Bone Marrow Transplantation (2008) 41, S58–S64

www.nature.com/bmt

ORIGINAL ARTICLE

Acute GvHD: pathogenesis and classification

LM Ball and RM Egeler, on behalf of the EBMT Paediatric Working Party

Department of Paediatric Immunology, Hematology, Oncology, Bone Marrow Transplantation, and Auto-immune Diseases, Leiden University Medical Center, Leiden, the Netherlands

Allogeneic hematopoietic SCT (HSCT) is an established treatment for some children with life-threatening hematological disease, immune deficiencies and inborn errors of metabolism. Despite advances in prevention and post transplant immuno-suppressive strategies, acute GvHD (aGvHD) remains a major cause of morbidity and mortality in children undergoing SCT. Although reported incidence rates differ, it has been estimated that, depending upon the patient and donor cohort studied, 20–50% of all transplanted patients will experience grade 2 or more aGvHD despite immuno-suppressive prophylaxis. aGvHD occurs when transplanted donor T lymphocytes recognize antigenic disparities between the host and recipient. Pathways other than direct T-cell-mediated cytotoxicity have been shown to be important in the pathogenesis. Inflammatory cytokine release has been implicated as the primary mediator of aGvHD and activation of T cells is one step in the complex process. Deregulated cytokine release by cells other than T cells leads to tissue damage associated with aGvHD. GvHD is a factor that compromises the overall success rate of allogeneic HSCT and remains a challenge, which, in turn, requires an understanding of the pathophysiology, clinical presentation and management of this complication. The authors concentrate on the most recent knowledge of the pathogenesis as well as the classification of aGvHD.
Bone Marrow Transplantation (2008) **41,** S58–S64; doi:10.1038/bmt.2008.56
Keywords: graft versus host disease; hematopoietic SCT; pathogenesis; children

Introduction

Hematopoietic SCT (HSCT) has been a successful therapy in use since the 1960s and a proven cure for pediatric patients suffering from hematological disorders as well as immune deficiencies and metabolic disorders. For many of these children, SCT is the only curative option.

Correspondence: Dr LM Ball, Department of Paediatrics, Leiden University Medical Center, PO Box 9600, Leiden 2300 RC, the Netherlands.
E-mail: L.M.Ball@lumc.nl

Despite advances in donor HLA-typing methods (and thus donor selection) and post transplant immune suppression, acute GvHD (aGvHD) remains a significant cause of transplant-related mortality and morbidity following allogeneic HSCT, even in the matched HLA-identical sibling setting.[1,2]

Billingham, in his historic Harvey Lecture, described the fundamentals of aGvHD over 30 years ago.[3] The first requirement is that the graft must contain sufficient numbers of immunologically competent cells. Second, the host must have important transplant isoantigens lacking in the graft. Finally, the host immune system must be incapable of mounting an effective immune response against the graft.

Acute GvHD occurs most frequently after engraftment, and this has led to an arbitrary period of 100 days post HSCT that has defined the acute versus chronic manifestation of this disease. However, as transplant practice has changed, so too has the timing of GvHD occurrence, and clinical manifestations are now a better definition than timing alone. The use of non-myeloablative or so-called reduced-intensity conditioning regimens has reduced the hematological toxicities of allogeneic HSCT, but GvHD remains a problem.[4,5] Donor lymphocyte infusions, especially in the context of reduced-intensity HSCT, are becoming more commonplace in protocols designed to induce graft versus leukemia effect and treat relapse or mixed chimeric populations after HSCT.[6–10] These strategies are associated with the risk of inducing GvHD. Clinical manifestations depend on the degree of donor/recipient HLA incompatibility and graft alloreactivity to major host antigens. The primary organs affected in the acute process are skin, liver and gastrointestinal (GI) tract, although other sites may be affected.

The aim of this review is to update the most recent knowledge of pathophysiology and clinical manifestations of aGvHD.

Genetic basis of GvHD

HLA-dependent factors

1. Major incompatibility antigens have a major effect on the biology and occurrence of GvHD in the HSCT setting. The encoding loci, so-called MHC, have a central role in both humoral and cell-mediated immune responses. MHC

is located on the short arm of chromosome 6 (p21) and encodes for HLA. Class I and II HLAs are cell surface molecules that not only determine histocompatibility but also control T-cell recognition.[11,12] Class I (HLA-A, B and C) are expressed on all nucleated cells. Sibling donors and recipients who share HLA antigens have better engraftment and reduced rates and severity of GvHD.[13] Class II (DR, DQ and DP) are more selectively expressed on cells of the immune system. CD4+ T cells are able to recognize foreign antigens through the presentation of class II HLA molecules. Class II HLA is found abundantly in skin and GI tract epithelium and may contribute to the specific organ sites of aGvHD.[11,14]

2. mHAs are peptides derived from intracellular proteins presented by specific MHC molecules to donor T cells.[15] These minor antigens express genetic polymorphisms encoded by a wide range of genes and are important in the initiation of GvHD in the identical sibling and sex-mismatched allogeneic transplant setting.[16] Human mHAs are mostly, but not exclusively, restricted to class I HLA. Tissue expression of some mHAs is limited to the hematopoietic system (HA-1 and HA-2), whereas other minor antigens are more widely expressed (HA-Y and HA-3). Mismatches between donor and recipient for HA-1, HA-2 and HA-5 are associated with an increased risk of GvHD.[17]

Non-HLA-dependent factors

1. Cytokine gene polymorphisms. Cytokine gene polymorphisms may have an important role in the afferent phase of aGvHD. Studies suggest that high levels of tumor-necrosis factor α (TNF-α) and low levels of IL-10 in patients pre-transplant result in more GvHD. These results, in turn, correlate with further investigations into the gene polymorphisms of TNF-α and IL-10 in donor and recipient HSCT cohorts. Candidate gene polymorphisms, which have been linked to the risk of aGvHD, that have so far been described are TNF-α, IL-10, IL-6, IFN-γ, the IL-1 family and transforming growth factor β genes.[17] Other candidate genes are Th1 and Th2 associated with immunopathology of GvHD, for example, IL-2, IL-13 and IL-4. It is important to interpret these genetic polymorphisms and their roles in GvHD susceptibility, as the relevance of these polymorphisms varies depending upon the donor, recipient and stem cell source (see Table 1). As more knowledge is available from gene mapping of the pro- and anti-inflammatory genes, the influence of polymorphisms of neighboring genes and the effects of cytokine release on the outcome of HSCT, interpretation will undoubtedly become more complex.

2. NOD2/CARD15 polymorphisms. The NOD2/CARD15 gene is involved in the innate immune response to bacterial infections in the GI tract and mediates nuclear factor-κB activation in response to bacterial cell wall products. Three single-nucleotide polymorphisms (8, 12 and 13) in the NOD2/CARD15 gene have been associated with a diminished nuclear factor-κB production and were first described with an increased risk of acute inflammatory bowel disease (Crohn's disease).[18] Recently, the same single-nucleotide polymorphisms have been implicated in both the incidence and severity of a GvHD following HLA-identical sibling donor HSCT. Transplant-related mortality rose from a cumulative incidence of 20% at 1 year post transplant for donor/recipient pairs without mutations to 49% for those with a recipient mutation to

Table 1 GvHD risk related to donor/recipient cytokine gene polymorphisms

Gene	*Polymorphism*	*Recipient/donor*	*Donor type*	*aGvHD outcome*
TNFα	d3/d3	Recipient	Identical sibling	↑ grade II–IV, GvHD, increased mortality
	TNF-863, TNF-857	Donor and/or recipient	MUD	Increased
	TNFα-238, TNFβ-252	Donor and/or recipient	Unrelated donor	Increased grade II–IV, increased mortality
	TNFd4, TNFα-1031C and TNFa5	Donor and/or recipient	Unrelated donor	Increased mortality
	TNFd4	Recipient	Identical sibling	↑ moderate aGvHD
	TNFRII-196R	Donor	MUD	↑ grade severe aGvHD
	TNFRII-196M	Homozygous donor	MUD	Reduced risk aGvHD
	TNFRII-196R	Recipient	Identical sibling	↑ grade severe aGvHD
IL-10	Low ACC producer	Recipient	Identical sibling	↑ grade severe aGvHD
	Intermediate ATA	Recipient	Identical sibling	↑ grade severe aGvHD, increased mortality
	R3-GCC	Recipient	MUD	Reduced aGvHD and mortality
IL-6	Il-6-174	Recipient	MUD	↑ grade severe aGvHD
INFγ	INFγ 2/2	Recipient	Identical sibling	Reduced aGvHD
	INFγ 3/3	Recipient	Identical sibling	↑ aGvHD
IL-1 family	IL-Ra	Donor	Identical sibling	Reduced aGvHD
		Recipient	Identical sibling	↑ Chronic GvHD
	IL-1α 889 (pediatrics)	Donor and recipient	MUD	Improved survival, less TRM
TGFβ	TGFβ-509	Donor and recipient	Identical sibling	No effects
	TGFβ codon 10 (pediatric)	Donor	Identical sibling	↑ aGvHD
	TGFβ codon receptor II (pediatric)	Recipient	Identical sibling	↑ aGvHD

Abbreviations: MUD = matched unrelated donor; TGF = transforming growth factor.
Adapted from Dickinson and Charron.[17]

59% for transplants with a donor-only mutation, whereas the worst-case scenario was where both donor and recipient had detectable single-nucleotide polymorphism mutations of the NOD/CARD15 gene (83%). Similar incidences were seen for overall and severe GI GvHD, which were prominent in matched sibling identical transplants.[19,20] These results have been demonstrated in patients undergoing T-cell-depleted donor grafts, suggesting that the detrimental effect of the NOD/CARD15 gene polymorphism is produced via the innate rather than the adaptive immune system.[21]

3. Gene expression profiles. Recently, the development of high-throughput methodologies, such as single-nucleotide polymorphism arrays, has enabled the analysis of hundreds of thousands of genetic markers throughout the genome and copy number variations.[22] Gene expression profiling, using quantitative PCR methodology, of CD4+ and CD8+ T cells from donors has been undertaken in an attempt to identify those donors who may present a greater risk of inducing GvHD (strong alloreactive type).[23] Analysis of a cohort of patients undergoing allogeneic identical sibling HSCT, controlled for conditioning and post transplant immune suppression, elucidated a donor gene expression profile, which had a dominant influence on the occurrence of both acute and chronic GvHD in the recipient. The authors suggested that predictive models limited to a set of 10–20 genes could achieve approximately 80% accuracy and the robustness desired for donor selection. However, before such selection criteria could be used to select suitable donors and modify post transplant immune suppression, extensive validation would be required in a large number of donor-recipient pairs and in alternative donor as well and non-myeloablative stem cells transplants.[22,23]

Similarly, polymorphisms influencing the pharmocokinetics of the widely used immuno-suppressive drug methotrexate have been implicated in the occurrence of aGvHD. Two recent studies have detected associations between MTHFR (methylenetetrahydrofolate reductase) 677 T and thymidylate synthase genotypes with a reduced rate of GvHD, possibly reflecting the increased sensitivity to methotrexate associated with these alleles.[24,25]

Pathophysiology of GvHD

GvHD can be described as a three-phase process.

1. Afferent phase. In this stage, as is seen with the conditioning of the patient, prior disease and comorbidity of the patient, damage to host tissue occurs (see Figure 1). For example, bacterial endotoxins (lipopolysaccharides) may translocate from the intestinal lumen into the circulation and induce the release of inflammatory cytokines, including IL-1, TNF-α, IL-6 and IFN-γ.[26–28] These act to upregulate the expression of MHC antigens and cell surface adhesion molecules on host APCs, which mediate an alloimmune response by mature donor T cells. This 'cytokine storm' is an important mediator of the occurrence and severity of aGvHD, and the above-mentioned polymorphism of cytokine genes directly influences this scenario. However, the balance between pro- and anti-inflammatory cytokine release in determining GvHD is complex and most probably influenced by many transplant variables including the type of conditioning regimen, stem

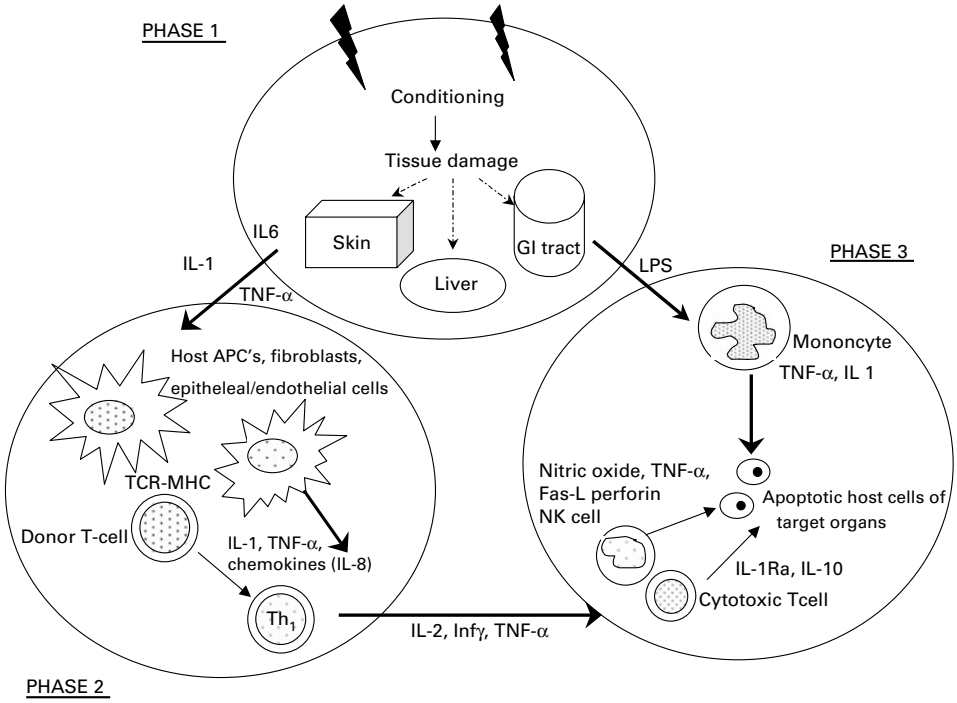

Figure 1 Diagrammatic representation of the 'cytokine storm', central to the pathogenesis of GvHD. Adapted from Dickinson and Charron,[17] and Hill and Ferrara.[26]

cell source and number of T cells within the graft as well as the type of GvHD prophylaxis. This is illustrated in a recent study of 113 patients undergoing non-myeloablative HSCT, where an increase of circulating IL-12 but no other cytokines was strongly associated with the development of aGvHD.[29] This is in sharp contrast to patients undergoing myeloablative conditioning, suggesting that the pathogenesis of GvHD is different in these two settings.

2. Induction and expansion phase. The second step is the triggering and activation of donor-derived T cells by recipient and donor APC as well as the inflammatory cytokines.[9] Activated T cells result in the production of IL-2 and IFN-γ (or Th1 response).[30] IL-2 controls and amplifies the allogeneic immune response,[31] activating further T-cell and natural killer cell responses, priming macrophages to release TNF-α and further inflammation damages skin and gut.

3. Effector phase. Finally, the effector phase is characterized by activated donor T-cell-mediated cytotoxic damage against host cells through Fas–Fas ligand interaction,[32,33] perforin–granzyme[33] and TNF-α.[34] The latter has a central role in the pathophysiology, stimulating cytokine production (IL-1, IL-6, IL-10, IL-12 and TNF-α). This dysregulation leads to the clinical manifestations of aGvHD.[1,28,35]

Cells involved in GvHD

1. T-cell subsets. It has been shown that CD4+ T cells are crucial for maintaining the expansion of CD8+ T cells that mediate GvHD.[36,37] However, in a recent clinical trial, attempts to reduce GvHD by eliminating CD8+ cells from the graft paradoxically showed a greater incidence of fever and rash and grade 2–4 GvHD.[38] These data suggest that although CD8+ cells are likely mediators of GvHD, CD4+ cells also have a crucial role in the pathogenesis of the disease.

2. T-regulatory cells (CD4+/CD25+ T_{reg}). T cells that may be capable of suppressing alloreactivity in the HSCT setting have been a recent focus of interest. A subset of CD4+ cells that coexpress CD25 is believed to suppress alloreactivity in a contact-dependent manner, with possible roles in cytokine production including IL-10 and transforming growth factor β.[39] Although murine models have demonstrated that infusing donor grafts rich in CD4+CD25+ T_{reg} cells decreases the incidence of severe GvHD,[40] it has proven difficult to exploit this in clinical practice. This is mainly due to the fact that CD25 expression is also upregulated in the setting of alloreactive T-cell stimulation. As such, two recent studies aimed at measuring the population of CD4+ CD25+ T cells infused in the graft showed a direct correlation between the quantity of these cells infused and the incidence of aGvHD and cGvHD. Further characterization and a better understanding of the role of this specific subset of T cells may assist in future therapies aimed at reducing GvHD.[41,42]

3. APCs. Recent studies have also implicated the role of residual host APCs in the initiation phase of aGvHD as described in murine models. In a model where donor CD8+ T cells recognized recipient minor HLA antigens, residual host APCs were essential for the initiation of GvHD.[20] It has also been demonstrated that the localization in various tissues may be relevant to the organ-specific manifestations of GvHD.[43–45] However, their precise role in the clinical setting in humans remains to be determined.

4. Natural killer cells. Natural killer cells can contribute to tissue damage in the effector phase by the release of inflammatory cytokines and nitric oxide. However, natural killer cells mediated cell death by two important contact-dependent pathways: Fas–Fas-ligand-mediated apoptosis and perforin–granzyme-B-mediated cytolysis.[32,33,46] Although they are important pathways of effective cell-mediated cytotoxicity, they are not the only mechanisms involved in GvHD. Murine models using anti-Fas antibodies or perforin-deficient T cells, where the induced organ-specific changes associated with aGvHD were not pronounced, suggest that natural killer cells are more likely to have a role in the affector than the effector phase.[11]

Trafficking of alloreactive T cells to target organs

To induce GvHD, alloreactive T cells must migrate to the specific tissue where they can exert their effector function.

Table 2 Organ staging of acute GvHD

Stage	*Skin*	*Liver*	*GI tract*
0	No rash due to GvHD	Bilirubin <2 mg per 100 ml or 35 μmol/l	None (<280 ml/m^2)
I	Maculopapular rash <25% of body surface area without associated symptoms	Bilirubin from 2 to <3 mg/100 ml or 35–50 μmol/l	Diarrhea >500–1000 ml/day (280–555 ml/m^2); nausea and emesis
II	Maculopapular rash or erythema with puritis or other associated symptoms ⩾25% of body surface area or localized desquamation	Bilirubin from 3 to <6 mg/100 ml or 51–102 μmol/l	Diarrhea >1000–1500 ml/day (556–833 ml/m^2); nausea and emesis
III	Generalized erythroderma; symptomatic macular, papular or vesicular eruption with bullous formation or desquamation covering ⩾50% of body surface area	Bilirubin 6 to <15 mg/100 ml or 103–225 μmol/l	Diarrhea >1500 ml/day (>833 ml/m^2); nausea and emesis
IV	Generalized exfoliative dermatitis or bullous eruption	Bilirubin >15 mg/100 ml or >225 μmol/l	Diarrhea >1500 ml/day (>833 ml/m^2); nausea and emesis. Abdominal pain or ileus

Abbreviation: GI = gastrointestinal.
Adapted from Przepiorka *et al.*[55]

Migration of immune cells is regulated by a complex system of chemokines and their receptors. The elevated expression of a number of pro-inflammatory chemokines has been demonstrated in target organs of GvHD in various murine models.[47] Their expression is influenced by the conditioning regimen as well as genetic factors and can be amplified by the occurrence of GvHD.[47] Most recently, the involvement of CCL27/CTACK–CCR10 interaction in recruiting T cells to the skin was demonstrated in 15 pediatric patients with skin GvHD.[48] During GvHD, circulating T cells isolated from these patients demonstrated a high proportion of CD4+ CD10+ T cells, which disappeared upon resolution of the skin GvHD. They expressed, in addition, skin homing markers (cutaneous lymphoid-associated antigen and CCR4) and produced Th1 cytokines, that is TNF-α and IL-2. These cells were absent in patients without skin GvHD. Skin biopsies showed infiltration of these cells and correlated with an increased epidermal expression of the ligand for CCL27/CTACK–CCR10.[48]

Predictors of GvHD

HLA differences between donor and recipient are the major predictor of GvHD.[49] Other factors implicated include age,[50] gender mismatch between donor and recipient,[50] mHA in otherwise identical HSCT,[16] donor age,[51] source and dose of stem cells (PBSCT greater risk than BM),[52] intensity of conditioning and GvHD prophylaxis.[53]

Administration of unmanipulated doses of donor lymphocytes (so-called DLI) has an increased risk of GvHD, especially following reduced-intensity conditioning for the treatment of mixed chimerism or relapse of solid tumors.[5–9] Again, this is related to dose and timing following HSCT.

Consideration has been given to an index of post transplant factors that may predict the severity of GvHD,[54] and new insights are being evaluated to determine what role, if any, genetic screening may have in the risk assessment of donor and recipient pairs.

Clinical manifestations of aGvHD

Acute GvHD affects predominately affects the skin, upper and lower GI tract, liver and occasionally the eye and oral mucosa. Clinical grading is determined by the site and severity of the manifestation (see Tables 2 and 3).[55]

Biopsy of involved tissues, although lacking sensitivity, when positive may be helpful in confirming the diagnosis, especially if the signs are relatively nonspecific.[1]

The characteristic rash of skin aGvHD is maculopapular, sometimes pruritic or painful. The distribution is typically on the palms of the hands and soles of the feet, which later progress's to the face, neck, upper chest and trunk. The severe stage III shows generalized erythroderma with progression to bullae formation and desquamation of the epidermal layers of the skin (stage IV—see Figure 2).

Acute liver GvHD is characterized by an isolated hyperbilirubinemia. The increase in alkaline phosphatase is seen more frequently than liver enzyme abnormalities.

Table 3 Overall clinical grading of aGvHD

Grade	*Skin*	*Liver*	*GI tract*	*Performance status*
0	0	0	0	0
I	1–2	0	0	0
II	1–3	1	1	1
III	2–3	2–3	2–3	2
IV	2–4	2–4	2–4	2–4

Abbreviation: GI = gastrointestinal.
Adapted from Przepiorka *et al.*[55]

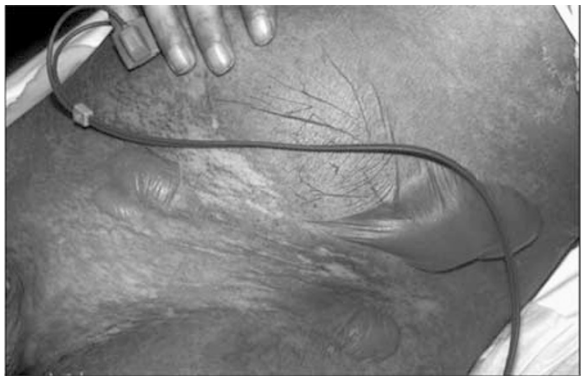

Figure 2 Stage IV skin acute GvHD. Characteristic erythroderma, generalized exfoliation of the superficial dermis with widespread bullous eruption.

Figure 3 Enteroscopic view of small bowel in stage IV acute GvHD (same patient as Figure 2). There is a striking atrophy of the villae, ulceration and bleeding. Biopsy of affected gut may lead to GI tract perforation and/or sepsis. These appearances were associated with severe diarrhea, malabsorption, intestinal ileus and severe pain.

Gastrointestinal GvHD symptoms are profuse diarrhea accompanied by anorexia and sometimes nausea. Progression with abdominal pain, GI bleeding and ileus is associated with later stages of GvHD (see Figure 3).

Conclusion

To date, our understanding of the pathogenesis of GvHD has dramatically improved and has led to new modalities of treatment and management. However, it is hoped that as new methodologies evolve and further insights are gained

into the underlying risk factors associated with the development of GvHD, it may be possible to construct not only clinical but also genetic paradigms, that may reduce the impact of GvHD as a major cause of transplant morbidity and mortality.

Conflict of interest

Neither author declared any financial interests.

References

1 Couriel A, Caldera H, Champlin R, Komanduri K. Acute graft versus host disease: pathophysiology, clinical manifestations, and management. *Cancer* 2004; **101**: 1936–1946.
2 Goker H, Haznedaroglu IC, Chao NJ. Acute graft-vs-host disease: pathobiology and management. *Exp Hematol* 2001; **29**: 259–277.
3 Billingham RE. *The Biology of Graft-versus-host Disease*. Academic Press Harvey Lecture: New York, 1966; **62**: 21.
4 McCarthy NJ, Bishop MR. Non-myeloablative allogeneic stem cell transplantation: early promise and limitations. *Oncologist* 2000; **5**: 487–496.
5 Marks DI, Lush R, Cavenagh J, Milligan DW, Schey S, Parker A *et al*. The toxicity and efficacy of donor lymphocyte infusions given after reduced-intensity conditioning allogeneic stem cell transplantation. *Blood* 2002; **100**: 3108–3114.
6 Barge RM, Osanto S, Marijt WA, Starrenburg CW, Fibbe WE, Nortier JW *et al*. Minimal GVHD following *in-vitro* T cell-depleted allogeneic stem cell transplantation with reduced-intensity conditioning allowing subsequent infusions of donor lymphocytes in patients with hematological malignancies and solid tumors. *Exp Hematol* 2003; **31**: 865–872.
7 Peggs KS, Thomson K, Hart DP, Geary J, Morris EC, Yong K *et al*. Dose-escalated donor lymphocyte infusions following reduced intensity transplantation: toxicity, chimerism, and disease responses. *Blood* 2004; **103**: 1548–1556.
8 Massenkeil G, Nagy M, Lawang M, Rosen O, Genvresse I, Geserick G *et al*. Reduced intensity conditioning and prophylactic DLI can cure patients with high-risk acute leukaemias if complete donor chimerism can be achieved. *Bone Marrow Transplant* 2003; **31**: 339–345.
9 Bader P, Kreyenberg H, Hoelle W, Dueckers G, Handgretinger R, Lang P *et al*. Increasing mixed chimerism is an important prognostic factor for unfavorable outcome in children with acute lymphoblastic leukemia after allogeneic stem-cell transplantation: possible role for pre-emptive immunotherapy? *J Clin Oncol* 2004; **22**: 1696–1705.
10 Slavin S. Smarter rather than stronger treatment of hematological malignancies and non-malignant indications for stem-cell transplantation. *Lancet* 2004; **364**: 122–124.
11 Goker H, Haznedaroglu IC, Chao NJ. Acute graft-vs-host-disease: Pathobiology and management. *Exp Hematol* 2001; **29**: 259–277.
12 Chao NJ. *Graft-vs-host disease*. 2nd edn. RG Landes and Co: Austin, USA, 1999.
13 Martin PJ, Gooley T, Anasetti C, Petersdorf EW, Hansen JA. HLAs and risk of acute graft-versus-host disease after marrow transplantation from and HLA identical sibling. *Biol Blood Marrow Transplant* 1998; **4**: 128–133.
14 Bland PW, Whiting CV. Induction of MHC class II gene products in rat intestinal epithelium during graft-vs-host disease and effects o the immune system of the epithelium. *Immunology* 1992; **75**: 366–371.
15 den Haan JM, Meadows LM, Wang W, Pool J, Blokland E, Bishop TL *et al*. The minor histocompatibility antigen HA-1: a diallelic gene with a single amino acid polymorphism. *Science* 1992; **279**: 1054–1057.
16 Goulmy E, Schipper R, Pool J, Blokland E, Falkenburg JH, Vossen J *et al*. Mismatches of minor incompatibility antigens between HLA-identical donors and recipients and the development of graft-versus-host disease after bone marrow transplantation. *N Engl J Med* 1996; **334**: 281–285.
17 Dickinson AM, Charron D. Non HLA immunogenetics in hematopoietic stem cell transplantation. *Curr Opin Imunnol* 2005; **17**: 517–525.
18 Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R *et al*. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001; **411**: 603–606.
19 Holler E, Rogler H, Herfarth H, Brenmoehl J, Wild PJ, Hahn J *et al*. Both donor and recipient NOD2/CARD15 mutations associate with transplant-related mortality and GvHD following allogeneic stem cell transplantation. *Blood* 2004; **104**: 889–894.
20 Holler E, Rogler G, Brenmoehl J, Hahn J, Herfarth H, Greinix H *et al*. Prognostic significance of NOD2/CARD15 variants in HLA-identical sibling hematopoietic stem cell transplantation: effect on long-term outcome is confirmed in 2 independent cohorts and may be modulated by the type of gastrointestinal decontamination. *Blood* 2006; **107**: 4189–4193.
21 Granell M, Urbano-Ispizua A, Aróstegui JI, Fernández-Avilés F, Martínez C, Rovira M *et al*. Effect of NOD2/CARD15 variants in T cell depleted allogeneic stem cell transplantation. *Hematologica* 2006; **91**: 1372–4193.
22 Mullighan CG, Bardy PG. New directions in the genomic of allogeneic stem cell transplantation. *Biol Blood Marrow Transplant* 2007; **13**: 127–144.
23 Baron C, Somogyi R, Greller LD, Rineau V, Wilkinson P, Cho CR *et al*. Prediction of graft versus host disease in humans by donor gene expression profiling. *PLoS Med* 2007; **4**: e23.
24 Murphy N, Diviney M, Szer J, Bardy P, Grigg A, Hoyt R *et al*. Donor methylenetetra-hydrofolate reductase genotype is associated with graft versus host disease in hematopoietic stem cell transplant patients treated with methotrexate. *Bone Marrow Transplant* 2006; **377**: 773–779.
25 Robien K, Bigler J, Yasui Y, Potter JD, Martin P, Storb R *et al*. Methylenetetra-hydrofolate reductase and thymidylate synthase genotypes and risk of acute graft versus host disease following hematopoietic stem cell transplantation for chronic myelogenous leukemia. *Biol Blood Marrow Transplant* 2006; **12**: 973–980.
26 Hill GR, Ferrara JL. The primacy of the gastrointestinal tract as a target organ of acute graft-versus-host disease: rationale for the use of cytokine shields in allogeneic bone marrow transplantation. *Blood* 2000; **95**: 2754–2759.
27 Ferrara JL, Cooke KR, Teshima T. The pathophysiology of acute graft-versus-host disease. *Int J Hematol* 2003; **78**: 181–187.
28 Zeiser R, Marks R, Bertz H, Finke J. Immune pathogenesis of acute graft-versus-host disease: implications for novel preventive and therapeutic strategies. *Ann Hematol* 2004; **83**: 551–565.
29 Mohty M, Blaise D, Faucher C, Vey N, Bouabdallah R, Stoppa AM *et al*. Inflammatory cytokines and acute graft versus host disease after reduced intensity conditioning allogeneic stem cell transplantation. *Blood* 2005; **106**: 4407–4411.
30 Mossmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman R. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986; **136**: 2348–2357.

31 Via CS, Finkelman FD. Critical role of interleukin-2 in the development of acute graft-versus-host disease. *Int Immunol* 1993; **5**: 565–572.
32 Schmaltz C, Alpdogan O, Horndasch KJ, Muriglan SJ, Kappel BJ, Teshima T *et al*. Differential use of Fas ligand and perforin cytotoxic pathways by donor T cells in graft-versus-host disease and graft-versus-leukemia effect. *Blood* 2001; **97**: 2886–2895.
33 Wasem C, Frutschi C, Arnold D, Vallan C, Lin T, Green DR *et al*. Accumulation and activation-induced release of pre-formed Fas (CD95) ligand during the pathogenesis of experimental graft-versus-host disease. *J Immunol* 2001; **167**: 2936–2941.
34 Rembereger M, Jaksch M, Uzunel M, Mattson J. Serum level of cytokines correlate to donor chimerism and acute graft-versus-host disease after hematopoietic stem cell transplantation. *Blood* 2003; **101**: 2440–2445.
35 Antin JH, Ferrara JL. Cytokine dysregulation and acute graft-versus-host disease. *Blood* 1992; **80**: 2964–2968.
36 Kalams SA, Walker BD. The critical need for CD4 help in maintaining effective cytotoxic T lymphocyte responses. *J Exp Immunol* 2002; **169**: 7111–7118.
37 Jansssen EM, Lemmens EE, Wolfe T, Christen U, von Herrath MG, Schoenberger SP. CD4+ T cells are required for secondary expansion and memory in CD8+ T lymphocytes. *Nature* 2003; **421**: 852–856.
38 Ho VT, Kim HT, Li S, Hochberg EP, Cutler C, Lee SJ *et al*. Partial CD8+ T-cell depletion of allogeneic peripheral blood stem cell transplantation is insufficient to prevent graft-versus-host disease. *Bone Marrow Transplant* 2004; **34**: 987–994.
39 Dieckman D, Bruett CH, Ploettner H, Lutz MB, Schuler G. Human CD4+CD25+ regulatory contact-dependent T cells induce interleukin 10-producing, contact independent type I like regulatory T cells. *J Exp Med* 2002; **196**: 247–253.
40 Nakaruma K, Kitani A, Strober W. Cell contact-dependent immunosuppression by CD4+CD25+ regulatory T cells is mediated by cell surface transforming growth factor beta. *J Exp Med* 2001; **194**: 629–644.
41 Stanzani M, Martins SL, Saliba RM, St John LS, Bryan S, Couriel D *et al*. CD25 expression on donor CD4+ or CD8+ T cells is associated with an increased risk of graft versus host disease after HLA identical stem cell transplantation in humans. *Blood* 2004; **103**: 1140–1146.
42 Clark FJ, Gregg R, Piper K, Dunnion D, Freeman L, Griffiths M *et al*. Chronic graft versus host disease is associated with increased numbers of peripheral blood CD4+CD25 high regulatory T cells. *Blood* 2004; **103**: 2410–2416.
43 Shlomchik WD, Couzens MS, Tang CB, McNiff J, Robert ME, Liu J *et al*. Prevention of graft versus host disease by inactivation of host antigen presenting cells. *Science* 1999; **285**: 412–415.
44 Duffner UA, Maeda Y, Cooke KR, Reddy P, Ordemann R, Liu C *et al*. Host dendritic cells alone are sufficient to initiate acute graft-versus-host disease. *J Immunol* 2004; **172**: 7393–7398.
45 Matte CC, Liu J, Cormier J, Anderson BE, Athanasiadis I, Jain D *et al*. Donor APC's are required for maximal GvHD but not for GVL. *Nat Med* 2004; **10**: 987–992.
46 Via CS, Nguyen P, Shustov A, Drappa J, Elkon KB. A major role for the Fas pathway in acute graft versus host disease. *J Immunol* 1996; **157**: 5387–5393.
47 Mapara MY, Leng C, Kim YM, Bronson R, Lokshin A, Luster A *et al*. Expression of chemokines in GvHD target organs is influenced by conditioning and genetic factors and amplified by GVHR. *Biol Blood Marrow Transplant* 2006; **12**: 623–634.
48 Faaij CM, Lankester AC, Spierings E, Hoogeboom M, Bowman EP, Bierings M *et al*. A possible role for CCL27/CTACK-CCR10 interaction in recruiting CD4+ T cells to skin in human graft verusus host disease. *Br J Haematol* 2006; **133**: 538–549.
49 Beatty PG, Clift RA, Mickelson EM, Nisperos BB, Flournoy N, Martin PJ *et al*. Marrow transplantation from related donors other than HLA-identical siblings. *N Engl J Med* 1985; **313**: 765–771.
50 Weisdorf D, Hakke R, Blazar B, Miller W, McGlave P, Ramsay N *et al*. Risk factors for acute graft versus host disease in histocompatible donor bone marrow transplantation. *Transplantation* 1991; **51**: 1197–1293.
51 Kollman C, Howe CW, Anasetti C, Antin JH, Davies SM, Filipovich AH *et al*. Donor characteristics as risk factors in recipients after transplantation of bone marrow from unrelated donors. *Blood* 2001; **98**: 2043–2051.
52 Cutler C, Giri S, Jeyapalan S, Paniagua D, Viswanathan A, Antin JH. Acute and chronic graft versus host disease after allogeneic peripheral blood stem cell and bone marrow transplantation: a meta-analysis. *J Clin Oncol* 2001; **19**: 3685–3691.
53 Przepiorka D, Smith TL, Folloder J, Khouri I, Ueno NT, Mehra R *et al*. Risk factors for acute graft-versus-host disease after allogeneic blood stem cell transplantation. *Blood* 1999; **94**: 1465–1470.
54 Leisenring WM, Martin PJ, Petersdorf EW, Regan AE, Aboulhosn N, Stern JM *et al*. An acute graft versus host disease activity index to predict survival after hematopoietic stem cell transplantation with myeloablative conditioning regimens. *Blood* 2006; **108**: 749–755.
55 Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J *et al*. 1994 Consensus conference on acute GvHD grading. *Bone Marrow Transplant* 1995; **15**: 825–828.