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Clinical and laboratory features of mesenchymal stromal cells in pediatric stem cell transplantation

Ball, L.M.

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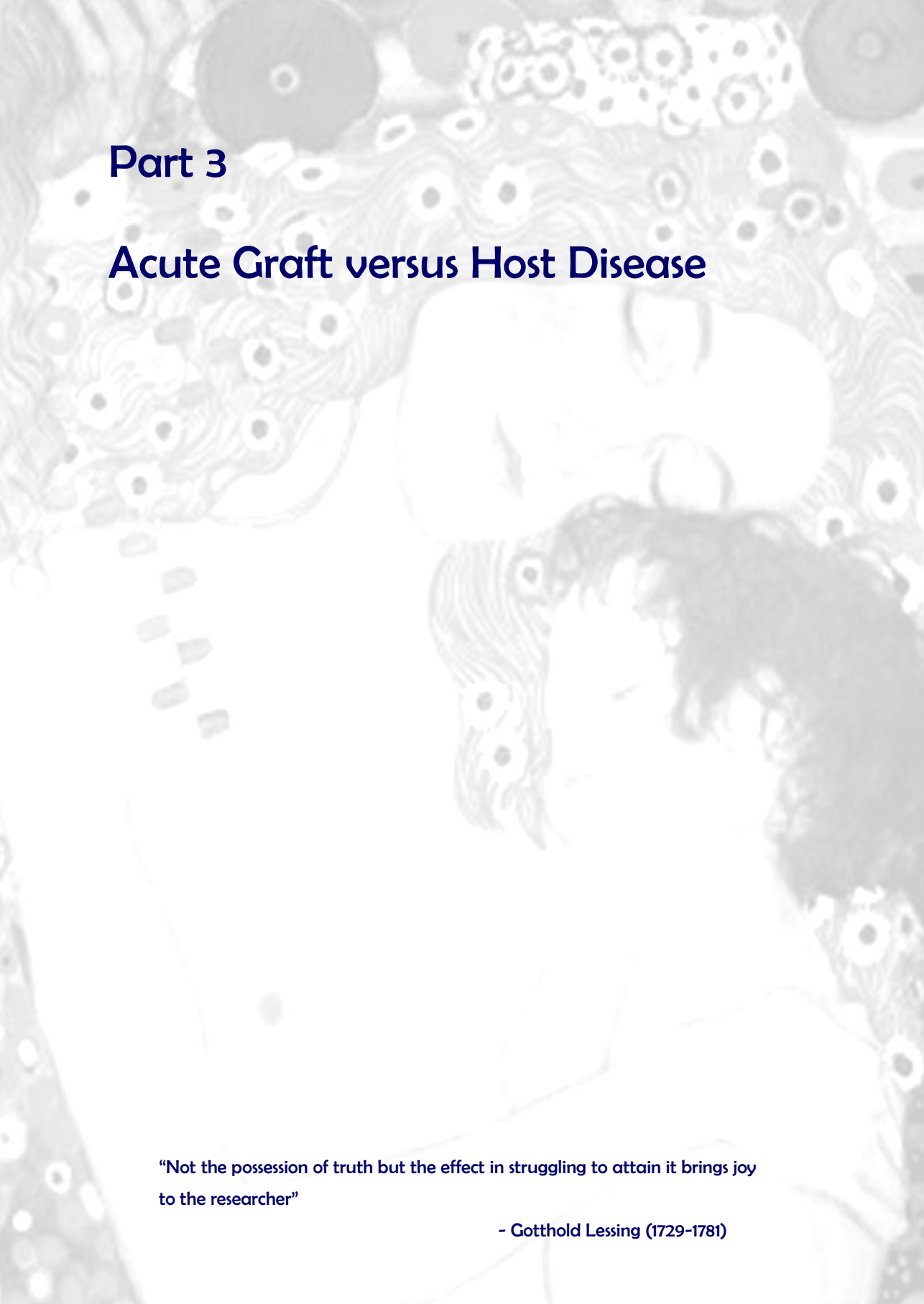
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Part 3

Acute Graft versus Host Disease

“Not the possession of truth but the effect in struggling to attain it brings joy to the researcher”

- Gotthold Lessing (1729-1781)

Chapter 6

Acute Graft-versus-Host Disease: Pathogenesis and classification.

LM Ball

RM Egeler

On behalf of the Pediatric Working Party of the EBMT

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Summary

Allogeneic hematopoietic stem cell transplantation (HSCT) is an established treatment for some children with life threatening hematological disease, immune deficiencies and in-born errors of metabolism. Despite advances in prevention and post transplant immune suppressive strategies, acute graft versus host disease (aGvHD) remains a major cause of morbidity and mortality in children undergoing stem cell transplantation. 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 immune suppressive prophylaxis.

Acute GvHD occurs when transplanted donor T lymphocytes recognize antigenic disparities between host and recipient. Methods 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 that activation of T cells is one step in a 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 acute GvHD.

Background

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

Despite advances in donor HLA typing methods (and thus donor selection) and post-transplant immune suppression, acute (α)GvHD 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 α GvHD over 30 years ago.³ The first requirement is that the graft must contain sufficient numbers of immunologically competent cells. Secondly, 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 a 100 days post HSCT that has defined the acute versus chronic manifestation of this disease. However, as transplant practice has changed, so to 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 have 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 common place in protocols designed to induced 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 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 α GvHD.

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 major histocompatibility complex or MHC, play 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 HLA 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 acute GvHD.^{11,14}

2. Minor histocompatibility antigens (mHag) 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 mHags are mostly but not exclusively restricted to class I HLA. Tissue expression of some mHags 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 may play an important role in the afferent phase of aGvHD. Studies suggest that high levels of TNF- α and low levels of IL-10 in patients pre-transplant results in more GvHD. These results in turn correlate with further investigations into the gene polymorphisms of TNF- α and IL-10 in donors and recipients 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, INF- γ , IL-1

family and TGF- β genes.¹⁷ Other candidate genes are Th1 and Th2 associated with immunopathology of GvHD e.g. 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 vary depending upon the donor, recipient and stem cell source. (See table 1). As more knowledge becomes available from gene mapping of the pro and anti-inflammatory genes, the influence of polymorphisms of neighboring genes and their effects of cytokine release on outcome of HSCT, interpretation will undoubtedly become more complex.

Table 1. GVHD risk related to donor/recipient cytokine gene polymorphisms

Gene	Polymorphism	Recipient /donor	Donor type	aGvHD outcome
TNF α	d3/d3	Recipient	Identical sibling	↑ grade II-IV GvHD Increased mortality
	TNF-863, TNF -857	Donor and/or recipient	Matched unrelated donor (MUD)	Increased
	TNF α -238, TNF β -252	Donor and/or recipient	Unrelated donor	Increased grade II-IV Increased mortality
	TNFD4, TNF α -1031C and TNFa5	Donor and/or recipient	Unrelated donor	Increased mortality
	TNFD4	Recipient	Identical sibling	↑ moderate aGvHD
	TNFR1-196R	Donor	MUD	↑ grade severe aGvHD
	TNFR1-196M	Homozygous donor	MUD	Reduced risk aGvHD
	TNFR1-196R	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-CCC	Recipient	MUD	Reduced aGvHD and mortality
IL-6	II-6-174	Recipient	MUD	↑ grade severe aGvHD
INF γ	INF γ 2/2	Recipient	Identical sibling	Reduced aGvHD
	INF γ 3/3	Recipient	Identical sibling	↑ aGvHD
IL-1 family	IL-Ra	Donor	Identical sibling	Reduced aGvHD
		Recipient	Identical sibling	↑ chronic GvHD
	IL-1 α 889 (pediatric)	Donor and Recipient	MUD	Improved survival Less TRM
TGF β	TGF β -509	Donor and Recipient	Identical sibling	No effects
	TGF β codon 10 (pediatric)	Donor	Identical sibling	↑ aGvHD
	TGF β codon receptor II (pediatric)	Recipient	Identical sibling	↑ aGvHD

Adapted from: Dickinson AM, Charron D. Current Opinions in Immunology, 2005; 17: 517-525.¹⁷

2. NOD2/CARD15 polymorphisms

The NOD2/CARD15 gene is involved in the innate immune response to bacterial infections in the gastrointestinal tract and mediated nuclear factor- κ B (NF- κ B) activation in response to bacterial cell wall products. Three single nucleotide polymorphisms (SNPs 8, 12 and 13) in the NOD2/CARD15 gene have been associated with a diminished NF- κ B production and were first described with an increased risk of acute inflammatory bowel disease (Crohn's disease).¹⁸ Recently, the same SNPs have been implicated in both the incidence and severity of a GvHD following HLA identical sibling donor HSCT. Transplanted 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 59% for transplants with a donor only mutation whereas the worst case scenario was where both donor and recipient had detectable SNP mutations of the NOD/CARD15 gene (83%). Similar incidences were seen for overall and severe gastrointestinal 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 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 are enabling analysis of hundreds of thousands of genetic markers throughout the genome and copy number variations to be investigated.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 off 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 large number of donor recipient pairs and in alternative donor as well and non-myeloablative stem cells transplants.^{22,23}

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

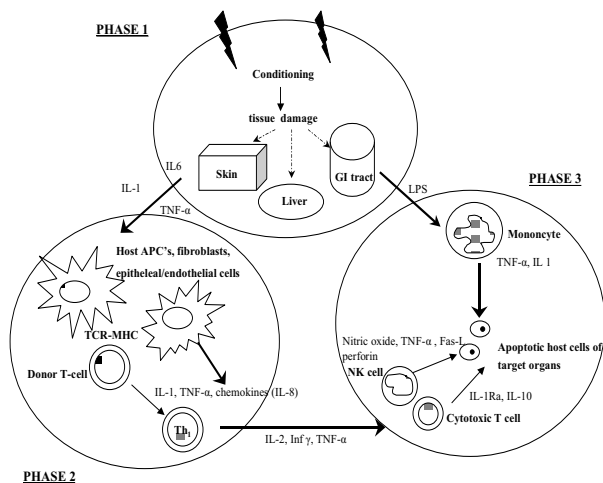
Pathophysiology of GvHD

GvHD can be described as a three-phase process.¹ (See figure 1)

1. Afferent phase

In this stage, as is seen with the conditioning of the patient, prior disease and co-morbidity of the patient, damage to host tissue occurs. For example, bacterial endotoxins (lipopolysaccharides- LPS) may translocate from the intestinal lumen into the circulation and induces the release of inflammatory cytokines, including IL-1, TNF- α , IL-6 and interferon- γ ,²⁶⁻²⁸. These act to up-regulate the expression of MHC antigens and cell surface adhesion molecules on host antigen presenting cells (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 influence 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 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 stark contrast to patients undergoing myeloablative conditioning, suggesting that the pathogenesis of GvHD is different in these two settings.

Figure 1. Diagrammatic representation of the “cytokine storm”; central to the pathogenesis of graft versus host disease



Adapted from: Dickinson AM, Charron D. *Curr Opin Immunol* 2005; 17: 518²⁷, Hill GR, Ferrara JL. *Blood* 2000; 95: 2755.²⁶

2. Induction and expansion phase

The second step is the triggering and activation of donor-derived T-cells by recipient and donor antigen presenting cells as well as the inflammatory cytokines.⁹ Activated T-cells result in the production of IL-2 and interferon- γ (or Th1 response).³⁰ IL-2 controls and amplifies the allogeneic immune response,³¹ activating further T and natural killer (NK) 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-granzyme³³ and TNF- α .³⁴ The latter plays 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 in fever and rash and grade 2-4 GvHD.³⁸ This data suggests that although CD8+ cells are likely mediators of GvHD CD4+ cells also play 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 co-express CD25 is believed to suppress alloreactivity in a contact-dependent manner, with possible roles for 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 decrease the incidence of severe GvHD,⁴⁰ it has proved difficult to exploit this in clinical practice. This is mainly due to the fact that CD25 expression is also up regulated 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 acute and chronic GvHD. 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. Antigen presenting cells (APCs)

Recent studies have also implicated the role of residual host APCs in the initiation phase of aGvHD has 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 (NK cells)

NK cells can contribute to the tissue damage in the effector phase by the release of inflammatory cytokines and nitric oxide. However, NK cells mediated cell death by two important contact dependent pathways, Fas-Fas ligand (Fas-L) mediated apoptosis and perforin-granzyme B mediated cytotoxicity.^{32,33,46} Though 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 NK cells are more likely to play a role in the effector than the effector phase.¹¹

Trafficking of alloreactive T cells to target organs

In order to induce GvHD alloreactive T cells must migrate to the specific tissue where they can exert their effector function. 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 i.e. 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 CCR10 (CCL27/CTACK).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 minor histocompatibility 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 un-manipulated doses of donor lymphocytes (so-called DLI's) have 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 GvHD⁵⁴ and new insight are being evaluated to determine what role if any genetic screening may play in the risk assessment of donor and recipient pairs.

Clinical manifestations of aGvHD

Acute GvHD 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 Table 1 and 2)⁵⁵

Biopsy of involved tissues, although lacking sensitivity, when positive may be helpful in confirming the diagnosis, especially if the signs are relatively non-specific.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, that later progress 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.

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

Table 1. Organ staging of acute GvHD

Stage	Skin	Liver	GI tract
Organ staging 0	No rash due to GvHD	Bilirubin < 2 mg/dl or 35 µmol/l	None
I	Maculopapular rash < 25% of body surface area without associated symptoms	Bilirubin from 2 mg/dl to < 3 mg/dl or 35-50 µmol/l	Diarrhea > 500-1000 ml/day; 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 mg/dl to < 6 mg/dl or 51-102 µmol/l	Diarrhea 1000 to 1500 ml/day; nausea and emesis
III	Generalized erythroderma or symptomatic macular, papular, or vesicular eruption with bullous formation or desquamation covering > 50% of body surface area	Bilirubin 6 mg/dl to < 15 mg/dl or 103-225 µmol/l	Diarrhea > 1500 ml/day; nausea and emesis
IV	Generalized exfoliative dermatitis or bullous eruption	Bilirubin > 15 mg/dl or > 225 µmol/l	Diarrhea > 1500 ml/day; nausea and emesis. Abdominal pain or ileus

Adapted from: Przepiorka D, Weisdorf D, Martin P *et al.* 1994 Consensus conference on acute GvHD grading. *Bone Marrow Transplant* 1995. 15: 825-828.⁵⁵

Table 2. 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

Adapted from: Przepiorka D, Weisdorf D, Martin P *et al.* 1994 Consensus conference on acute GvHD grading. *Bone Marrow Transplant* 1995. 15: 825-828.⁵⁵

Figure 2. Stage IV skin aGvHD



Characteristic erythroderma, generalized exfoliation of the superficial dermis with widespread bullous eruption.

Figure 3. Enteroscopic view of small bowel in stage IV acute Graft versus host disease
(same patient as figure 2)



There is 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

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 genetic paradigms, which may reduce the impact of GvHD as a major cause of transplant morbidity and mortality.

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