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1083-8791/06/1202-0004$32.00/0
doi:10.1016/j.bbmt.2005.09.018

Skin Explant Model of Human Graft-versus-Host Disease: Prediction of Clinical Outcome and Correlation with Biological Risk Factors

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Received September 5, 2005; accepted September 26, 2005

ABSTRACT

A human skin explant model has been used to predict the clinical outcome and to study the immunopathology of human graft-versus-host disease (GVHD). Whether the model gives the same predictive effect for GVHD in different hematopoietic stem cell transplantation (HSCT) settings has not been assessed. It is also unknown whether the skin explant result reflects the known biological risk factors for clinical GVHD. In this study, the skin explant model was used to detect graft-versus-host reactions (GVHR) in vitro for 225 eligible patient/donor pairs. The predicted skin GVHR grade was correlated with the outcome of clinical GVHD, as well as HLA matching status, sex mismatches, and patient age. In sibling HSCT under either myeloablative or reduced-intensity conditioning, a significant correlation was observed between the predicted skin GVHR and clinical GVHD ($P < .001$ and $P = .033$, respectively). In HSCT using unrelated donors, the involvement of T-cell depletion led to a sharp increase in false-positive GVHR results, and no correlation was observed between the predicted skin GVHR and clinical GVHD. The skin GVHR grade correlated significantly with the HLA matching status (HLA-matched sibling pairs, HLA-matched unrelated pairs, and HLA-unmatched unrelated pairs). Furthermore, HLA-matched sibling pairs with a female-to-male sex mismatch had a significantly higher overall skin GVHR grade and a higher ratio of high- versus low-grade skin GVHR than the sibling pairs with all other sex combinations. Patient age was not reflected in the skin explant result. In conclusion, the predictive value of the skin explant model for aGVHD varies depending on the clinical transplant protocols, such as the type of GVHD prophylaxis used. Nevertheless, the skin explant model remains a unique in vitro system that provides an in situ histopathologic readout for studying alloreactivity and human GVHD. The model has also the potential to aid the development of novel prophylaxis and treatment for GVHD.

KEY WORDS

Skin explant model • Graft-versus-host disease • Hematopoietic stem cell transplant

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is increasingly used to treat a variety of malignant and nonmalignant diseases. Despite improvements in the ability to type and match HLA antigens between donor and recipient, graft-versus-host disease (GVHD) remains a major obstacle to successful allogeneic HSCT, contributing substantially to morbidity and transplant-related mortality. Because GVHD is frequently refractory to treatment, great effort and attention have been focused on the early identification

of high-risk patients [1-6]. The accurate prediction of the occurrence and severity of GVHD could help direct the application of treatment strategies before the appearance of clinical manifestations so that the complication can be prevented by alternative or additional prophylactic immunosuppression. An in vitro human skin explant model was originally described by Vogelsang et al. [7] to predict the occurrence of acute GVHD (aGVHD) in bone marrow transplant recipients. The model has since been established and used successfully in our center for predicting aGVHD in conventional myeloablative HSCT from HLA-matched siblings, with an 80% overall correct prediction rate [8,9]. The skin explant model has also frequently been used to study the mechanisms and immunopathology of human GVHD. Previous studies have demonstrated the roles of $CD4^+/CD8^+$ T-cell subsets and minor histocompatibility antigen–specific cytotoxic T lymphocyte clones, as well as the levels of cytokines, including interferon γ, tumor necrosis factor α, and interleukin 10, in the severity of graft-versus-host reactions (GVHR) in skin [10-13]. A novel use of a human skin explant model has also been highlighted in a recent investigation for the immunobiological consequences of conditioning regimens on resident host cells [14]. Over the last decade, the trend in allogeneic HSCT has shown an increased use of reduced-intensity conditioning regimens and HLA-matched unrelated donors (MUD). These changes in clinical transplantation practice have inevitably influenced the immunobiological process of GVHD. Whether the skin explant model retains its predictive power for aGVHD in HSCT with reduced-intensity conditionings or MUDs has not yet been assessed. It is also unknown whether the biological risk factors for GVHD can be reflected by the skin explant results.

In this study, we evaluated the use of the skin explant model for predicting aGVHD in HLA-matched sibling HSCT with either conventional myeloablative or reduced-intensity preparative regimens and in HSCT with MUDs. This was the first attempt to investigate and compare the predictive value of the skin explant model for aGVHD in different allogeneic HSCT settings. To further test the validity of the skin explant as a model of human GVHD, the skin explant result was correlated, for the first time, with the biological risk factors of clinical GVHD, including the HLA matching status, female-to-male sex mismatch, and older patient age.

MATERIALS AND METHODS

Ethics Committee approval was obtained for all aspects of this study. Written informed consent was obtained from all patients and donors for blood and skin biopsy samples. Ethics approval and informed consent were also obtained for using blood donations from healthy volunteer blood donors.

A total of 225 eligible patient/donor pairs were collected from 4 HSCT centers as a European collaborative project (Newcastle, UK; Regensburg, Germany; Vienna, Austria; and Prague, Czech Republic). The patients were divided into 3 cohorts. Cohort 1 (n = 126) included patients who had HSCT from HLA-matched sibling donors under conventional myeloablative conditioning regimens. Cohort 2 (n = 44) included patients who received a transplant from HLA-matched sibling donors under reduced-intensity conditioning regimens. Cohort 3 (n = 55) included patients who received a transplant from MUDs. The myeloablative conditioning regimen used for the HLA-matched sibling transplants was mainly fractionated total body irradiation (TBI) combined with either melphalan (Mel) or cyclophosphamide (Cy) (n = 85). A proportion of patients were conditioned with busulfan (Bu) + Cy or Bu + Mel without TBI (n = 41). The reduced-intensity conditioning regimens used for the HLA-matched sibling transplantations consisted of fludarabine (Flu) + Mel (n = 15), Flu + Mel + Campath (Schering Health Care Ltd, West Sussex, UK) (n = 18), Flu + Mel + carmustine (n = 4), or Flu + cytosine arabinoside + Mel (n = 7). Patients in the MUD transplant cohort received either myeloablative conditioning regimens (n = 39; TBI + Cy, TBI + Mel, Bu + Cy, Bu + Mel, Flu + Cy, or TBI + Cy + Flu) or reduced-intensity conditioning (n = 16; Flu + Mel). The vast majority of MUD transplant patients (48/55) had T-cell depletion by in vivo application of either Campath or antithymocyte globulin. GVHD prophylaxis for all cohorts was cyclosporin A (CsA) alone, CsA + methotrexate (MTX), or CsA + mycophenolate mofetil. The clinical characteristics of all patients are shown in Table 1. Acute GVHD was graded by using the Glucksberg criteria [15]. Patients were considered assessable for aGVHD if they survived for more than 30 days after a myeloablative transplantation. Because of the nature of delayed onset of aGVHD in nonmyeloablative HSCT [16], patients were considered assessable for aGVHD if they survived more than 100 days after transplantation. Patients were also considered assessable if they died before 30 or 100 days with significant GVHD for myeloablative and nonmyeloablative transplantation, respectively.

HLA Typing

The HLA typing was performed by using medium- to high-resolution molecular typing with polymerase chain reaction sequence-specific primers for HLA-A, -B, -Cw, -DRB1*, -DQB1*, and DPB1*. For the sibling transplant cohort, all patient/donor pairs were fully matched for HLA-A, -B, -Cw, -DRB1*, and -DQB1*. For the unrelated donor transplant cohort,

Table 1. *Characteristics of Patients in Different Transplantation Cohorts*

Characteristic	Cohort 1 (n = 126)	Cohort 2 (n = 44)	Cohort 3 (n = 55)
Median age, y (range)	35 (17-60)	44 (21-59)	34 (18-56)
Sex (male/female)	77/49	28/16	33/22
Stem cells (BM/PBMCs)	106/20	8/36	38/17
Diagnosis			
AML + ALL	78	14	30
CML	34	4	14
NHL + HD + MM	11	23	9
Others	3	3	2
Campath or ATG			
Yes	0	20	48
No	126	24	7
GVHD prophylaxis			
CsA alone	61	20	18
CsA + MTX	65	19	30
CsA + MMF	0	5	7

Cohort 1 indicates HLA-matched sibling HSCT with myeloablative conditioning; cohort 2, HLA-matched sibling HSCT with reduced-intensity conditioning; cohort 3, MUD transplantation with myeloablative or reduced-intensity conditioning; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; NHL, non-Hodgkin lymphoma; HD, Hodgkin disease; MM, multiple myeloma; ATG, antithymocyte globulin; CsA, cyclosporin A; MTX, methotrexate; MMF, mycophenolate mofetil; BM, bone marrow; PBMC, peripheral blood mononuclear cells.

>90% of patient/donor pairs were fully matched at HLA-A, -B, -Cw, -DRB1*, and -DQB1*. Five out of 55 patient/donor pairs had 1 mismatch at the HLA-B or -Cw allele. HLA-DPB1* was typed for 17 MUD transplant patient/donor pairs. Four out of 17 had 1 or 2 -DP mismatches.

Cell Preparation and Culture Conditions

Peripheral blood was obtained from patients and donors 4 weeks before transplantation. Peripheral blood mononuclear cells (PBMCs) were separated by density gradient centrifugation over Lymphoprep (Nycomed Pharma AS, Oslo, Norway). RPMI 1640 medium (GIBCO, Paisley, UK) supplemented with penicillin, streptomycin, L-glutamine (GIBCO, Paisley, UK), and 10% heat-inactivated human AB serum was used for mixed lymphocyte reactions (MLRs). The same medium supplemented with 20% heat-inactivated patient autologous serum was used for skin coculture. All the cultures were incubated at 37°C in a humidified 5% carbon dioxide in 95% air incubator.

Skin Explant Model

The skin explant model has been described in detail previously [17,18]. The model consists of 3 main steps, including a primary MLR to activate donor-allospecific T cells, a coculture of patient skin with activated donor T cells to induce graft-versus-host (GVH)–type tissue damage, and an in situ histopathologic evaluation of the severity of skin tissue damage. Briefly, the MLR was set up in the GVH direction by using patient PBMCs as stimulator cells (20 Gy of irradiation) and an equal number of donor PBMCs as responder cells. At day 7 of MLR, standard 4-mm punch skin biopsy samples were obtained from patients. The skin biopsy samples were trimmed of excess dermis, dissected into small sections of equal size, and cocultured with MLR-primed donor responder cells. The skin sections cultured with medium alone were used as background controls. After 3 days of coculture, skin sections were fixed in 10% buffered formalin and stained with hematoxylin and eosin. The histopathologic evaluation of the skin sections was performed blindly by 2 observers and confirmed by an independent histopathologist. On the basis of the severity of histopathologic changes, skin GVHR was defined as grades I to IV (Figure 1) according to the Lerner grading system [19]. All background controls displayed a skin GVHR of grade I or less. A skin GVHR of grade II or above was considered as a high grade or as positive. In the case of the HLA-unmatched situation, the MLR was set up by using PBMCs from patients who underwent autologous HSCT or plastic surgery as stimulators and PBMCs from an unrelated healthy blood donor as responders. The MLR-primed cells were then cocultured with skin sections taken from the corresponding stimulator. The statistical significance for the association between predicted skin GVHR and clinical aGVHD was analyzed by using the χ^2 test. The differences in overall skin GVHR grade between groups with various HLA matching statuses, different sex combinations, and various age ranges were analyzed by using the Mann-Whitney test. Multivariate analysis was undertaken by using binomial logistic regression with a forward stepwise model and an inclusion cutoff of 0.15 (SPSS software version 11; SPSS Inc., Chicago, IL). The model was built by using skin GVHR grade (grade 0-I versus grades II-IV), patient age (>40 versus <40 years), sex disparity (female to male versus others), cytomegalovirus (CMV) status (negative for both patient and donor versus others), and GVHD prophylaxis (CsA alone versus CsA + MTX).

RESULTS

Correlation of Skin GVHR and Clinical aGVHD in HLA-Matched Sibling Transplantations with Conventional Myeloablative Conditioning

A total of 126 patient/donor pairs were included in this cohort. The results were initially analyzed as a whole regardless of the type of GVHD prophylaxis. A significant association was observed between the predicted skin GVHR grade and the outcome of clinical aGVHD, with a total correct prediction rate of 64%

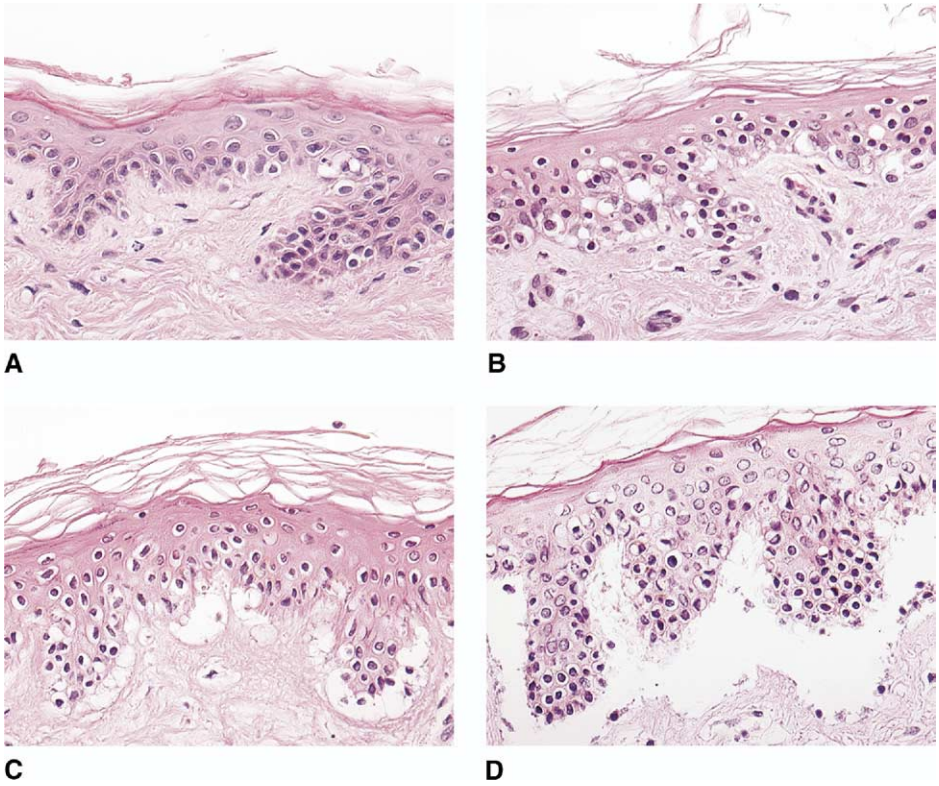

Figure 1. Histopathologic changes for different grades of skin GVHR. A, Grade I skin GVHR showing very mild vacuolization of epidermal cells. B, Grade II skin GVHR showing diffuse vacuolization of epidermal cells. C, Grade III skin GVHR showing cleft formation between the epidermis and dermis caused by confluent vacuolar damage to basal keratinocytes. D, Grade IV skin GVHR showing the complete separation of the epidermis and dermis.

($P < .001$; Table 2). The negative predictive value was stronger than the positive predictive value (72% versus 58%, respectively). Further analysis of subgroups of patients with different types of GVHD prophylaxis revealed that variation in GVHD prophylaxis could significantly affect the GVHD prediction value. The predictive power of the skin explant model was significantly improved in the cohort that received CsA only as GVHD prophylaxis, in which the overall correct prediction rate was 79% ($P < .0001$; Table 2), corresponding to a positive predictive value of 75% and a negative predictive value of 83%. No correlation, however, was then found in the remaining cohort, which had received CsA + MTX prophylaxis ($P = .864$; Table 2). This means that the predictive effect of the skin explant model observed here is mainly due to the cohort of patients who received CsA alone as GVHD prophylaxis. To determine whether the skin explant result was able to predict aGVHD independently, the multivariate analysis was undertaken for the sibling myeloablative transplant cohort, in which the largest number of patient/donor pairs was contained. In a model built by using skin GVHR grade, patient age, sex disparity, CMV status, and GVHD prophylaxis, only the skin GVHR result was significantly predictive of aGVHD (grade II and above; odds ratio, 4.77; $P < .001$). When the skin GVHR data were excluded from the model, sex disparity showed a trend, although its predictive effect did not reach statistical significance (odds ratio, 1.88; $P = .10$).

Table 2. *Correlation of Predicted Skin GVHR and Clinical aGVHD in HLA-Matched Sibling HSCT with Conventional Myeloablative Conditioning*

Skin GVHR	Acute GVHD Grade	
	0–I	II–IV
All patients (n = 126; $P < .001$)		
Grade 0–I	34	13
Grade II–IV	33	46
Patients with CsA along as GVHD prophylaxis (n = 61; $P < .0001$)		
Grade 0–I	24	5
Grade II–IV	8	24
Patients with CsA + MTX as GVHD prophylaxis (n = 65; $P = .864$)		
Grade 0–I	10	8
Grade II–IV	25	22

Correlation of Skin GVHR and Clinical aGVHD in HLA-Matched Sibling Transplantations with Reduced-Intensity Conditioning and in MUD Transplantations

The skin GVHR results and clinical GVHD outcomes for the sibling transplantation cohort with re-

Table 3. *Correlation of Predicted Skin GVHR and Clinical aGVHD in Sibling Transplantations with Reduced-Intensity Conditioning and in MUD Transplantations*

Skin GVHR	Acute GVHD Grade 0–I	Acute GVHD Grade II–IV
Sibling transplant patients with reduced-intensity conditioning (n = 44; *P* = .033)		
Grade 0–I	**13**	**6**
Grade II–IV	**9**	**16**
MUD transplant patients with myeloablative or reduced-intensity conditioning (n = 55; *P* = .183)		
Grade 0–I	**6**	**7**
Grade II–IV	**28**	**14**

duced-intensity conditioning are shown in Table 3. The results demonstrated a significant correlation ($P = .033$) between the predicted skin GVHR and the occurrence of clinically significant aGVHD (grade II and above). The overall correct prediction rate was 66% (29/44), with a positive predictive value of 64% (16/25) and a negative predictive value of 68% (13/19). In the MUD transplant cohort, 42 of 55 patients were predicted to have high-grade skin GVHR (grade II or higher). Only a third (14/42) of them developed aGVHD of grade II or above after transplantation. Clinically significant aGVHD also occurred in 7 of 13 patients with predicted low-grade skin GVHR. The overall correct prediction rate was as low as 36% (20/55), with a positive predictive value of 33% (14/42) and a negative predictive value of 46% (6/13). Statistical analysis confirmed that there was no significant association between the predicted skin GVHR grade and the outcome of clinical aGVHD for this particular cohort of patients ($P = .183$; Table 3). Taking the conditioning regimens and GVHD prophylaxis into consideration, the analysis was also performed for separate subgroups of patients defined either by the type of conditioning as myeloablative (n = 39) versus reduced intensity (n = 16) or by the type of GVHD prophylaxis as CsA alone (n = 18) versus CsA + MTX or CsA + mycophenolate mofetil (n = 37). No correlation between the predicted skin GVHR grades and clinical aGVHD was observed in any of these subgroups (data not shown).

Correlation of Skin GVHR and Biological Risk Factors for aGVHD

To explore whether the skin explant result could reflect the HLA matching status, the skin GVHR grade was analyzed for 3 groups of allogeneic stimulator/responder pairs representing 3 different degrees of HLA matching status. The HLA-matched sibling group mainly demonstrated grade I and II skin GVHR, whereas grade III and IV skin GVHR dominated the HLA-unmatched unrelated group ($P < .0001$; Mann-Whitney test). The overall skin GVHR grade of the HLA-matched unrelated group was significantly higher than that of the HLA-matched sibling group ($P = .017$) but was significantly lower than that of the HLA-unmatched unrelated group ($P = .004$; Figure 2a). The ratios of high-grade (II or higher) versus low-grade (less than II) skin GVHR were 1.6 (104:66), 3.2 (42:13), and 19.5 (39:2) for the HLA-matched related, HLA-matched unrelated, and HLA-unmatched unrelated groups, respectively. The

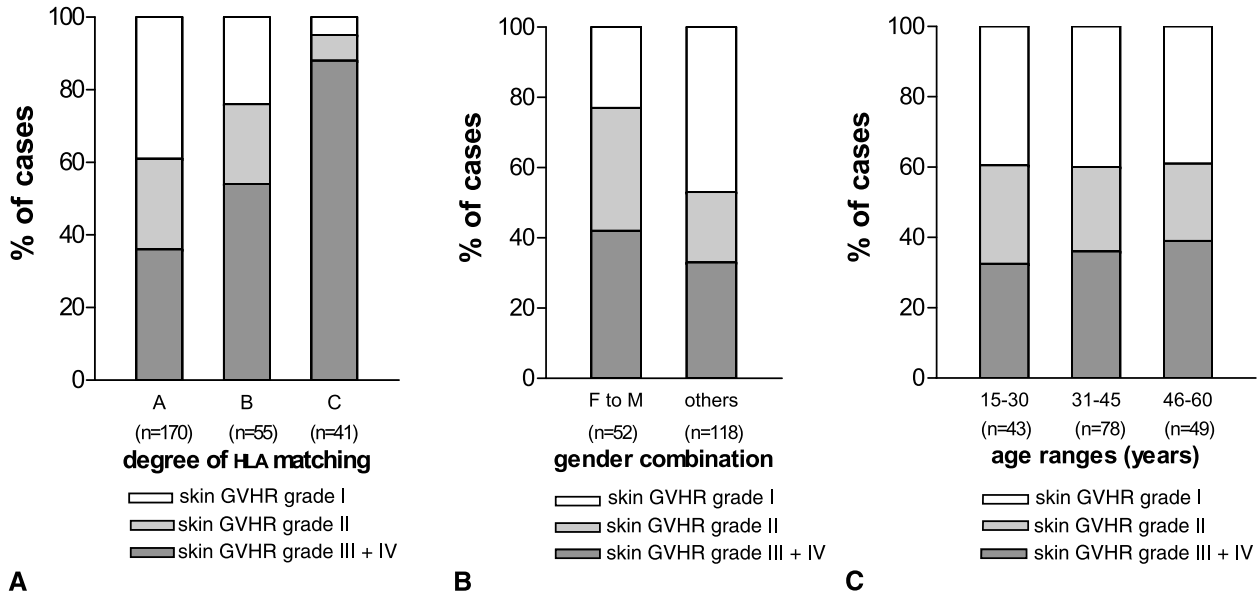

Figure 2. Association of skin GVHR grade with HLA matching status, sex combinations, and patient age. In Figure 2A, A, B, and C represent HLA-matched related, HLA-matched unrelated, and HLA-unmatched unrelated allopairs, respectively.

differences between any of the 2 groups were statistically significant ($P = .04$, $P = .012$, and $P < .0001$, respectively; χ^2 test).

Another significant clinical risk factor for GVHD is transplantation from a female donor to a male recipient [20,21]. The skin GVHR results from HLA-matched siblings were then analyzed in 2 subgroups with different sex combinations. One group consisted of the sex combination of female as responder and male as stimulator (female to male; n = 52). Another group consisted of sex combinations of either both male or both female as stimulator and responder (male to male or female to female) or male as responder and female as stimulator (male to female; n = 118). The analysis revealed that the overall skin GVHR grade in the female-to-male sex mismatch group was significantly higher compared with the results for the comparison group with all other forms of sex combinations ($P = .031$; Mann-Whitney test; Figure 2b). The ratio of high-grade (II or higher) versus low-grade (less than II) skin GVHR was 3.3 (40:12) in the female-to-male mismatch group and 1.1 (63:55) in the comparison group. The difference between the 2 groups was statistically significant ($P = .004$; χ^2 test). Furthermore, compared between HLA-matched sibling pairs with female-to-male sex mismatch and HLA-matched unrelated pairs, there were no significant differences in terms of either overall skin GVHR grade or the ratio of high- versus low-grade skin GVHR (3.3 versus 3.2, respectively).

Patient age was an important issue in HSCT until the introduction of the reduced-intensity conditioning regimens established recently [22,23]. An older patient age has been reported to contribute to increased severity of GVHD and to be a poor prognostic factor for survival after HSCT [21,24]. The skin GVHR results from HLA-matched siblings were therefore also analyzed in 3 groups defined by the age ranges of 15 to 30, 31 to 45, and 46 to 60 years. The age factor was, however, not reflected in the skin explant result. There were no significant differences in the overall skin GVHR grade between any of the age groups (Figure 2c). The ratios of high-grade (II or higher) versus low-grade (less than II) skin GVHR were virtually the same for 3 groups (1.53, 1.55, and 1.58, respectively).

DISCUSSION

A human skin explant model has been used to predict the clinical outcome and to study the immunopathology of human GVHD. Whether the model gives the same predictive effect for GVHD in different HSCT settings has not been assessed. It is also unknown whether the skin explant result reflects the relevant biological risk factors for clinical GVHD. In this study, the predictive value of the human skin explant model for aGVHD was evaluated in 3 cohorts of patients who received different forms of allogeneic HSCT in terms of conditioning regimens, GVHD prophylaxis, and donor sources. The results revealed that the skin explant model predicts aGVHD to a varying degree depending on the clinical procedure of the transplantation.

For HLA-matched sibling HSCT with conventional myeloablative conditioning, a previous report of 56 patients demonstrated that the predicted skin GVHR grades correlated with aGVHD occurrence in 80% of cases [8,9]. A further observation from a small (n = 19) mixed cohort of adults and children indicated a reduced predictive effect of the skin explant model for clinical aGVHD when the patients were given increased GVHD prophylaxis of CsA + MTX [25]. In this study, we have confirmed, in a larger European multicenter cohort (n = 126) containing only adult patients, that the skin explant results could be highly predictive of aGVHD in HLA-matched sibling HSCT with conventional myeloablative conditioning regimens. The accuracy of the prediction was, however, GVHD prophylaxis dependent, and the significance of the correlation between the predicted skin GVHR grade and the occurrence of clinical aGVHD lay only in a cohort of patients who received CsA alone as GVHD prophylaxis. In multivariate analysis, the skin explant results retained independent predictive power over sex disparity, patient age, CMV status, and GVHD prophylaxis. This may suggest that the skin GVHR grade is a more precise predictive factor and probably reflects other unknown complex biological factors, such as the influence of cutaneous minor histocompatibility antigens. It was unexpected that none of the known clinical risk factors included in the analysis demonstrated a significant predictive effect for aGVHD except a trend in female-to-male sex disparity. This is very likely due to the inadequate sample size. Clinical risk factors are usually reflected in large registry studies and can be modulated by therapeutic interventions.

A significant correlation between predicted skin GVHR and clinical aGVHD after sibling HSCT with reduced-intensity conditioning suggested that the skin explant model has the potential to be used in this cohort to identify patients who might be at a higher risk of developing aGVHD so that alternative or additional GVHD prophylaxis can be considered. Meanwhile, the effect of different GVHD prophylaxis and in vivo T-cell depletion in this particular cohort is worthy of further investigation when a larger cohort of patients is available. The observation from the MUD transplant cohort demonstrated a total lack of correlation between the predicted skin GVHR grade and clinical GVHD. Clinically, the number of alloreactive T cells present in the donor graft is undoubt-

edly the most significant risk factor for the development of aGVHD after transplantation. The vast majority of MUD transplant patients received in vivo donor T-cell depletion, and this could have resulted in a significant reduction in the occurrence of clinical aGVHD [26,27]. It is likely that T-cell depletion, by significantly reducing the incidence of clinical GVHD, will decrease the predictive power of the skin explant model as a result of a sharp increase in the false-positive skin GVHR results. The removal of donor T cells could therefore be the most important factor influencing the predictive value of the skin explant model for aGVHD, regardless of the type of GVHD prophylaxis.

Although the value of the skin explant model for GVHD prediction can be influenced and limited by the clinical transplantation procedures, the model remains a valuable tool for studying the immunopathology of human GVHD. Previous studies using the model have demonstrated the roles of $CD4^+/CD8^+$ T-cell subsets and minor histocompatibility antigen–specific cytotoxic T-lymphocyte clones, as well as the levels of cytokines, including interferon γ, tumor necrosis factor α, and interleukin 10, in the severity of GVH reactions [10-13]. The immunobiological consequences of conditioning regimens on resident host cells have also been demonstrated by using a skin explant model system [14]. This study shows, for the first time, a significant correlation between the skin GVHR result and known biological risk factors for aGVHD. A total of 61% of HLA-matched sibling pairs and 76% of HLA-matched unrelated pairs gave rise to positive skin GVHR (grade II and above), thus indicating a significant role of the alloresponses induced by the disparity of undetected major and/or minor histocompatibility antigens. The correlation between high-grade skin GVHR and female-to-male sex mismatch was in line with our recently published observations showing that direct cellular infiltration of H-Y minor histocompatibility antigen–specific cytotoxic T cells caused severe GVH reactions in male skin [11]. Indeed, the data presented here have revealed for the first time in vitro that the ratio of high- versus low-grade skin GVHR for HLA-matched sibling pairs with female-to-male sex mismatch was as high as that for HLA-matched unrelated pairs. This has highlighted the importance of alloreactivity induced by the disparity of minor histocompatibility antigens in HSCT and provided novel in vitro biological evidence for clinically observed increased GVHD incidence and severity in female-to-male HSCT [20,21]. Furthermore, the direct correlation between the skin GVHR grade and the frequency of alloreactive cytotoxic T-lymphocyte precursors [28] mirrors the clinical fact that the dose of alloreactive T cells present in the donor graft is one of the most significant risk factors for the development of aGVHD after transplantation. One of the significant clinical risk factors for aGVHD, namely, older patient age, has not been reflected in the skin explant result. This is not surprising. The mechanisms related to the higher risk of GVHD and poor outcome after transplantation in older patients are apparently not entirely based on alloreactive T-cell responses. It could be influenced by complex overall effects, including an advanced stage of disease, modification of the immune system associated with aging, general fitness, altered pharmacokinetics, and the capacity of multiorgan function to cope with the transplantation procedure [24,29,30].

In conclusion, the predictive value of the skin explant model for aGVHD varies depending on the clinical procedure of the transplantation. The model can be highly predictive of aGVHD, but only in selected cohorts of patients. Increased GVHD prophylaxis with CsA + MTX and the use of donor T-cell depletion could diminish the predictive power of the skin explant model. Because of the changes in the clinical transplantation procedures over recent years, it was necessary to reevaluate the predictive power of the model for GVHD in different transplantation settings. Currently the clinical risk factors and pathology of aGVHD have been well described, but the cellular mechanisms are more difficult to study without an intact tissue environment. The skin explant model is an informative in vitro system that most closely mimics the in vivo mechanisms and pathology of human aGVHD. The model gives a functional indication for the immune responses induced by the disparities in major and/or minor histocompatibility antigens and provides a unique in situ histopathologic readout for studying human GVHD. The model has also the potential to aid the development of novel prophylaxis and treatment for GVHD.

ACKNOWLEDGMENTS

This work was supported by European Commission FP5 grant no. TRANSEUROPE QLK3-CT-2002-01936 and a Tyneside Leukaemia Research Association Programme grant.

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