



Review

Predicting Outcome in Hematological Stem Cell Transplantation

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Abstract. This review summarizes recent results investigating the role of certain cytokine gene polymorphisms, including those of TNF- α , IFN- γ , IL-6, IL-10 and IL-1 receptor antagonist (IL-1Ra), in allogeneic stem cell transplantation. It discusses their role in predicting outcome and the development of a genetic risk index for graft versus host disease (GvHD) in HLA-matched sibling transplants. By the comparative use of an *in vitro* human skin explant model, initial results suggest that certain cytokine gene polymorphisms may be associated with more severe disease.

Key words: cytokine gene polymorphisms; predicting GvHD; an *in vitro* human skin explant model.

Introduction

Acute graft versus host disease (GvHD) remains a major complication of allogeneic stem cell transplantation (SCT), leading to chronic disease and transplant-related morbidity^{19, 21}. GvHD incidence following allogeneic SCT is 30–80% even in patients undergoing conventional prophylaxis^{5, 46} and can be fatal in 50% of cases. In recent years, methods to predict the occurrence and severity of GvHD have been described. These include a human skin explant assay for GvHD and genotyping for non-HLA-coded genes, including

polymorphisms within cytokine genes. Recently, other non-HLA-coded genes, such as the vitamin D receptor, have also been associated with outcome and survival^{17, 39}. This review will summarise some of the most recent results in this area of transplant risk assessment and describe a multivariable analysis of a large, multicentre cohort.

Graft versus Host Disease

GvHD has been described as occurring in at least 3 phases and involving a “cytokine storm”^{17, 33}. The

Abbreviations used: SCT – stem cell transplantation, TNF- α – tumour necrosis factor α , IFN- γ – interferon γ , IL-1 – interleukin 1, IL-1 Ra – interleukin 1 receptor antagonist, VNTR – variable number of tandem repeats, SNP – single nucleotide polymorphism, IDDM – insulin dependant diabetes mellitus, SLE – systemic lupus erythematosus, GvHD – graft versus host disease, GvHR – graft versus host reactions, GvL – graft versus leukemia, IP – interstitial pneumonitis, VOD – veno-occlusive disease, CyA – cyclosporin A, ATG – anti-thymocyte globulin, NK – natural killer, CML – chronic myeloid leukemia.

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first phase, initiated by host transplant-conditioning regimens (total body irradiation and cytotoxic chemotherapy), gives rise to proinflammatory cytokine and chemokine release (e.g. IL-1, IL-6, IL-8, TNF- α) and initial histopathological damage in GvHD target organs: skin, liver and gut^{1, 11, 24, 62}. This initial cytokine release is amplified in the second phase via the activation of donor T cells within the marrow inoculum reacting to upregulated host tissue antigens, including HLA and adhesion molecule expression^{29, 34, 45}. The final phase involves further host tissue damage at the level of effector cells (activated T cells and NK cells) and the release of mainly Th1 type cytokines (IL-2, IFN- γ , TNF- α)^{17, 32, 56}.

Recent results in the mouse model have demonstrated the role of host-derived dendritic cells⁴⁹ in the development of GvHD. Results from TESHIMA et al.⁵⁷ have also further shown that allorecognition by specific T cells need not necessarily cause the pathological lesions in GvHD and that proinflammatory mediators (initiated by dendritic cell action) such as IL-1 and TNF- α can cause direct damage. This challenges current dogma of the direct role of the alloimmune T cell response.

Predicting GvHD in Allogeneic Hematopoietic Stem Cell Transplants

A skin explant model

For over a decade we have used a human skin explant assay for predicting GvHD in HLA-matched sibling cohorts (for a recent review see SVILAND and DICKINSON⁵⁴). The assay, originally described for predicting GvHD in HLA-matched sibling transplants by VOGEL-SANG et al.⁶¹ consists of a mixed culture of patient (stimulator) and donor (responder) lymphocytes which elicits graft versus host type reactions (GvHR) in a patient skin explant (see Fig. 1). The grades of GvHR reflect the degree and severity of GvHD which the patient may encounter post transplant. The GvHR grades I–IV (see Table 1 and Figs. 2–4) observed in the bio-

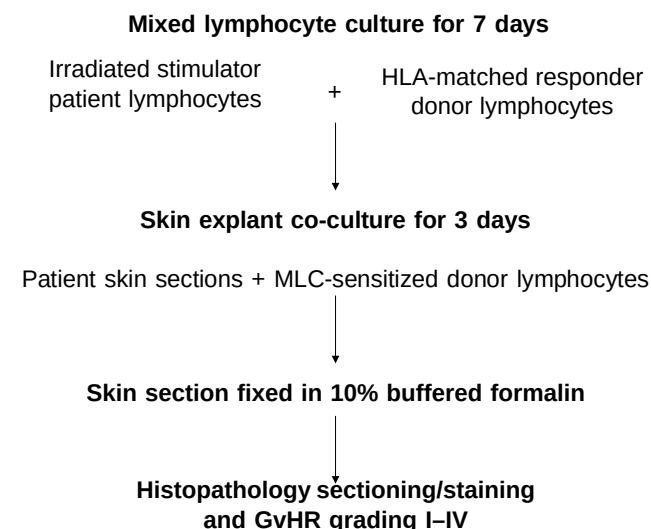


Fig. 1. Flow chart of the skin explant assay

psies demonstrate increasing severity to grade IV GvHR. Skin biopsies graded II, III or IV are considered positive, and in HLA-matched sibling transplants on cyclosporin alone as prophylaxis, the assay has a positive or negative predictive value of 80%. The degree of predictability of the assay depends on the degree of GvHD prophylaxis given post transplant. Studies of pediatric and adult cohorts showed that the skin explant assay was not as predictive (50–60%) in patients given cyclosporin and additional methotrexate¹³.

This knowledge therefore allows clinicians to modify GvHD prophylaxis, depending on the skin explant result, on an individual patient basis. For example, in our BMT centre, where HLA-matched sibling transplants are routinely given cyclosporin alone as prophylaxis, if their skin explant assay is positive they are given increased prophylaxis in the form of cyclosporin plus additional methotrexate. Patients who are not at such a risk of developing GvHD, having grade I or negative GvHR skin explant results, remain on cyclosporin alone. Similarly, a more recent study of a matched, unrelated donor cohort demonstrated that the skin explant assay was 100% predictive in pediatric patients given cyclosporin and methotrexate but was not as predictive in patients who were T cell depleted with anti-thymocyte globulin (ATG) or Campath⁽²⁸⁾ plus unpublished data).

Nevertheless, the skin explant assay remains a clear positive indicator of grades II–IV GvHD and indicates that appropriate or increased GvHD prophylaxis needs to be given. Conversely, patients with negative skin explant results may benefit from a reduced form of prophylaxis and this may aid in the development of a beneficial graft versus leukemia (GvL) effect²⁸.

Table 1. Histopathological evaluation of GvHR

Grade	Severity of GvHR
0	Normal skin
I	Few histopathological changes; some vacuolization of epidermal basal cells
II	Diffuse vacuolization of basal cells, with dyskeratotic bodies
III	Subepidermal cleft formation
IV	Complete epidermal separation

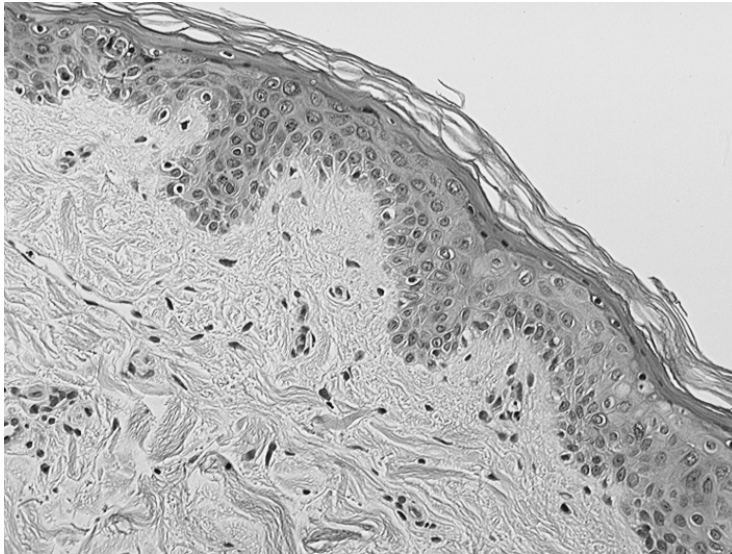


Fig. 2. Grade I GvHR-negative skin explant showing few histopathological changes. Hematoxylin and eosin $\times 200$

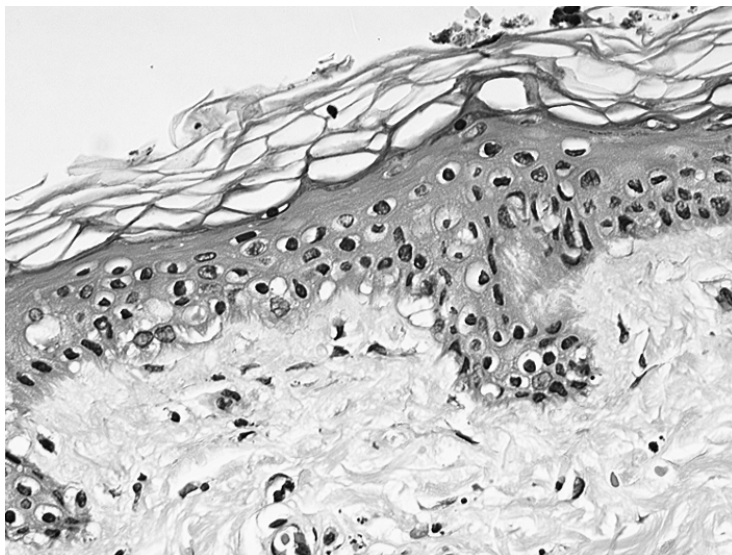


Fig. 3. Grade II GvHR-positive skin explant showing vacuolization and dyskeratosis. Hematoxylin and eosin $\times 200$

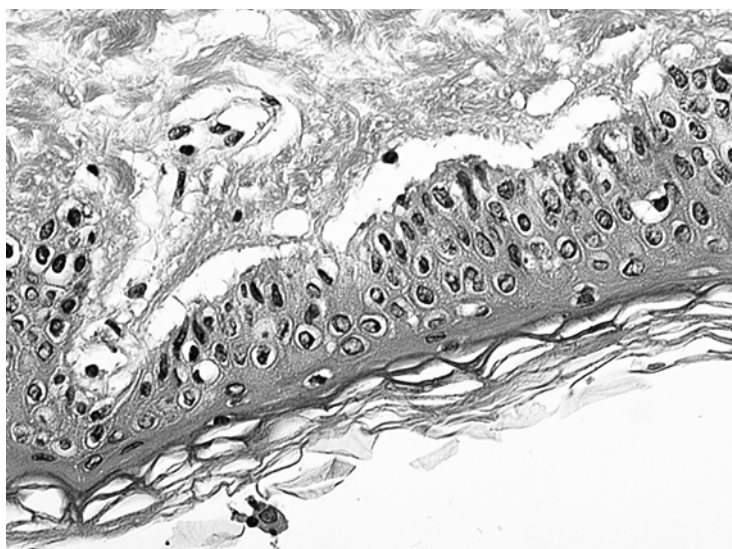


Fig. 4. Grade III GvHR-positive skin explant showing extensive vacuolization and subepidermal cleft formation. Hematoxylin and eosin $\times 200$

The skin explant assay is currently being used as a comparative test in cytokine gene polymorphism studies and as a risk factor indicator in combined analyses.

Cytokine gene polymorphisms

HOLLER et al.^{23, 25, 26} have demonstrated the pathophysiological involvement of inflammatory cytokines, including TNF- α , not only in GvHD but also in other transplant-related complications, such as interstitial pneumonitis (IP) and veno-occlusive disease (VOD)²⁷. Patients characterized by high spontaneous IL-10 production (an antagonist of TNF- α) were protected from GvHD as well as IP and VOD²⁷. Analysis of the results suggested a genetic background was associated with the protection. Furthermore, within normal populations, high or low producers of TNF and IL-10 exist and this is genetically determined due to inherent cytokine gene regulation associated with cytokine genetic polymorphisms. Polymorphisms within the 5' or 3' regulatory sequences of genes may alter the structure of the transcription factor binding sites within gene promoters, and many of the reported cytokine gene polymorphisms occur within apparent regulatory regions of the gene^{6, 7}. In line with these observations, we recently demonstrated the role of cytokine gene polymorphisms of TNF- α (TNFd3/d3 genotype) and IL-10 (IL-10⁽¹²⁻¹⁶⁾ genotypes) in predicting GvHD in HLA-matched sibling transplants. The results suggest that the patients' genotype genetically predispose them to the development of GvHD. Other cytokines, such as IL-1, IL-2, IL-6 and IFN- γ , all play a role in the alloimmune response, and our current studies include a comparative analysis of

acute GvHD-predictive skin-explant-assay results together with cytokine gene polymorphism data as predictive indicators of GvHD in HLA-matched sibling transplants.

All the cytokine gene polymorphisms examined in our studies were selected on the basis of either their association with altered cytokine production *in vivo*, and/or their demonstrated association with transplant-related pathology, and/or their association with diseases possessing autoimmune or immune-definite characterization.

Polymorphisms within or adjacent to regulatory regions are quite common in cytokine genes, whereas coding-region polymorphisms and variations in protein structure have not been widely studied or reported (see Table 2). TNF- α and IL-10 polymorphisms have been associated with renal transplant rejection and systemic lupus erythematosus (SLE)^{16, 20, 31, 47, 53, 58-60}. Polymorphism in the IFN- γ intron-1 microsatellite (CA)_n repeat has been associated with *in vitro* IFN- γ production⁴⁴, lung transplant fibrosis³, renal transplant rejection², insulin-dependent diabetes mellitus (IDDM)⁴ and Graves' disease⁵⁰. The IL-6⁻¹⁷⁴ (G/C) single nucleotide polymorphism (SNP) has been linked to *in vivo* and *in vitro* IL-6 production and juvenile chronic arthritis¹⁸ and associated with acute renal rejection³⁸, osteoporosis⁴² and SLE³⁶. All of the above polymorphisms in our study on GvHD have been associated in the recipient with acute or, in the case of IL-6⁻¹⁷⁴, chronic GvHD, suggesting that the recipient genotype for cytokine production and release may play a role in the severity of subsequent classical GvHD involving the T cell response. The exception is IL-1Ra, where in our studies the donor genotype was associated with GvHD

Table 2. Cytokine gene polymorphism; disease association

Cytokine gene polymorphism	Disease association	References
TNF- α d3/d3	Cardiac transplant rejection	58
TNF- α ⁻³⁰⁸	Cardiac and renal transplant rejection	59
TNF- α ⁻³⁰⁸	SLE	31
		53
		20
IL-10 ⁻¹⁰⁶⁴	SLE	16
IL-10 ⁻¹⁰⁸²	Cardiac and renal transplant rejection	58, 60
IFN- γ (CA) _n		47
	Lung allograft fibrosis	3
	Renal transplant rejection	2
Intron 1	IDDM	4
	Graves' disease	50
IL-6 ⁻¹⁷⁴	G/C juvenile chronic arthritis	18
IL-6 ⁻¹⁷⁴ (donor)	Renal transplant rejection	38
IL-1Ra VNTR	Bone loss	30
Intron 2	IDDM	43
	Inflammatory bowel disease	37
	SLE	8

outcome. The IL-1 α variable number of tandem repeats (VNTR) in intron 2 has been associated with SLE⁸, inflammatory bowel disease³⁷, bone loss³⁰, IDDM⁴³ and increased IL-1 β production⁴⁸.

In vivo disease association may not necessarily correlate with *in vitro* expression or production. This could be due to a number of factors including, for example, the fact that genetic differences between populations can also give rise to differences in *in vivo* or *in vitro* cytokine production due to genetic variation in cytokine/chemokine gene polymorphisms. These differences, therefore, make comparisons between populations difficult unless local population allele frequency is simultaneously adequately assessed.

Recent studies in our laboratories⁴¹ and others¹⁵ have shown an association of the presence of an ACC IL-10 haplotype not only with low IL-10 production¹⁵, but with susceptibility to GvHD and disease⁵². This may also be shown to be more prevalent in certain populations than others, such as in Japan, where the GCC haplotype is of a very low frequency compared with Europe⁵⁵.

Our initial studies on the TNF α 3 allele showed that with increased *in vitro* TNF production and recipient TNF α 3 homozygosity correlated with increased severity of acute GvHD in cyclosporin A (CyA) treated patients and was associated with severe GvHD grades III–IV. Similarly, the IL-10 microsatellites (alleles IL-10^(12–15) with a larger number of CA repeats) were significantly associated with more severe GvHD grades III–IV. These results were confirmed by others⁵² and the allele frequency overlaps with the GCA haplotype frequency also associated with low IL-10 production¹⁵. Others have demonstrated a role of donor rather than recipient haplotype⁵².

Recent studies from our laboratory¹⁰ confirmed the initial studies on the involvement of recipient TNF α 3/d3 and IL-10^(12–16) on GvHD occurrence and severity, and data collected on the role of cytokine gene polymorphisms of IFN- γ (intron 1; 3,3), IL-6⁻¹⁷⁴GG, together with TNF α 3/d3 and IL-10^(12–16) suggest that a risk index for GvHD may be developed. This risk analysis would include other known clinical risk factors, such as gender, age, cytomegalovirus status and minor histocompatibility mismatches, and may aid in future clinical policy on prophylaxis and therapy for GvHD. Results on 80 HLA-matched siblings using multivariate analysis of known cytokine gene polymorphisms as risk factors demonstrated that recipient IFN- γ , IL-10⁻¹⁰⁶⁴ and TNF α genotype associate with severe acute GvHD. The IL-6⁻¹⁷⁴ genotype also associates with chronic GvHD⁹ and, more recently, in a larger

cohort, IL-6⁻¹⁷⁴ may associate with acute GvHD (unpublished observations). Logistic regression analysis⁹ confirmed the independent association of recipient IFN- γ intron 1–3/3 homozygous genotype with severe acute GvHD. Other independent factors included the recipient possession of one or more IL-10⁻¹⁰⁶⁴ (12–16) alleles, TNF α 3/d3 homozygous genotype and age as acute GvHD risk factors. The IL-6⁻¹⁷⁴ GG genotype was also confirmed, by forward step-wise modelling, as a risk factor for chronic GvHD together with age, gender mismatch and disease (chronic myeloid leukemia – CML). Other recent reports support the hypothesis of cytokine gene polymorphisms associating with bone marrow transplantation complications both in GvHD and multiorgan dysfunction^{22, 35, 51, 52, 55}.

Extended studies in a multicentre European cohort (unpublished results) have confirmed these findings and have further identified as “risk factors” cytokine gene polymorphisms which may occur in either the patient or the donor genotype.

In multivariate analysis cytokine gene polymorphism risk-factor analysis appears as important as that of other known clinical risk factors for GvHD. The influence of cytokine gene polymorphism on outcome also appears to be influenced by such factors as the size of the cohort and diagnosis, e.g. CML, age and prophylaxis.

The presence of certain cytokine gene polymorphisms, including IL-1 α VNTR (absence of allele 2), TNF α 3/d3, IL-10⁻¹⁰⁶⁴ and IL-6⁻¹⁷⁴, also correlate with positive skin explant results (unpublished results) and this can aid in risk-factor analysis and the potential development of a risk index for GvHD. By analysing the presence or absence of recipient or donor cytokine gene polymorphisms together with skin explant assay results, the risk factors associating more with grades 0–I and II–IV GvHD in contrast to grades 0–II and the more severe grades III–IV GvHD can be compared and distinguished.

By multivariate analysis, skin explant assay positive grades II–IV GvHD results were shown to be associated with GvHD grades II–IV in patients treated with CyA alone, and the assay is a useful model for studying the functional role of cytokines in the immunopathology of GvHD via, for example, *in vitro* blocking studies with antibody¹² and also the role of minor histocompatibility antigens in the degree of GvHD¹⁴.

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