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Mouse models of graft-versus-host disease: advances and limitations

Mark A. Schroeder1 and John F. DiPersio1,*

The limiting factor for successful hematopoietic stem cell transplantation (HSCT) is graft-versus-host disease (GvHD), a post-transplant disorder that results from immune-mediated attack of recipient tissue by donor T cells contained in the transplant. Mouse models of GvHD have provided important insights into the pathophysiology of this disease, which have helped to improve the success rate of HSCT in humans. The kinetics with which GvHD develops distinguishes acute from chronic GvHD, and it is clear from studies of mouse models of GvHD (and studies of human HSCT) that the pathophysiology of these two forms is also distinct. Mouse models also further the basic understanding of the immunological responses involved in GvHD pathology, such as antigen recognition and presentation, the involvement of the thymus and immune reconstitution after transplantation. In this Perspective, we provide an overview of currently available mouse models of acute and chronic GvHD, highlighting their benefits and limitations, and discuss research and clinical opportunities for the future.

Introduction

A major complication and limitation to the broad application of hematopoietic stem cell transplantation (HSCT) is graft-versus-host disease (GvHD), which results from immune-mediated attack of recipient tissue by donor T cells contained in the transplant. GvHD accounts for 15-30% of deaths that occur following allogeneic HSCT that is carried out to treat malignant diseases, and is a major cause of morbidity in up to 50% of transplant recipients (Ferrara et al., 2009). This high incidence is despite the use of aggressive immunosuppressive therapies after transplantation, as well as allele-level human leukocyte antigen (HLA) matching between donor and recipient. Despite recent advances to reduce the incidence of GvHD through altering prophylactic regimens and reducing the intensity of conditioning prior to transplantation, effective treatments for GvHD are lacking. Thus, accurate and reproducible experimental models of GvHD are essential for advancing our fundamental understanding of this disorder and for developing new treatments. In this Perspective, we provide an overview of available mouse models of acute and chronic GvHD, discuss their uses and limitations, and provide guidance on selecting an appropriate model. We also briefly discuss the graft-versus-leukemia (GVL) effect, although readers are referred to other recent reviews for more detail on this topic (Bleakley and Riddell, 2004; Ringden et al., 2009). We begin by describing the clinical symptoms and immunopathology of GvHD.

GvHD immunopathology in humans and mice

In humans, GvHD manifests as acute GvHD (aGvHD), which occurs within 100 days of transplant, or as chronic GvHD (cGvHD), which typically develops 100 days after transplantation and can take 2-5 years to become clinically evident. This temporal distinction does not necessarily translate to mouse models of the disease, which can differ in time of onset and are mainly defined by their clinical phenotype: cGvHD develops within weeks after transplantation in most mouse models. In addition, in humans, aGvHD typically precedes cGvHD, although in some cases cGvHD can occur without the occurrence of clinically obvious aGvHD. One factor that confounds the translation of findings in mouse models to the human disease is that most patients are given immunosuppressive therapy to prevent aGvHD, and these medications might influence the development of cGvHD. Interestingly, interventions to prevent aGvHD in the clinic have, in general, not significantly decreased rates of cGvHD in humans (Lee, 2010).

The sequence of events that lead to the development of GvHD has largely been defined using mouse models. Early work established that T-cell alloreactivity is the underlying cause of the disease (Korngold and Sprent, 1978; Sprent et al., 1986). The pathology of both acute and chronic mouse models of GvHD relies on T-cell alloreactivity, but each form has a different phenotype owing to differential involvement of cytotoxic (CD8+) or helper (CD4+) T-cell subsets. Donor CD8+ T cells are activated when their T-cell receptor (TCR) binds to recipient peptides presented in the context of recipient class I major histocompatibility complex (MHC) molecules (see Box 1 for a glossary of immunological terms). T-cell activation also requires the engagement of co-stimulatory receptors on activated donor T cells by their cognate ligands expressed on donor antigen-presenting cells (APCs). CD8+ T-cell cytotoxicity is mediated by perforin, granzymes and Fas ligand, and inflammatory cytokines augment this response. Donor CD4+ T cells are activated when their TCR binds to exogenous

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Mouse models of GvHD

Key events involved in the development of aGvHD in mice are illustrated in Fig. 1. First, priming of the immune response establishes a milieu for enhanced T-cell activation and expansion, and occurs as a direct result of ‘conditioning’. Conditioning suppresses the recipient’s immune system and is applied to allow engraftment of donor hematopoietic stem cells. It can be achieved with cytokotic agents such as irradiation or chemotherapy, or with immunosuppressive agents such as anti-thymocyte globulin or drugs that target recipient T cells, such as fludarabine. The most common method of conditioning in mouse models is total body irradiation, which ablates the recipient’s bone marrow, allowing donor stem cell engraftment and preventing rejection of the graft by limiting the proliferation of recipient T cells in response to donor cells. Subsequent activation of donor T cells depends on cognate antigen recognition. T cells are also activated by non-cognate stimulation by cytokines such as IL-1 and TNFα that are released during priming by many different cell types (Ferrara et al., 1993). Subsequent expansion of donor T cells and release of cytokines such as IFNγ leads to the activation of additional effector cell types, including neutrophils, monocytes and natural killer (NK) cells, which can result in further donor T-cell proliferation independent of their interactions with cognate antigen (Teshima et al., 2002).

In addition, proinflammatory macrophages are activated, which can release IL-1β, TNFα and nitric oxide (Nestel et al., 1992; Cooke et al., 1998).
The final step leading to end organ damage in aGVHD is T-cell-mediated tissue toxicity, which involves soluble mediators, including TNFα, perforin, granzymes, Fas and Fas ligand (Baker et al., 1996; Braun et al., 1996; Graubert et al., 1997; Jiang et al., 2001; Maeda et al., 2005). In addition, T helper 17 (Th17) cells are a subset of T cells that have recently been shown to contribute to this final cytotoxic phase and can cause increased inflammation and cytotoxicity when supplemented in the graft in an experimental setting. A recent report demonstrated that Th17 cells were sufficient but not necessary to cause GvHD (Iclozan et al., 2009). Surprisingly, however, some studies have reported that their absence from the graft can also worsen GvHD (Yi et al., 2008; Carlson et al., 2009; Kappel et al., 2009). It is hoped that future studies will resolve this discrepancy to clarify the role of Th17 cells in GvHD pathology. A crucial counterpart to Th17 cells are regulatory T (TReg) cells, which have been shown to suppress a wide range of immune responses, including alloreactive and autoreactive T-cell responses (Edinger et al., 2003; Sakaguchi et al., 2006).

**cGVHD immunopathology**

Unlike aGVHD, cGVHD typically presents as an autoimmune-like syndrome. As well as effects mediated by T cells, cGVHD involves B-cell stimulation, autoantibody production and systemic fibrosis. Mouse models of cGVHD are associated with prolonged survival compared with mouse models of aGVHD. Additional clinical characteristics depend on the mouse model and can include splenomegaly and lymphadenopathy resulting from B-cell expansion, glomerulonephritis, biliary cirrhosis and scleroderma.

The immunopathology underlying cGVHD is less well understood than that of aGVHD, in part because of the limitations of currently available mouse models. Unlike aGVHD, there is no unifying theory for the development of cGVHD, and the pathological mechanisms differ depending on the model system. Mouse models of cGVHD typically involve three main pathological mechanisms: autoantibody production, pro-fibrotic pathways and defective thymic function. Here, we describe the main events that underlie the phenotype, although models exploring the three main mechanisms will be discussed in detail later.

A summary of our current knowledge of the presumed sequence of events leading to end organ damage in cGVHD is outlined in Fig. 2. First, unlike in aGVHD, immune priming in cGVHD models is not dependent on tissue damage from conditioning: compared with aGVHD models, the initial inflammatory milieu is reduced or absent in cGVHD models, which involve reduced levels of radiation or no conditioning at all. The outcome of transplantation is mixed T-cell chimerism in most models. Thymic damage can result in dysfunctional negative selection of autoreactive T cells, and there can be decreased TReg cell number or function. In addition, donor and recipient B cells recognize and process extracellular antigens for presentation in the context of MHC. All of these processes can contribute to the initiation of cGVHD. Second, unlike in aGVHD, T-cell activation is not dependent on recipient APCs, but is a mix of cognate antigen recognition and cross-priming of donor T cells. CD4+ T cells are necessary and sufficient to cause cGVHD in mouse models, whereas cytotoxic CD8+ T cells are not required, in contrast to aGVHD (Rolink and Gleichmann, 1983; Rolink et al., 1983; Zhang et al., 2006). Recipient B cells are activated by minor antigens and Th2 cytokines, and mature to present processed antigens in the context of MHC to donor CD4+ T cells, which in turn co-stimulate B cells to produce autoantibodies and additional stimulatory cytokines (Morris et al., 1990a; Shimabukuro-Vornhagen et al., 2009). Third, T and B cells clonally expand in response to antigenic stimulation and co-stimulation, and form memory and effector cells. In addition, epitope spreading can occur, resulting in oligoclonal T-cell expansion. Fourth, clonally expanded T and B cells can traffic to target organ sites and mediate damage through cytokine-mediated mechanisms.
Mouse models of aGvHD

Most mouse models of aGvHD (summarized in Table 1) involve the transplantation of T-cell-depleted bone marrow supplemented with varying numbers and phenotypic classes of donor lymphocytes (either splenocytes or lymph node T cells) into lethally irradiated recipients. The bone marrow provides donor stem cells that allow hematopoietic reconstitution after transplant; T-cell depletion is carried out to control the dose and type of immune cells that are delivered.

The discovery of the MHC and minor histocompatibility antigens (miHAs) has been integral to advancing the field of HSCT. Differences in MHC antigens are largely responsible for immune-mediated rejection of foreign tissues and cells; however, even in an MHC-matched setting, differences in miHAs can cause grafts to be slowly rejected. Unlike humans, inbred mouse strains are closely related with respect to MHC antigens (note that class I MHC is referred to as H2 in mice). Although two strains of mice might share the same H2 (i.e. they are MHC-I-matched), differences in miHAs can result in GvHD in the context of experimental HSCT. MHC-mismatched and miHA-mismatched models are discussed below. In addition, we describe more recently developed models involving the xenotransplantation of human cells into immunodeficient mice.

The severity of aGvHD depends on several factors. First, the dose and type of T-cell subsets (i.e. CD4+, CD8+ or TReg cells) that are transferred with donor bone marrow can affect the severity of the disease (Sprent et al., 1988; Korngold, 1992; Edinger et al., 2003). Second, irradiation dose is proportional to the degree of tissue damage and the subsequent cytokine storm, and thus is directly proportional to aGvHD-related mortality in the mouse (Hill et al., 1997; Schwarte and Hoffmann, 2005; Schwarte et al., 2007). In addition, the dose of irradiation that is myeloablative varies by mouse strain: inbred strains are generally more susceptible to radiation than their F1 progeny, and different strains show susceptibility differences (e.g. BALB/c>C3H>C57Bl/6). Third, genetic disparities play a major role in disease. For example, even mice that are MHC matched can vary by miHAs that influence aGvHD severity. Fourth, variation in environmental pathogens between labs and in mice from different suppliers can affect GvHD (Nestel et al., 1992). Therefore, the same model of aGvHD can vary between investigators because of differences in radiation dose and delivery rate, differences in pathogens between animal facilities, and differences in T-cell dose and sources of T cells.

Fig. 2. Steps to cGvHD in mice. The progression of events occurring in the development of cGvHD in the mouse is illustrated. The five steps illustrate immune priming (A), activation of T and B cells (B), T-cell expansion and B-cell autoantibody production (C), trafficking to sites of tissue damage (D), and end organ damage through chronic inflammation and fibrosis (E). See main text for full details.
Table 1. Mouse models of aGvHD

| Donor strain | Recipient strain | Conditioning regimen | Genetics | Main T-cell type contributing to phenotype | Cell type and dose | Outcome | Reference example(s) |
|--------------|------------------|----------------------|----------|----------------------------------|-------------------|---------|---------------------|
| C57/Bl6 (H2^b) | BALB/c (H2^d) | 900 cGy | Mismatched for MHC I, MHC II and miHAs | CD4^+ and CD8^+ | Splenic T cells (0.5-2x10^6) and TCD donor BM cells | Systemic disease by 10-21 days; lethal | van Leeuwen et al., 2002 |
| C3H/HeJ (H2^b) | C57/Bl6 (H2^b) | 900 cGy | Mismatched for MHC I, MHC II and miHAs | CD4^+ and CD8^+ | 2x10^6 BM cells and 5-25x10^6 spleen cells or 0.1-1x10^6 lymph node cells | Systemic disease by 10-30 days; lethal | Blazar et al., 1991 |
| C57/Bl6 (H2^b) | B6C3F1 (H2^k/b) | 600-1050 cGy | Mismatched for MHC I, MHC II and miHAs | CD4^+ and CD8^+ | 7x10^6 spleen cells and 1x10^7 TCD BM | Systemic disease; lethal | Kanamaru et al., 1984; Fowler et al., 1996 |
| C57/Bl6 (H2^b) | B6D2F1 (H2^k/b) | No XRT or 1100-1300 cGy | Mismatched for MHC I, MHC II and miHAs | CD4^+ and CD8^+ | Whole splenocytes: 1-10x10^6 cells if no XRT; 2.5x10^5 purified T cells with XRT | Systemic disease; lethal depending on T-cell dose and source; symptoms by 30-50 days if no XRT | Pickel and Hoffmann, 1977; Via et al., 1996a; Krenger et al., 2000a; Hildebrandt et al., 2004; Blaser et al., 2005; Niculescu et al., 2005 |
| C57/Bl6 (H2^b) | B6AF1 (H2^k/a) | No XRT | Mismatched for MHC I, MHC II and miHAs | CD4^+ and CD8^+ | Whole lymphoid cells (2-6x10^7) | Systemic disease; lethal depending on T-cell dose | Ghayur et al., 1988; Nestel et al., 1992; Gendelman et al., 2004 |
| C57/Bl6 (H2^b) | B10.8R (H2^b) | 750-900 cGy +/- Cytoxan 120 mg/kg | Mismatched for MHC I, MHC II and miHAs | CD4^+ and CD8^+ | 2.5x10^5 to 2x10^7 TCD BM cells and 5-25x10^6 spleen cells or 0.5-1x10^6 lymph node cells | Systemic disease; lethal depending on T-cell dose and source; symptoms by 14-28 days | Valera et al., 1981; Blazar et al., 1991; Panoskaltsis-Mortari et al., 1998 |
| C57/Bl6 (H2^b) | B6.C-H2^bm1 (bm1) (H2^b background with mutation at MHC I) | 950 cGy or sublethal XRT | MHC I mismatch | CD8^+ | 4x10^6 TCD BM cells and 1.5-7.5x10^5 CD8^- T cells | Systemic disease if CD8^- T cells infused with death by 30-80 days | Rolink et al., 1983; Sprent et al., 1986 |
| C57/Bl6 (H2^b) | B6.C-H2^bm12 (bm12) (H2^b background with mutation at MHC II) | 950 cGy or sublethal XRT | MHC II mismatch | CD4^+ | 4x10^7 TCD BM cells and 1-5x10^6 CD4^+ T cells | Systemic disease if CD4^+ T cells infused with death by 20-40 days | Rolink et al., 1983; Sprent et al., 1986 |

miHA mismatched

| Donor strain | Recipient strain | Conditioning regimen | Genetics | Main T-cell type contributing to phenotype | Cell type and dose | Outcome | Reference example(s) |
|--------------|------------------|----------------------|----------|----------------------------------|-------------------|---------|---------------------|
| B10.8R (H2^b) | CBA or BALB.K (H2^d) | 750 cGy | miHA mismatches | CD8^+ | 8x10^6 TCD BM cells and 1x10^6 whole splenocytes or 5x10^6 CD4^- or CD8^- T cells | Systemic disease; symptoms include tail scaling, ear erosions, diarrhea, hunching | Korngold and Sprent, 1987; Korngold, 1992; Cooke et al., 1996; Kaplan et al., 2004; Fanning et al., 2006 |
| B10.D2 (H2^b) | DBA/2 (H2^b) | 800 cGy | miHA mismatches | CD4^- (minor contribution by CD8^-) | 4x10^7 TCD BM cells and 1x10^6 CD4^- T cells, or purified CD4^- or CD8^- T cells | Systemic disease; lethal by day 80 | Korngold and Sprent, 1987; Korngold, 1992 |

Table 1 continued on next page.
### Table 1. Continued

| Donor strain | Recipient strain | Conditioning regimen | Genetics | Main T-cell type contributing to phenotype | Cell type and dose | Outcome | Reference example(s) |
|--------------|------------------|----------------------|----------|--------------------------------------------|--------------------|---------|----------------------|
| miHA mismatched | B10.D2 (H2d)     | BALB/c (H2a)         | Sub-lethal 600-750 cGy or Fractionated 1000 cGy | miHA mismatches   | CD4 (minor contribution by CD8+) | 1×10^7 BM cells and 1×10^8 whole splenocytes | Systemic disease | Korngold and Sprent, 1987; Eyrich et al., 2005 |
|               | B10 (H2^d)       | BALB.b (H2b)         | 775 cGy | miHA mismatches | CD4^+ (with some contribution from CD8^+) | 8×10^7 TCD BM cells and 3×10^7 unfractionated spleen cells or 1.4×10^7 CD4^+ or 6×10^7 CD8^+ T cells | Systemic disease; no cutaneous involvement | Kaplan et al., 2004 |
|               | C57/B16 (H2b)    | BALB.b (H2b)         | 850–1000 cGy | miHA mismatches | CD4^+ | 5×10^6 TCD BM cells and 2×10^6 T cells | Systemic disease | Berger et al., 1994; Zhang et al., 2005 |
|               | D8A/2 (H2^b)     | B10.D2 (H2^d)        | 820 cGy | miHA mismatches | CD8^+ | 4×10^6 TCD BM cells and 1×10^6 T cells, or purified CD4^+ or CD8^+ T cells | Minimal systemic disease with whole spleen cells but increased with purified CD8^+ T cells; up to 50% mortality at day 25 | Korngold and Sprent, 1987 |

### Xenotransplantation models

| Human PBMCs | NOD/SCID IL2rγ-null (NSG) | No XRT or 200-250 cGy | Mismatched for MHC I, MHC II and miHAs | CD4^+ | 1×10^7 or 5-10×10^6 | Death from GvHD in 30-50 days | Ito et al., 2009; King et al., 2009 |
|-------------|----------------------------|-----------------------|----------------------------------------|--------|---------------------|-----------------------------|-----------------------------------|
| Human PBMCs | BALB/c-RAG2^{-/-} IL2rγ^-/- | 350 cGy | Mismatched for MHC I, MHC II and miHAs | CD4^+ and CD8^+ | 5-30×10^6 | Systemic disease | van Rijn et al., 2003 |
| Human peripheral blood T cells: naive or CD3/CD28 bead expanded | NOD/SCID Bl2m-null | 250-300 cGy | Mismatched for MHC I, MHC II and miHAs | - | 1×10^7 human T cells RO injection | Systemic disease | Nervi et al., 2007 |

BM, bone marrow; cGy, centigray; RO, retro-orbital injection; TCD, T-cell depleted; XRT, radiation conditioning.
MHC-mismatched models

The most commonly studied MHC-mismatched model of aGVHD is C57/B6 (H2b) → BALB/c (H2d) (transplantation of cellular isolates from C57/B6 (H2b) donors into BALB/c (H2d) recipients). MHC-mismatched models of aGVHD also contain a number of miHA mismatches, although the impact of miHA mismatches on the pathophysiology of the clinical phenotype observed is limited. All models incorporate myeloablative radiation conditioning either as a single dose or, when radiation doses of more than 1100 cGy are delivered, as a fractionated dose separated by 3-8 hours, which decreases gut toxicity. Most MHC-mismatched models are dependent on both CD8+ and CD4+ T cells. To dissect the mechanisms by which these T-cell subsets induce aGVHD, transgenic mice that have a mutant MHC I (which is involved in CD8+ T-cell activation) [B6.C-H2bm12 (bm1)] or MHC II (which is involved in CD4+ T-cell activation) [B6.C-H2bm12 (bm12)] have been used. The H2bm1 and H2bm12 transgenic mouse models have been important in dissecting the interaction of T cells with recipient and donor APCs (Sprent et al., 1990). CD8+ T cells induce aGVHD by engagement of the TCR and release of perforin, granzymes and Fas ligand (Via et al., 1996b; Graubert et al., 1997; Maeda et al., 2005). Only recipient APCs can stimulate CD8+ T cells in CD8+ T-cell-dependent models (Shlomchik et al., 1999). Conversely, either recipient or donor APCs can stimulate CD4+ T-cell responses (Anderson et al., 2005). TNFα mediates the cytotoxic effects of alloreactive CD4+ T cells. The target of these cytotoxic T-cell responses is epithelial epithelium, although the epithelium is not required to present antigen to T cells (Teshima et al., 2002).

Another MHC-mismatched model of aGVHD is the ‘parent-to-F1’ model. Parent-to-F1 transplants have been studied using either lethal irradiation regimens or no irradiation conditioning. The latter model allows the study of aGVHD without the effects of radiation, thus mimicking the situation in humans that undergo non-lethal irradiation regimens or no irradiation conditioning. The former model allows the study of aGVHD without the effects of radiation, thus mimicking the situation in humans that undergo non-lethal irradiation regimens or no irradiation conditioning. The cells transplanted into these models are either whole splenocytes (in the non-irradiated models) or purified T cells with T-cell-depleted bone marrow (in the irradiated models). The development of aGVHD in these models depends on differences in class I and class II MHC antigens, and involves the effects of CD4+ and CD8+ T cells (Hakim et al., 1991). The characteristics of aGVHD differ in the irradiated and non-irradiated parent-to-F1 model: whereas the irradiated model exhibits weight loss and skin problems, the non-irradiated model has relatively limited skin pathology or weight loss, and aGVHD mainly manifests as immune deficiency and mixed chimerism, with lower mortality. Importantly, not all parental donor strains cause aGVHD. Some can cause cGVHD (e.g. DBA/2 → B6D2F1), possibly because of the natural bias of some strains to mount either a Th1- or Th2-cell response (De Wit et al., 1993; Nikolic et al., 2000; Tawara et al., 2008).

miHA-mismatched models

miHA-mismatched models of aGVHD represent human HSCT more closely than MHC-mismatched models, because MHC-mismatched transplants in humans are not commonly performed. Similar to the human setting, miHA-mismatched models exhibit less morbidity than MHC-mismatched models, but still result in lethal aGVHD. An example of a miHA-mismatched transplant model is B10.D2 (H2d) → BALB/c or DBA/2 (H2b). The aGVHD that occurs in this model is primarily dependent on CD4+ T cells and less on CD8+ T cells (Kornfeld and Sprent, 1987). However, transplants between congenic strains illustrate that differences in MHC (which means that alloantigens are presented differently in vivo) influence the type and specificity of the T-cell response underlying the GvHD phenotype (Kaplan et al., 2004). For example, in other miHA-mismatched transplant models, such as C3H.SW → B16 (H2d) and DBA/2 → B10.D2 (H2b), GvHD depends primarily on CD8+ T cells.

Xenogeneic transplant models

These models have been developed to generate a system whereby human T-cell-mediated aGVHD can be studied and manipulated in vivo. However, transplanting across species (xenotransplantation) requires significant immunosuppression to prevent graft rejection, because the immune response to xenografts is typically much more robust than to allografts. Initial attempts to xenotransplant human cells into mice were conducted using non-obese diabetic severe combined immunodeficiency (NOD/SCID) mice and resulted in a 1-20% rate of engraftment (Mosier et al., 1988; Hoffmann-Fezer et al., 1993; Christianson et al., 1997). Low engraftment was due to the fact that NOD/SCID mice lack functional T and B cells but retain NK cell function and can therefore respond to foreign antigens. Subsequently, better engraftment of xenotransplanted cells was obtained using RAG2-deficient and IL-2-receptor-γ-deficient mice (BALB/c-RAG2-/-/IL2γ-/-), which lack functional T, B and NK cells (van Rijn et al., 2003). Our group has used the NOD/SCID β2-microglobulin-null mouse (Christianson et al., 1997; Nervi et al., 2007) to image the trafficking of xenotransplanted human T cells in vivo. Using this system, we demonstrated that retro-orbital injection of human T cells into sublethally irradiated NOD/SCID β2m-null recipients resulted in consistent engraftment and expansion of human T cells, the trafficking of T cells to target organs including the lung, skin, liver and kidney, and clinical symptoms consistent with severe aGVHD. T cells could be detected 1 hour after retro-orbital injection and trafficked to secondary lymphoid organs, resulting in lethal GvHD; by contrast, intravenous injection of T cells caused them to traffic first to the lungs, and was associated with the development of less severe aGVHD and less morbidity and mortality (Nervi et al., 2007).

The most permissive mouse model for human stem-cell and T-cell engraftment was derived by back-crossing mice carrying a homozygous deletion of the common γ-chain of the IL-2 receptor onto a NOD/SCID background [referred to as NOD/SCID IL2γ-null (NSG) mice]. NSG mice lack T, B and NK cells, and also have reduced function of macrophages and dendritic cells. Transplantation of human peripheral blood mononuclear cells (PBMCs) causes an aGVHD-like syndrome that results in death by 20-50 days, and is manifested by overt human T-cell infiltration of mouse skin, liver, intestine, lungs and kidneys (Ito et al., 2009). Xenogeneic transplants of human PBMCs into immunodeficient mice require human APCs to process mouse antigens and present them in the presence of class II MHC. Because T-cell recognition of MHC molecules is restricted by species, human TCRs do not recognize mouse MHC, making this a primarily CD4+ T-cell-dependent model, at least in the case of NOD/SCID (Lucas et al., 1990).
Antigen-specific transgenic TCR models
A major limitation of all MHC- and miHA-mismatched transplant models of aGvHD is that it is difficult to determine the specific alloantigen that the responding T cells recognize. In addition, the cytokine storm induced by conditioning can lead to antigen-independent bystander activation of T cells. One solution to this problem has been to use mouse strains that have T cells expressing TCRs of defined specificity (TCR-transgenic mice); these strains were generated initially to study thymic selection, but have more recently been useful for understanding T-cell alloresponses in GvHD. The specificity of the T cells in a TCR-transgenic mouse is generally restricted to a single peptide epitope that is either constitutively expressed by the transplanted allogeneic APCs or is administered as an exogenous antigen to stimulate a T-cell response. Examples of TCR-transgenic mice include H-Y, TEa, 2C, TS1, D011.10 and OT-II (Kisielow et al., 1988; Sha et al., 1988; Murphy et al., 1990; Grubin et al., 1997; Barnden et al., 1998; Wilkinson et al., 2009). These mice have been used to investigate several aspects of GvHD, including the role of antigen affinity (Yu et al., 2006), antigen-induced T-cell activation and proliferation, Treg cell function (Albert et al., 2005; Damdinsuren et al., 2010) and, more recently, cross presentation of antigens (Wang et al., 2009).

Mouse models of cGvHD
Although the clinical phenotype and kinetics of cGvHD are distinct from aGvHD, with fibrosis and chronic inflammation predominating in a manner resembling autoimmune disease, most of the same organs are affected. There are five factors that can contribute to the pathology of cGvHD in humans and mouse models after HSCT: cytokine-mediated fibrosis, defective negative selection of T cells, deficiency of Treg cells, genetic polymorphisms and dysregulated B-cell responses. However, no mouse model developed thus far recapitulates all of these characteristics or exactly mimics what is seen in human cGvHD.

Current mouse models of cGvHD (summarized in Table 2) can be broadly divided into sclerodermatous (pro-fibrotic) models, autoantibody-mediated (lupus-like) models and aGvHD models after HSCT: cytokine-mediated fibrosis, defective negative selection of T cells, deficiency of Treg cells, genetic polymorphisms and dysregulated B-cell responses. However, no mouse model developed thus far recapitulates all of these characteristics or exactly mimics what is seen in human cGvHD.

Sclerodermatous models
Sclerodermatous (pro-fibrotic) cGvHD models are characterized by fibrotic changes in the dermis, which can involve the lung, liver and salivary glands (Jaffee and Claman, 1983; Claman et al., 1985; Howell et al., 1989; McCormick et al., 1999; Levy et al., 2000). Transplantation results in full donor chimerism, and the resulting phenotype resembles scleroderma in humans, which occurs in up to 15% of patients with cGvHD (Skert et al., 2006). However, fibrosis in the mouse models begins within 30 days of transplantation, which is atypical of human cGvHD, which occurs months to years after transplantation.

These models are MHC matched but differ in numerous miHAs. The pathology is dependent on CD4+ T cells that release Th2 cytokines, which can stimulate other cells to release fibrosing cytokines (such as IL-13 and TGFβ) (for a review, see Wynn, 2004), resulting in the sclerodermatous changes (Hamilton, 1987; Korngold and Sprent, 1987). In addition, other cellular mediators have also been implicated: macrophages are responsible for TGFβ production, whereas other cell types such as eosinophils and mast cells have been implicated in the pathogenesis of fibrosis in target organs. Mediators secreted by eosinophils and mast cells can cause fibroblast proliferation and collagen production (Levi-Schaffer and Weg, 1997). Two examples of sclerodermatous models are B10.D2 – BALB/c and LP/J – Bl6, both of which require total body irradiation conditioning (Jaffee and Claman, 1983; DeClerck et al., 1986). The dose of radiation can also affect the severity of disease (Jaffee and Claman, 1983).

The sclerodermatous cGvHD models provide important insights into the pathogenesis of fibrosis that occurs in cGvHD; however, there are a number of limitations of these models. First, the fibrosis in human cGvHD can be systemic or pleiotropic, unlike mouse models, which mainly involve the skin, suggesting other mechanisms might be at play in the development of systemic sclerosis. Second, the time course of development of skin changes is less rapid in humans than in mouse models. Finally, these mouse models fail to reproduce the full clinical spectrum seen in humans, which can involve target organs including the intestine, lungs, liver, salivary glands, eyes, oral mucosa, muscles, tendons and nerves.

Autoantibody-mediated models
Autoantibody-mediated (lupus-like) cGvHD models, which exhibit a pathology similar to that of lupus, are manifested by nephritis, biliary cirrhosis, salivary gland fibrosis, lymphadenopathy, splenomegaly, autoantibody production and, to a lesser extent, skin pathology. These models are MHC mismatched and classically involve parent-to-F1 transplants that result in mixed chimerism. The most commonly studied autoantibody model is the DBA/2 – B6D2F1 model. This model results in expansion of recipient B cells, leading to lymphadenopathy, splenomegaly and autoantibody production. Both MHC I and II are mismatched in these models, but the pathology is dependent on donor CD4+ T cells (Rolkink and Gleichmann, 1983; Rolkink et al., 1983; Hakim et al., 1991) and is associated with the production of Th2 cytokines. Manipulating the cytokine balance by skewing the CD4+ T-cell response to a Th1 phenotype, by exposure to IL-12, causes a shift to a more aGvHD-like phenotype (Via et al., 1994). Importantly, the choice of parental donor determines the phenotypic outcome. For example, if C57/Bl6 is the T-cell donor for a B6D2F1 recipient, an aGvHD phenotype results. This might be due to naturally high proportions of cytotoxic CD8+ T cells in the C57/Bl6 mouse strain (Via et al., 1987).

Unlike models of aGvHD, which depend primarily on donor T cells, pathology in autoantibody models of cGvHD depends on recipient B cells (Morris et al., 1990a). Specifically, the recipient B cells interact with donor T cells (Morris et al., 1990b) via cell-surface MHC and are also stimulated by cytokines, including IL-4 and IL-10 (De Wit et al., 1993; Durie et al., 1994; Via et al., 1996a; Kim et al., 2008). Recipient B cells act as potent APCs that stimulate donor T cells, perpetuating the cycle that leads to autoantibody production.

Mouse autoantibody cGvHD models are similar to cGvHD that occurs in humans in that they also involve the production of autoantibodies, although there are a number of distinctions. First,
### Table 2. Mouse models of cGVHD

| Donor strain | Recipient strain | Conditioning regimen | Genetics | Main T-cell type contributing to phenotype | Cells and dose | Outcome | Reference example(s) |
|--------------|------------------|----------------------|----------|--------------------------------------------|---------------|---------|----------------------|
| **Scleroderma (pro-fibrotic models)** | | | | | | | |
| B10.D2 (H2d) | BALB/c (H2d) | 700-900 cGy | miHA mismatched | CD4⁺ | TCD BM cells and 1x10⁷ whole spleenocytes or purified T cells | Scleroderma; progressive fibrosis of organs | Korngold and Sprent, 1978; Jaffee and Claman, 1983; Claman et al., 1985; Hamilton, 1987; Korngold and Sprent, 1987; McCormick et al., 1999; Levy et al., 2000; Kaplan et al., 2004; Zhou et al., 2007 |
| LP/J (H2d) | C57Bl6 (H2b) | 900-1100 cGy | Mismatched for miHAs and MHC | ND | 20-50x10⁷ spleen cells and 5x10⁷ BM | Scleroderma occurring by day 28 | Hamilton and Parkman, 1983; DeClerck et al., 1986 |
| B10.D2 (H2d) | BALB/c Rag–/– (H2d) | None | H2d matched but miHA mismatched | ND | Whole spleen cells (2.5x10⁷) | Scleroderma; progressive fibrosis of organs; autoantibodies | Ruzek et al., 2004 |
| **Autoantibody-mediated (lupus-like) models** | | | | | | | |
| DBA/2 (H2d) | B6D2F1 (H2b/d) | No conditioning | Mismatched for MHC I, MHC II and miHAs | CD4⁺ | 1-10x10⁷ | Lupus Sjogren’s (Sialoadenitis); autoantibodies | Via et al., 1987; Fujiwara et al., 1991; De Wit et al., 1993; Via et al., 1996a; Slayback et al., 2000; Kim et al., 2006; Kim et al., 2008 |
| BALB/c (H2d) | (BALB/cxA)F1 (H2d/a) | No conditioning | Mismatched for MHC I, MHC II and miHAs | CD4⁺ | 8-12x10⁷ spleen cells | Polyarthritis, scleroderma, glomerulonephritis, Sjogren’s, sclorosing cholangitis | Pals et al., 1985; Vidal et al., 1996 |
| C57Bl6 (H2d) | (B6xBALB/c)F1 (H2b/d) | No conditioning | Mismatched for MHC I, MHC II and miHAs | CD4⁺ | 6x10⁷ whole spleen cells | Acute progressing to chronic GvHD | Tschetter et al., 2000 |
| DBA/2 (H2d) | BALB/c (H2d) | 650 cGy | miHA mismatch | – | 5x10⁷ whole spleen cells | Autoantibodies; skin sclerosis | Zhang et al., 2006 |
| C57Bl6 (H2d) | (C57Bl6 x B6C-H2b-H2d)F1 (H2b background with mutation at MHC I) | No conditioning | MHC I mismatched | CD8⁺ | 5x10⁷ spleen cells or 10⁷ spleen (2/3) and lymph node (1/3) cells; 2 doses of cells on day 0 and 7 | Glomerulonephritis; non-lethal in unirradiated mice; lethal in irradiated mice at 30-60 days | Rolink et al., 1983; Ito et al., 1992 |
| C57Bl6 (H2d) | (C57Bl6 x B6C-H2b-H2d)F1 (H2b background with mutation at MHC II) | None or 900 cGy | MHC II mismatched | CD4⁺ | No conditioning: 4-5x10⁷ spleen CD4⁺ T cells, 2-3x10⁷ BM cells; 2 doses of cells on day 0 and 7 | Systemic disease and death by 20-40 days | Rolink et al., 1983; Ito et al., 1992; Brown et al., 2002 |
| **Thymic dysfunction model** | | | | | | | |
| B6-H2-Abl–/– (H2b) | C3H/HeN (H2d) | 1300 cGy split in two fractions | Mismatched for MHC I, MHC II and miHAs | BM-derived CD4⁺ and CD8⁺ | 5x10⁷ TCD BM cells | Epidermal atrophy, fat loss, follicular drop out and dermal thickening; biliary duct loss and periportal fibrosis in liver; salivary gland fibrosis and atrophy; cytopenias | Sakoda et al., 2007 |

Abbreviations as for Table 1, plus: B6, C57Bl6; B6D2F1, (C57Bl6 x DBA/2)F1; ND, not determined.
nephritis in cGvHD in humans is rare. Second, the repertoire of autoantibodies associated with cGvHD is more diverse in humans than in mice. Finally, lymphadenopathy and splenomegaly do not occur in the human setting.

A cGvHD model involving both autoantibodies and scleroderma

To more accurately model cGvHD that occurs in humans, attempts have been made to combine the features of autoantibody-mediated and scleroderma models in a single mouse model. For example, transfer of splenocytes from DBA/2 (H2b) mice into sublethally irradiated BALB/c (H2k) recipients results in a lupus-like and sclerodermatous phenotype (Zhang et al., 2006). These mice develop proteinuria with basement membrane immune complex deposits, and skin fibrosis with collagen I deposition; the severity of these disorders depends on the number of donor cells transplanted. In addition, these mice have increased levels of autoantibodies specific for double-stranded DNA. In this case, donor CD4+ T cells are required for the pathology, whereas donor CD8+ T cells do not contribute. Unlike pathology in the parent-to-F1 autoantibody-mediated model, which involves recipient B cells, pathology in this model involves donor B cells. Finally, the transfer of Treg cells rescues the cGvHD phenotype, indicating that Treg cells are functional in treating cGvHD, as has been demonstrated in other models. In summary, this model indicates the importance of donor CD4+ T cells as well as B cells (from both donor and recipient) in the development of cGvHD. However, it does not support a role for the thymus in the pathophysiology of cGvHD, in contrast to the model discussed below.

Defective thymic function model

Given the similarity between the presentation of cGvHD and some autoimmune diseases, as well as the fact that thymectomy of 3-day-old mice results in symptoms similar to some aspects of cGvHD (Nishizuka and Sakakura, 1969; Bonomo et al., 1995), it has long been hypothesized that defects in thymic negative selection might have a role in the pathogenesis of cGvHD. The thymus is a crucial target of aGvHD (Fukushi et al., 1990; Krenger et al., 2000b; Hauri-Hohl et al., 2007), and it is known that aGvHD results in destruction of the normal thymic architecture (Ghayur et al., 1988), especially Hassall's corpuscles, which have been shown to be crucial for the development of Treg cells (Watanabe et al., 2005). The presentation of tissue-restricted antigens by thymic APCs and epithelial cells could result in their direct attack by cytotoxic donor T cells. Cytotoxic attack of these cell types, which are crucial for negative selection, could result in impaired central tolerance and subsequent autoreactivity (Krenger and Hollandar, 2008; Mathis and Benoist, 2009).

A potential role for thymic damage in GvHD was initially proposed by a group that demonstrated dysfunctional negative selection of autoreactive T-cell clones in mice with aGvHD (van den Brink et al., 2000). More recently, a transgenic mouse transplant model was developed: T-cell-depleted bone marrow from MHC-II-deficient mice on a C57/B16 background [H2-Ab1 +/- (H2b)] were transferred to lethally irradiated C3H/HeN (H2k) recipients. Recipient mice developed a phenotype similar to clinical cGvHD, with skin fibrosis characterized by epidermal atrophy, fat loss, follicular drop out and dermal thickening; bile duct loss and periportal fibrosis in the liver; salivary gland fibrosis and atrophy; and cytopenias (Sakoda et al., 2007). This phenotype developed within 6 weeks of donor cell transfer, and fewer than 20% of animals lived for more than 100 days. Importantly, thymectomized recipient mice abrogated the phenotype, highlighting the role of the thymus in the pathology seen in this model. Experiments involving chimeric mice showed that donor APCs were essential for the development of the cGvHD phenotype in this model: donor APCs that trafficked to the thymus after transplantation were principally responsible for negative selection. In a series of adoptive transfer studies, it was shown that CD4+ T cells isolated from C3H/HeN recipients of MHC-II-deficient bone marrow that develop cGvHD caused aGvHD when transferred to the C57/B16 donor strain, but caused cGvHD when transferred to established chimeric mice created from a C57/B16 → C3H/HeN transfer. This implies that donor APCs are essential for the development of the cGvHD phenotype not only through their role in negative selection but through peripheral antigen presentation and T-cell stimulation.

Although the mouse model described above (H2-Ab1 +/- → C3H/HeN) is the first that reproduces multiple clinical features of human cGvHD, it might not be clinically relevant. The model cannot rule out the possibility that additional insult to the thymus caused by GvHD might be the cause of dysfunctional negative selection. It is also difficult to determine the exact kinetics of cGvHD in this model, and whether there is a critical period of thymic damage that is necessary to cause the resulting phenotype. Nonetheless, this model suggests that a crucial component of cGvHD is dysfunctional thymic selection, which may or may not be caused by donor T-cell-induced damage of the recipient thymus as a consequence of aGvHD.

Linking acute and chronic GvHD in a mouse model

The high incidence of cGvHD that occurs following aGvHD in humans suggests that there is a direct causal link between these two disease states. If the mechanisms underlying this link could be identified, targeted interventions could be used to prevent the development of autoimmune-like responses that characterize cGvHD from the alloresponses that underlie aGvHD.

Zhang et al. attempted to link acute and chronic GvHD in a mouse model (Zhang et al., 2007). Donor bone-marrow-derived CD4+ T cells that arose from hematopoietic precursors following transplantation and were selected (educated) in the recipient thymus during the development of aGvHD could mediate a cGvHD phenotype when transferred to secondary allogeneic recipients, resulting in cGvHD affecting the skin, liver and gut. When transferred to syngeneic secondary recipients, the same cells resulted in a lethal aGvHD phenotype, suggesting that appropriate positive selection had taken place in the recipient thymus but that thymic negative selection was absent or dysfunctional. This illustrates a possible link between the cytotoxic CD8+ T-cell responses of aGvHD and the CD4+ T-cell-mediated responses demonstrated in these models of cGvHD. The exact mechanism is unknown, but these data suggest that the thymus-educated donor CD4+ T cells that should be self-tolerant are autoreactive, suggesting that the link between aGvHD and cGvHD is related to the targeting to the thymus by alloreactive T cells (both CD4+ and CD8+) and subsequent elimination or alteration of the function of thymic cellular elements that are essential for appropriate negative
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The effects of these autoreactive T cells can only be demonstrated by adoptive transfer experiments because of the highly lethal nature of aGvHD models.

Another attempt to investigate the link between aGvHD and cGvHD was performed using an F1 donor and F1 recipient [(B10 × B10.D2)F1 (H2b/d) → (BALB.b × BALB/c)F1 (H2b/d)] transplant: this resulted in both systemic symptoms of aGvHD and skin manifestations of cGvHD (Kaplan et al., 2004). In this model, MHC was matched between donor and recipient, but miHAs were mismatched. This same group investigated transplantation between multiple strain combinations matched at MHC loci, and demonstrated that certain MHC haplotypes were associated with the development of GvHD involving specific target organs, which they suspected was due to differences in MHC affinity for antigens found in certain target tissues. For example, immunodominant antigens in the skin might be bound with high affinity in only certain MHC strains. This illustrates the important role of MHC in selecting immunodominant antigens, and how this might influence cGvHD.

A final example of the link between alloreactive T-cell responses of aGvHD and the autoimmune responses of cGvHD involved the use of immunodeficient mice (Tivol et al., 2005; Chen et al., 2007). In this case, aGvHD was initiated through a C57/Bl6 → BALB/c lethally irradiated MHC-mismatched transplant. After 20 days, recipient animals were sacrificed and their splenic T cells were isolated. Transfer of these isolated T cells into unconditioned immunodeficient (Rag-/-) C57/Bl6 recipients (i.e. the same background as the original donor) resulted in inflammatory infiltration of the colon and liver (Chen et al., 2007). This autoimmune phenotype was dependent on CD4+ T cells (with a Th1 and Th17 cytokine profile) and could be rescued by the use of immunodeficient mice that have allelic differences at the CD45 locus (e.g. CD45.1 or CD45.2) to track cellular subsets from donor versus recipient when they have a common MHC.

Considerations when selecting a mouse model of GvHD

With the large number of GvHD models now available, it can be difficult to determine which model is appropriate for the research question at hand. We offer in this section a few suggestions.

(1) First, decide whether you are interested in studying acute or chronic GvHD. It is of course useful to first establish from published literature which models have been successfully validated for testing a given intervention.

(2) Next, determine what mechanism or T-cell population you want to be dominant in your model for testing a specific therapeutic intervention. For example, a drug that targets CD8? T cells would not be effective in a model in which CD4+ T cells mediate pathology. Conversely, if limiting the degree of cytokine storm produced from conditioning is important, then miHA-mismatched or TCR-transgenic models would be useful, because conditioning can be reduced or eliminated.

(3) Next, consider measurement outcomes such as: severity, coat color and the ability to track specific cell populations. If easy clinical scoring of GvHD severity and survival are the main outcomes measured, then a model that has consistent rapid kinetics and 100% penetrance is desirable. In this case, the C57/Bl6 → BALB/c transplant model could be a good choice. In general, the less the degree of MHC mismatch, the less severe the model. If bioluminescence imaging (BLI) will be used (see Box 2), consider the coat color of the mouse that will be imaged, because imaging is more difficult with mice that have dark coats (which require shaving prior to BLI). If the tracking of donor cells in the recipient is necessary, it is possible to use flow cytometry to track MHC disparity, to track fluorescent donor cells using BLI, or to use congenic mice that have allelic differences at the CD45 locus (e.g. CD45.1 or CD45.2) to track cellular subsets from donor versus recipient when they have a common MHC.

(4) It is also necessary to consider more complex issues such as the production of transgenic strains. Sophisticated modeling can allow for elegant mechanistic studies, but if you are going to use a transgenic strain as a donor or recipient, extensive backcrossing to a single background is essential to eliminate significant genetic disparity that could affect results.

(5) Finally, it is crucial to personally validate all new models in your lab. Even a basic model (such as Bl6 → BALB/c) and certainly any novel strain combinations require careful validation to investigate the influence of various factors – including pre-conditioning radiation dose, mouse strain and cleanliness of colonies (which varies between labs) – on, for example, disease incidence, severity and pathologic evidence of GvHD.

When working with any mouse model of GvHD, there are a number of caveats to be aware of when interpreting and translating experimental data, which are outlined in Box 3.

Measuring the GVL effect in GvHD models

The graft-versus-leukemia (GVL) effect, also referred to as graft-versus-tumor effect, describes the alloimmune responses of donor immune cells to residual leukemia or tumor, which are indications for which HSCT is frequently performed as a treatment. Although a complete review of models of GVL is beyond the scope of this article [the reader is instead referred to reviews on the mechanisms of GVL in blood and bone marrow transplantation (Contag and Contag, 2009)], we think it is important to consider the use of luciferase-based imaging to monitor the in vivo trafficking and persistence of T cells (Beilhack et al., 2005), Treg cells (Edinger et al., 2003) and other T-cell subsets, including Th17 cells (Izotov and Contag, 2009). It can also be used to measure the GVL effect by tracking the expansion or elimination of luciferase-expressing tumor cells over time after HSCT.
Mouse models of GvHD

Box 3. Caveats and considerations when interpreting findings from mouse models of GvHD and translating them to the clinic

Differences in conditioning regimens

Pre-transplant conditioning in humans mainly consists of chemotherapy regimens, given with or without radiation, which is delivered at a rate (typically fractionated) that differs from that used in mouse models of GvHD (usually single dose). Conditioning results in tissue damage and a proinflammatory reaction that might differ between mice and humans and therefore influence the GvHD phenotype. In addition, conditioning regimens and the timing of transplantation can impact experimental outcomes (Gendelman et al., 2004; Mabed et al., 2005; Schwarte and Hoffmann, 2005; Mapara et al., 2006). This can be directly related to the residual host-versus-graft (HVG) effect, which can decrease GvHD severity if the host immune system is not adequately suppressed. The HVG reaction is a rare complication after transplant in humans but increases as conditioning regimen intensity is decreased.

Genetic and immunological disparities between donor and recipient

In humans, HLA typing occurs via high-resolution DNA typing, and most transplants are matched at the allele level with respect to MHC loci, although they usually differ at the level of miHAs that lie outside of the MHC locus. By contrast, many mouse models of GvHD involve mismatches in both MHC antigens and miHAs. There are also key species differences between the mouse and human immune system that can make the direct translation of results difficult. [These have been reviewed elsewhere (Mestas and Hughes, 2004).] Some of these differences include: involution of the thymus in humans but not mice; expression of MHC II by human but not mouse T cells; and differences in the proportions of circulating lymphocytes and their effector mechanisms in the two species. Furthermore, strain-specific disparities in the proportion of lymphocyte subsets (such as CD4+, CD8+ and Treg cells) influence the phenotype between different mouse models of GvHD. Finally, recent reports suggest that MHC-matched mice can carry genetic polymorphisms outside of the MHC locus that can affect the clinical phenotype of GvHD, which might explain observed differences in results in different labs, even when the same strain combinations are used (Cao et al., 2003; Fanning et al., 2009).

Source of donor immune cells

Material for HSCT in humans is now most commonly derived from mobilized stem cell products and contains donor immune cells from the circulation. The number, origin and type of circulating immune cells can differ depending on the mobilization regimen used. By contrast, immune cells delivered together with transplants in mouse models are usually isolated from spleen or lymph nodes. It is important to consider that immune-cell populations from different sources might have different trafficking capacities and composition (i.e. different proportions of T-cell subsets, dendritic cells and early myeloid progenitors), and therefore have a different influence on the GvHD phenotype.

Differences in normal flora

Enteric pathogens can play a role in the kinetics and severity of GvHD, which targets the gut in many cases. Microorganisms express molecules that can activate the immune system and therefore can augment the severity of disease (Cooke et al., 1998). In line with this, changes in the environment of the mice, which in turn affect the composition of gut flora, have been shown to affect disease severity (Bennett et al., 1998; Gerbitz et al., 2004; Gorski et al., 2007). Given that the gut flora of a human is markedly different from that of a mouse kept in a pathogen-free facility, this should be taken into account when translating experimental data.

Age

HSCT is commonly carried out in adult humans, whereas mice used to study GvHD are typically 8- to 14-weeks old (and have a 2-year lifespan). Given that age can influence the efficiency of immune reconstitution after transplant, as well as susceptibility to GvHD (Ordemann et al., 2002), this factor must be taken into account when translating experimental results.

[Adapted, with permission, from Socié and Blazar (Socié and Blazar, 2009).]

It could be argued that any novel therapy designed in these models to target GvHD should be assessed for its effect on GVL because of the clinical implications for translation to humans. To test the GVL effect, a labeled congenic tumor cell line (i.e. genetically compatible with the recipient, such as the A20 cell line for a BALB/c background) or primary tumor cells [such as mouse acute promyelocytic leukemia cells from PML/RARα transgenic mice (Bl6/129 background) (Westervelt et al., 2003)] can be transduced to stably express a fluorescent marker or luciferase for bioluminescent studies. After a lethal injection of tumor cells at the time of allogeneic transplantation, mice that develop aGvHD generally eliminate the genetically compatible tumor, owing to the alloreactive response mounted by donor T cells. The ultimate goal of interventions has been to prevent GvHD while preserving GVL. There have been a number of recent articles demonstrating this (Liang et al., 2008; Nishimura et al., 2008; Zheng et al., 2009). The availability of BLI (Box 2) has improved the capacity to detect residual leukemia after transplantation and is used as a measure of response to treatments, together with survival and GvHD scoring, in these models.

Conclusion

Mouse models of GvHD are crucial for advancing our understanding of the disease in its acute and chronic forms. As our understanding grows, it is hoped that targeted therapies will be developed to treat GvHD and preserve GVL. Recent advances in treatment that have evolved owing to studies of mouse models of aGvHD include targeting mediators of the cytokine storm, such as TNFα and IL-1, with antagonists (Uberti et al., 2005; Alousi et al., 2009; Antin et al., 2002), as well as reducing the intensity of conditioning regimens to avoid excess inflammation. Other novel interventions include the proteasome inhibitor bortezomib (Sun of GvL (Bleichley and Riddell, 2004; Kolb et al., 2004) as well as a recent historical perspective on GVL (Kolb, 2008)], it is worth mentioning that separating GvHD from the GVL effect is a long-standing dilemma in transplantation, and is still an area of active investigation. In fact, separating the immunopathological aspects of GvHD from the curative effect of GvL is perhaps the most clinically meaningful endpoint of HSCT. However, this is a complicated issue because T cells mediate both GvHD and GVL.

Research in this area has focused on identifying the mechanisms that might differentiate cytotoxic T-cell responses in GvHD from cytotoxic T-cell responses in GVL, as well as identifying accessory cells that could block GvHD while allowing or augmenting GVL. The first evidence for GVL came from mouse transplant models (Barnes and Loutit, 1957; Weiss et al., 1983). Initial studies in the 1950s demonstrated that a mouse leukemia could not be eliminated by irradiation or by irradiation and syngeneic bone marrow, but irradiation plus allogeneic bone marrow prolonged survival of mice and eliminated leukemia from detection. However, transplanted mice developed a wasting syndrome that later became known as GvHD. Later efforts in the early 1980s were better able to define the immunological basis for this GVL effect, and subsequent work defined the importance of T cells and miHAs, as well as of innate immune cells, such as NK cells, on the GVL effect. The models discussed above can also be used to test GVL effects of allogeneic cellular therapies, as well as to test treatments that are designed to prevent GvHD while preserving the GVL effect.
et al., 2004; Sun et al., 2005), and the DNA methyltransferase inhibitors decitabine and azacitidine (Choi et al., 2010). The exact mechanism by which these agents work is not known, but they might involve suppression of alloreactive T-cell responses and/or an increase in T_{Reg} cell number or function following transplantation. In addition, cellular therapies, such as depleting T cells from the graft, or infusing T_{Reg} cells, have been developed based on studies performed in the mouse. Finally, cGVHD models have been instrumental in developing imatinib—a tyrosine kinase inhibitor that targets PDGFRα, which has been implicated in the pathogenesis of fibrosis (McCormick et al., 1999; Daniels et al., 2004; Abdollahi et al., 2005)—and rituximab, an antibody that targets potential autoreactive B cells (Cutler et al., 2006). Areas that warrant further study include the influence of reduced intensity conditioning regimens (Sadeghi et al., 2008), in vivo imaging, and the genomics and epigenomics of GvHD.

As discussed, advances in in vivo imaging techniques to monitor trafficking of immune cells have recently been developed and will be crucial for dissecting the temporal and spatial relationships of donor and recipient T cells, B cells and APCs in these models (Panoskaltsis-Mortari et al., 2004). Furthermore, recent advances in proteomics, gene profiling and whole genome sequencing will help to elucidate the genetic basis of GvHD, provide ways to better match donor and recipient to prevent GvHD, and improve our ability to predict the severity and kinetics of the disease (Baron et al., 2007; Hori et al., 2008; Paczesny et al., 2009).

Despite the advances made through preclinical modeling of GvHD in mice, many questions remain. Mouse models will continue to allow for the dissection of mechanistic pathways of GvHD through the use of informative genetic knock-out, knock-in and knock-down experiments that are only possible in the mouse. Fundamental research using mouse models of GvHD should enhance our understanding of GvHD in humans and result in improved and novel therapeutic approaches to minimize GvHD while optimizing the GVL effect, as well as immune reconstitution, following HSCT.

COMPETING INTERESTS

The authors have no relevant financial or competing interests to disclose.

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