td>
<td>CD4</td>
</tr>
<tr>
<td>LP/J</td>
<td>B6</td>
<td>mHAs</td>
<td>CD4</td>
</tr>
<tr>
<td>DBA/2</td>
<td>B6D2F1</td>
<td>I, II, mHAs</td>
<td>CD4</td>
</tr>
<tr>
<td>B6</td>
<td>(B6 × DBA/2)F1</td>
<td>I, II, mHAs</td>
<td>CD4</td>
</tr>
<tr>
<td>BALB/c</td>
<td>BALB/c × A)F1</td>
<td>I, II, mHAs</td>
<td>CD4</td>
</tr>
</tbody>
</table>

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 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 (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 alloimmune 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.
Mouse models of graft-versus-host disease

<table>
<thead>
<tr>
<th>T cell costimulation</th>
<th>T cell</th>
<th>APC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adhesion</strong></td>
<td>ICAMs</td>
<td>LEA-1</td>
</tr>
<tr>
<td></td>
<td>LEA-1</td>
<td>ICAM-~</td>
</tr>
<tr>
<td></td>
<td>CD2 (LEA-2)</td>
<td>LFA-3</td>
</tr>
<tr>
<td><strong>Recognition</strong></td>
<td>TCR/CD4</td>
<td>NIIIC hi</td>
</tr>
<tr>
<td></td>
<td>TCR/CD8</td>
<td>Mi-lcc 1</td>
</tr>
<tr>
<td><strong>Costimulation</strong></td>
<td>CD28</td>
<td>CD80/86</td>
</tr>
<tr>
<td></td>
<td>CD152 (CTLA-4)</td>
<td>CD80/86</td>
</tr>
<tr>
<td></td>
<td>ICOS</td>
<td>B7H/B7RP-1</td>
</tr>
<tr>
<td></td>
<td>PD-1</td>
<td>PD-L1, PD-L2</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>B7-H3</td>
</tr>
<tr>
<td></td>
<td>CD 154 (CD4OL)</td>
<td>CD40</td>
</tr>
<tr>
<td></td>
<td>CD134 (OX40)</td>
<td>CD13L(OX40L)</td>
</tr>
<tr>
<td></td>
<td>CD137 (4-IBB)</td>
<td>CD137L (4-1BB)</td>
</tr>
<tr>
<td></td>
<td>HVEM</td>
<td>LIGHT</td>
</tr>
</tbody>
</table>

Table 2. T-cell-APC Interactions that Regulate GVHD.

- **CD4+ and CD8+ cells**

CD4 and CD8 proteins are coreceptors for constant portions of MHC class II and class I molecules, respectively (Csencsits and Bishop, 2003). Therefore, MHC class I (HLA-A, -B, -C) differences stimulate CD8+ T cells and MHC class II (HLA-DR, -DP, -DQ) differences stimulate CD4+ T cells (Csencsits and Bishop, 2003; Ferrara et al., 1993; Korngold and Sprent, 1982; Korngold and Sprent, 1985; Korngold and Sprent, 1987). But clinical trials of CD4+ or CD8+ depletion have been inconclusive (Wu and Ritz, 2006). This may not be 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 (Goulmy, 2006). Because the manner of protein processing depends on genes of the MHC, two siblings will have many different peptides in the MHC groove (Goulmy, 2006). 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 (Wu and Ritz, 2006). The peptide repertoire for class I or class II MHC remains unknown and likely to be distinct between one individual to the next (Spierings et al., 2006). But it is plausible that only a few of these many peptides might behave as immunodominant “major minor” antigens that can potentially induce GVHD. In any event, such antigens remain to be identified and validated in large patient populations.

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 (Sykes, 2001; Wekerle et al., 1998; Wekerle et al., 2000). In contrast peripheral mechanisms to induce tolerance of CD4+ and CD8+ T cells appears to be distinct (Wells et al., 1999; Wells et al., 2001). The T cell apoptosis pathways by which peripheral deletion occurs can be broadly categorized into activation-induced cell death (AICD) and passive cell death (PCD; Lechler et al., 2003). Experimental data suggests that deletional tolerance by AICD is operative via the Fas (for CD4+) or TNFR (CD8+) pathways in Th1 cells and when there is a higher frequency of alloreactive cells (Combadiere et al., 1998; Min et al., 2004; Siegel et al., 2000; Zhang et al., 1997; Zheng et al., 1995). PCD or “death by neglect” is due to rapid downregulation of Bcl-2 and appears to be critical in non-irradiated, but not after irradiated BMT (Drobyski et al., 2002). 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 appear promising in reducing experimental acute GVHD (Bondanza et al., 2006; Bonini et al., 1997; Bordignon et al., 1995; Chen et al., 2002; Chen et al., 2002; Drobyski and Gendelman, 2002).

- **Naïve and Memory Subsets**

Several independent groups have intriguingly found that, unlike memory (CD62L−) T cells, the naïve (CD62L+) T cells were alloreactive and caused acute GVHD across different donor/recipient strain combinations (Anderson et al., 2003; Chen et al., 2004; Maeda et al., 2007; Zhang et al., 2004). Furthermore, expression of the naïve T cell
Donor CD4+ also show that activated donor NK cells can reduce GVHD through the elimination of host APCs or by secretion of acute GVHD in an IL-4 dependent manner. (Lowsky et al., 2005; Zeng et al., 1999; Hashimoto et al., 2005) By contrast, evaluated the relationship between donor CD4+ type myeloid APCs were also able to suppress acute GVHD (Sato et al., 2003). One of the clinical studies that including cytokines and their receptors. The Th1 cytokines (IFN-γ, IL-2 and TNF-α) have been implicated in the pathophysiology of acute GVHD (Antin and Ferrara, 1992; Ferrara and Krenger, 1998; Liu et al., 2007; Ratanatharathorn et al., 1998; Reddy, 2003). IL-2 production by donor T cells remains the main target of many current clinical therapeutic approaches, such as cyclosporine, tacrolimus and monoclonal antibodies (mAbs) against the IL-2 and its receptor to control acute GVHD (Ferrara and Krenger, 1998; Liu et al., 2007; Ratanatharathorn et al., 1998; Reddy, 2003; Ferrara, 1994). But emerging data indicate an important role for naturally occurring 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 (Edinger et al., 2003; Nguyen et al., 2007). CD4+CD25+ T cells induced/generated by immature or regulatory host type DCs and by regulatory donor type myeloid APCs were also able to suppress acute GVHD (Sato et al., 2003). 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 (Stanzani et al., 2003). These data suggest that coexpression 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 (Miura et al., 2004; Zorn et al., 2005). But Foxp3 expression in humans, unlike mice, may not be specific for T cells with a regulatory phenotype (Ziegler, 2006). It is likely that the precise role of regulatory T cells in clinical acute GVHD will, therefore, not only depend upon identifying 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 United States and Europe to substantially expand these cells ex vivo and use for prevention of GVHD.

Host NK1.1+ T cells are another T cell subset with suppressive functions that have also been shown to suppress acute GVHD in an IL-4 dependent manner. (Lowsky et al., 2005; Zeng et al., 1999; Hashimoto et al., 2005) By contrast, donor NKT cells were found to reduce GVHD (Morris et al., 2005; Morris et al., 2006) and enhance perforin mediated GVL in an IFN-γ dependent manner. Recent clinical data suggests that enhancing recipient NKT cells by repeated TLI conditioning promoted Th2 polarization and dramatically reduced GVHD (Lowsky et al., 2005). 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; Morris et al., 2006). 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 (Wang et al., 2002).

### 3.2.3. 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 (Antin and Ferrara, 1992; Ferrara and Krenger, 1998; Liu et al., 2007; Ratanatharathorn et al., 1998; Reddy, 2003). 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 (Ferrara and Krenger, 1998; Liu et al., 2007; Ratanatharathorn et al., 1998; Reddy, 2003; Ferrara, 1994). But emerging data indicate an important role for IL-2 in the generation and maintenance of CD4+CD25+ Foxp3+ Tregs, suggesting that prolonged interference with IL-2 may have an unintended consequence of preventing the development of long-term tolerance after allogeneic HCT (Gavin et al., 2007; Liston and Rudensky, 2007; Zeiser et al., 2006; Zhang et al., 2005).

Similarly the role of other Th1 cytokines IFN-γ or their inducers as 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 (Reddy, 2004; Sykes et al., 1999; Yang et al., 1997). Thus, although the “cytokine storm” initiated in phase 1 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

---

**Stembook.org**
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 (Reddy, 2003; Reddy, 2004; Reddy et al., 2001; Sykes et al., 1995). 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 (Fowler et al., 1994; Fowler and Gress, 2000; Hill et al., 1998; Jung et al., 2006; Krenger et al., 1995; Fan et al., 1995; Reddy et al., 2003). 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 (Nikolic et al., 2000). 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 suppressing immune responses and its role in regulating experimental acute GVHD is unclear (Blazar et al., 1998). Recent clinical data demonstrate an unequivocal association between IL-10 polymorphisms and the severity of acute GVHD (Lin et al., 2003). TGF-β, another suppressive cytokine, was shown to suppress acute GVHD, but to exacerbate chronic GVHD (Banovic et al., 2005). The roles of some other cytokines, such as IL-7 (that promotes immune reconstitution) and IL-13, remain unclear (Alpdogan et al., 2001; Alpdogan et al., 2003; Gendelman et al., 2004; Sinha et al., 2002). For example, interaction between α4β7 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 (Murai et al., 2003; Waldman et al., 2006). αLβ2/ICAM1, 2, 3 and α4β1/VCAM-2 interactions are important for homing to the lung and liver after experimental HCT (Wysocki et al., 2005). Expressing 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 (Beilhack et al., 2005). The migratory requirement of donor T cells to specific lymph nodes (e.g., Peyer’s patches) for the induction of GVHD might be dependent on other factors such as the conditioning regimen, inflammatory milieu etc. (Murai et al., 2003; Welniak et al., 2006). Furthermore, FTY720, a pharmacologic sphingosine-1-phosphate receptor agonist, inhibited GVHD in murine, but not in canine models of HCT (Kim et al., 2003; Lee et al., 2003). Thus, there might also be significant species differences in the ability of these molecules to regulate GVHD.

### 3.2.4. 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 causing tissue damage (Wysocki et al., 2005). 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 (Cyster, 2005). T-lymphocyte production of macrophage inflammatory protein-1α (MIP-1α) is critical to the recruitment of CD8+ but not CD4+ T cells to the liver, lung and spleen during acute GVHD (Serody et al., 2000). Several chemokines such as CCL2–5, CXCL2, CXCL9–11, CCL17 and CCL27 are overexpressed and might play a critical role in the migration of leukocyte subsets to target organs liver, spleen, skin and lungs during acute GVHD (Wysocki et al., 2005; Cyster, 2005; Serody et al., 2000; Mapara et al., 2006). CXCR3+ T and CCR5+ T cells cause acute GVHD in the liver and intestine (Wysocki et al., 2005; Duffner et al., 2003; Murai et al., 1999; Wysocki et al., 2004). CCR5 expression has also been found to be critical for Treg migration in GVHD (Wysocki et al., 2005). 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 (Murai et al., 2003; Cyster, 2005; Waldman et al., 2006). For example, interaction between α4β7 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 (Murai et al., 2003; Waldman et al., 2006). αLβ2/ICAM1, 2, 3 and α4β1/VCAM-2 interactions are important for homing to the lung and liver after experimental HCT (Wysocki et al., 2005). Expressing 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 (Beilhack et al., 2005). The migratory requirement of donor T cells to specific lymph nodes (e.g., Peyer’s patches) for the induction of GVHD might be dependent on other factors such as the conditioning regimen, inflammatory milieu etc. (Murai et al., 2003; Welniak et al., 2006). Furthermore, FTY720, a pharmacologic sphingosine-1-phosphate receptor agonist, inhibited GVHD in murine, but not in canine models of HCT (Kim et al., 2003; Lee et al., 2003). Thus, there might also be significant species differences in the ability of these molecules to regulate GVHD.

### 3.3. 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 (Sallusto et al., 2000). 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; Wysocki et al., 2005; Cyster, 2005; Serody et al., 2000; Mapara et al., 2006; Duffner et al., 2003; Murai et al., 1999; Wysocki et al., 2004; Waldman et al., 2006; Welniak et al., 2006; Kim et al., 2003; Lee et al., 2003; Sallusto et al., 2000; Sackstein, 2006; Wysocki et al., 2003).
3.3.1. Cellular effectors

Cytotoxic T cells (CTLs) are the major cellular effectors of GVHD (Kagi et al., 1994; Van Den Brink and Burakoff, 2002). 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 (Van Den Brink and Burakoff, 2002; Chicheportiche et al., 1997; Jiang et al., 1998; Jiang et al., 2004; Maeda et al., 2005; Pan et al., 1997). 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 (Voskoboinik et al., 2006). Ligation of Fas results in a death-inducing signaling complex (DISC) and also activates caspases (Chinnaiyan et al., 1996; Krammer, 2000).

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 (Zeng et al., 1999), and across isolated MHC Class I or Class II disparities (Zeng et al., 1999; Van Den Brink and Burakoff, 2002; Baker et al., 1997; Braun et al., 1996; Graubert et al., 1996; Graubert et al., 1997). 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 play 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 the Fas/FasL pathway in CD4-mediated GVHD (Graubert et al., 1996; Graubert et al., 1997; Ueno et al., 2000). 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 (Aggarwal, 2003). 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 (Van Den Brink et al., 2000; Via et al., 1996). FasL-defective T cells cause less GVHD in the liver, skin and lymphoid organs (Baker et al., 1997; Ueno et al., 2000; Van Den Brink et al., 2000; Via et al., 1996). 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 (Van Den Brink et al., 2000). 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 (Van Den Brink and Burakoff, 2002; Van Den Brink et al., 2000; Hattori et al., 2005). 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 (Lin et al., 1998). Elevated serum levels of soluble Fasl and Fas have also been observed in at least some patients with acute GVHD (Das et al., 1999; Liem et al., 1998).

Using 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 (Braun et al., 1996). However, subsequent studies demonstrated that cytotoxic effector mechanisms of donor T cells are critical in preventing host resistance to GVHD (Maeda et al., 2005; Martin et al., 1998). Thus, when recipient mice were treated with a lethal dose of irradiation, cdd (CD4+ T cells produced similar mortality to wild type CD4+ T cells; Maeda et al., 2005). These results were confirmed by a recent study demonstrating that GVHD target damage can occur in mice that lack allotengen expression on the epithelium, preventing direct interaction between CTLs and target cells (Maeda et al., 2005).

The participation of other 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 LTα/LIGHT, and are all assumed to play a role in GVHD and GVL responses (Welniak et al., 2007; Brown and Thiele, 2000; Brown et al., 2002; Brown et al., 2005; Sato et al., 2005; Schmaltz et al., 2002; Xu et al., 2006; Zimmerman et al., 2005). 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 (Zimmerman et al., 2005; Zimmerman et al., 2005). 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 (Van Den Brink and Burakoff, 2002). 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 there might be some distinction between the use of different lytic pathways for the specific GVHD target organs and GVL, the clinical applicability of these observations is as yet largely unknown

3.3.2. 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 (Antin and Ferrara, 1992). 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 (Ferrara et al., 1999). 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 (Hill and Ferrara, 2000; Iwasaki and Medzhitov, 2004). 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 (Iwasaki and Medzhitov, 2004; Fritz et al., 2006). Recent clinical studies of GVHD suggested the possible association with TLR/NOD polymorphisms and the severity of GVHD (Lorenz et al., 2001; Dickinson et al., 2004; Holler et al., 2006). 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 (Teshima et al., 2002). The severity of GVHD appears to be directly related to the level of innate and adaptive immune cell priming and release of proinflammatory cytokines such as TNF-α, IL-1 and nitric oxide (NO; Teshima et al., 2002; Hill and Ferrara, 2000; Hill et al., 1999; Krenger et al., 1996; Nestel et al., 1992).

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, pleiotropic, and redundant effects on both activation and effector phases of GVHD (Reddy, 2003). 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 (Piguet et al., 1987). Target organ damage could be inhibited by an infusion of anti-TNF-α MAbs, and mortality could be reduced from 100% to 50% by the administration of soluble TNF-α receptor (sTNFR), an antagonist of TNF-α (Xun et al., 1994; Hill et al., 1997; Hill et al., 1999). Accumulating experimental data further suggest that TNF-α is involved in a multi-step 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 in involves in donor T cell activation directly through its signaling via TNFR1 and TNFR2 on T cells. TNF-TNF1 interactions on donor T cells promote alloreactive T cell responses, and TNF-TNF2 interactions are critical for intestinal GVHD (Brown et al., 2002; Hill et al., 2000). TNF-α also seems to be an important effector molecule in GVHD in skin and lymphoid tissue (Piguet et al., 1987; Ferrara and Burakoff, 1990). 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 (Tanaka et al., 1993). An important role for TNF-α in clinical acute GVHD has been suggested by studies demonstrating elevated serum levels or TNF-α or elevated 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; Tanaka et al., 1993; Holler et al., 1993; Tanaka et al., 1993). 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 (Herve et al., 1992; Uberti et al., 2005).

The second major proinflammatory cytokine that appears to play an important role in the effector phase of acute GVHD is IL-1 (Antin and Ferrara, 1992). Secretion of IL-1 appears to occur predominantly during the effector phase
of GVHD of the spleen and skin, two major GVHD target organs (Abhyankar et al., 1993). 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 administering 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, intraperitoneal administration of IL-1ra starting on day 10 post-transplant reversed the development of GVHD in the majority of animals, giving treated animals a significant survival advantage (McCarthy et al., 1991). However, the attempt to use IL-1ra to prevent acute GVHD in a randomized trial was not successful (Antin et al., 2002). As a result of activation during GVHD, macrophages also produce Nitric Oxide (NO), which contributes to the deleterious effects on GVHD target tissues, particularly immunosuppression (Krenger et al., 1996; Falzarano et al., 1996). NO also inhibits the repair mechanisms of target tissue destruction by inhibiting proliferation of epithelial stem cells in the gut and skin (Nestel et al., 2000). In humans and rats, the development of GVHD is preceded by an increase in serum levels of NO oxidation products (Bogdan, 2001; Langrehr et al., 1992; Langrehr et al., 1992; Weiss et al., 1995).

Existing data derived from mouse models demonstrate important role for various inflammatory effectors in GVHD. Although some have shown to be of therapeutic relevance (TNF-α) and the role of some others (IL-1) has not been clinically validated. 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.

4. Conclusion

Studies from murine models have contributed to the outstanding progress in understanding the biological basis of GVHD. The three phase model, developed largely on the basis of murine studies, allows for an easy perspective of the complex process of GVHD but is not meant to suggest that GVHD actually occurs to such discrete phases or steps. The mouse models have allowed for a more refined understanding of the cellular interactions and networks that impact upon GVHD. Further improvements and greater sophistication in analysis of the existing mouse models over the next few years will undoubtedly bring better refinement and greater understanding of this complex immunological process.

5. References


Mouse models of graft-versus-host disease


Mouse models of graft-versus-host disease


Mouse models of graft-versus-host disease


Mouse models of graft-versus-host disease


Mouse models of graft-versus-host disease


Mouse models of graft-versus-host disease


Mouse models of graft-versus-host disease


Mouse models of graft-versus-host disease


