Table 4 Multivariate analysis for OS, RFS and relapse

Factors selected	P-*value*	*Hazard ratio*
For OS		
PS = 2, 3 or 4	0.174	3.39
aGVHD (+) (any grade)	0.068	4.43
Related donor	0.238	0.41
Donor HTLV-I (+)	0.553	0.61
For RFS		
Age ⩾43	0.123	2.34
PS = 2, 3 or 4	0.528	1.70
aGVHD (+) (any grade)	0.033	4.10
Related donor	0.022	0.24
For relapse		
Related donor	0.040	0.09
Donor HTLV-I (+)	0.076	6.00

patient achieved CR after transplantation, suggesting the efficacy of myeloablative conditioning to ATLL. In this analysis, there was no difference in the incidence of relapse or survival among patients treated with busulfan-based regimens and TBI-containing regimens. It seemed that chemotherapy was as effective as TBI for ATLL in terms of a conditioning regimen for allo-HSCT.

The major difference between autologous and allo-HSCT was the incidence of relapse of the primary disease after transplantation. We reported previously that relapse occurred in all four evaluable cases after autologous HSCT.[9] In contrast, 10 out of 39 cases (25.6%) suffered ATLL relapse after allo-HSCT. The immunological reaction induced only by allo-HSCT could control the residual ATLL after high-dose therapy, which may be called the GvATLL effect. In this study, among 10 patients in whom ATLL relapsed after transplantation, five patients achieved second CR. Interestingly, three achieved CR only by the reduction or cessation of immunosuppressive agents. This observation further supported the idea that the GvATLL effect actually worked and contributed to long-term RFS. One of the clinical findings supporting the idea of graft-versus-leukemia (or lymphoma) effect is the benefit of GVHD in the prevention of disease relapse,[18–20] for example, as shown in NHL in which grades II–IV aGVHD were associated with a lower incidence of disease progression after transplantation from an unrelated donor.[21] In this study, however, we found no positive effect of GVHD on freedom from relapse, or long-term survival by univariate and multivariate analysis. On the other hand, the existence of aGVHD, even grade I, negatively affected the OS and RFS. Of note, the negative impact of GVHD on the survival did not change by the analysis with Cox's proportional hazard model (in the analysis of aGVHD1, $P = 0.06$ for OS and $P = 0.02$ for RFS), which treats GVHD as a time-dependent covariate, although nine cases lacking some clinical data were missed in this analysis. The possible GvATLL effect (second CR obtained by the reduction or cessation of immunosuppressive agents) but no benefit of aGVHD and cGVHD on the survival or the prevention of relapse (demonstrated by the univariate and multivariate analysis) suggests that the GvATLL effect could work on those patients even without clinically obvious GVHD. This hypothesis is in concordance with our recent observation[22] that showed the development of cytotoxic T cells specific for Tax, one of the HTLV-I products, in patients after nonmyeloablative allo-HSCT for ATLL. In some patients, the HTLV-I-infected cell-specific immune reaction may contribute to the eradication of ATLL.

A total of 21 patients died after allo-HSCT, and 15 of those 21 patients were lost within 6 months after HSCT mostly due to treatment-related adverse events. We assume that both intensive conditioning before transplantation and the immunological reaction between graft and host after transplantation were effective for ATLL; however, these two factors also had a negative impact on the survival of patients at the same time. As shown in the univariate analysis, the general status of patients but not the disease status at transplantation was significantly related with the survival after transplantation. The indication of myeloablative allo-HSCT for ATLL needs to be determined based on the general status rather than disease status to reduce TRM. Furthermore, because patients with ATLL are immunocompromised,[4,7] the strengthening of supportive care for infection and the development of less toxic conditioning could contribute to a better outcome. In this respect, nonmyeloablative conditioning will also be tested for ATLL. As shown by multivariate analysis, it seems important to control aGVHD for patients with ATLL. Transplantation from a related donor will lead to the reduction of aGVHD and TRM.

The median age of 44 years old of the patients in this series is apparently less than that of general ATLL patients (about 60 years old in Japan). Although there was some bias in case selection, our results demonstrated that allo-HSCT can provide apparent long-term RFS in some patients, the GvATLL effect was actually observed. In a patient who was transplanted from a seronegative donor, the original ATLL population in peripheral blood was not detected by Southern blot analysis 6 years after transplantation. However, anti-HTLV-I antibody was positive (data not shown). It is necessary to analyze the precise virological status of recipients after allo-HSCT using techniques such as PCR to understand how it works on ATLL. Further prospective controlled studies are needed to assess the efficacy of allo-HSCT for ATLL and the GvATLL effect.

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bjh research paper

Impact of human leucocyte antigen mismatch on graft-*versus*-host disease and graft failure after reduced intensity conditioning allogeneic haematopoietic stem cell transplantation from related donors

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Summary

The impact of human leucocyte antigen (HLA) incompatibility between donor and recipient on graft-*versus*-host disease (GVHD) and graft failure after reduced-intensity conditioning stem cell transplantation (RICT) remains to be elucidated. We retrospectively analysed outcome in 341 patients who underwent RICT from related donors for haematological malignancies. The overall cumulative incidence of grade II–IV acute GVHD (aGVHD) was 40% for all subjects; 39% in recipients with HLA-matched donors, 44% in those with one-locus-mismatched donors, and 50% in those with two- to three-loci-mismatched donors. In a Cox regression model adjusted for potential confounders, the tendency for grade II–IV aGVHD ($P = 0{\cdot}01$), chronic GVHD (cGVHD) ($P = 0{\cdot}05$) and graft failure ($P = 0{\cdot}033$) increased with HLA disparity. Use of peripheral blood grafts instead of marrow was a risk factor for cGVHD. Use of antithymocyte globulin was associated with reduced aGVHD and cGVHD. Overall survival (OS) in recipients of two- to three-loci-mismatched RICT at 2 years (18%) was significantly worse than that in patients who received one-locus-mismatched RICT (51%) and HLA-matched RICT (48%) ($P < 0{\cdot}0001$). A two- to three-loci mismatch was identified as an independent risk factor for OS ($P < 0{\cdot}001$), but there was no significant difference in OS between HLA-matched and one-locus-mismatched RICT. HLA incompatibility between the donor and recipient is an important risk factor for graft failure, aGVHD, cGVHD and OS after RICT. RICT from a one-locus-mismatched donor may represent an effective alternative approach in patients with high-risk malignancies who lack HLA-matched related donors.

Keywords: human leucocyte antigen, graft-*versus*-host disease, rejection, reduced intensity conditioning, antithymocyte globulin.

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Allogeneic haematopoietic stem cell transplantation (SCT) is a potentially curative treatment for haematologic malignancies. A growing body of evidence suggests that allogeneic SCT is also useful for the treatment of bone marrow failure, congenital metabolic disorders, non-haematologic disorders including solid tumours and autoimmune diseases (Burt *et al*, 2003; Slavin *et al*, 2004). However, high transplant-related mortality (TRM) precludes the wider application of allogeneic SCT for these diseases. Recently, several investigators have reported encouraging results with allogeneic SCT using a reduced-intensity conditioning (RIC) transplant regimen (RICT) (Bacigalupo, 2004). These regimens have been designed to reduce TRM and provide a platform for durable donor cell engraftment to exploit a graft-*versus*-tumour effect.

Graft-*versus*-host disease (GVHD) is still a major obstacle to allogeneic SCT. Human leucocyte antigen (HLA) disparity between the SCT donor and recipient is the most critical factor that governs the severity of GVHD after conventional allogeneic SCT. Studies in patients who have been transplanted from a related donor other than an HLA-identical sibling after myeloablative conditioning have shown that HLA incompatibility increases the incidence and severity of acute GVHD (aGVHD), as well as the incidence of graft failure (Beatty *et al*, 1985). Although it was initially assumed that RIC may reduce the incidence of GVHD, GVHD appears to be a significant clinical problem following RICT (Khouri *et al*, 1998; Slavin *et al*, 1998; Nagler *et al*, 2000; Schetelig *et al*, 2002; Mielcarek *et al*, 2003; Bacigalupo, 2004; Diaconescu *et al*, 2004). While most studies on RICT have been performed in an HLA-matched related setting, alternative donor grafts are increasingly used in RICT (Kottaridis *et al*, 2000; Giralt *et al*, 2001; Maris *et al*, 2003; Niederwieser *et al*, 2003; Wong *et al*, 2003; Bacigalupo, 2004; Goggins & Rizzieri, 2004). Our current knowledge regarding the association of HLA incompatibility with GVHD, graft failure and survival is based primarily on results obtained in the setting of conventional and myeloablative allogeneic SCT. However, the risk factors that affect the transplant outcome after RICT, including engraftment, GVHD and survival, are still poorly defined. The present study was performed to analyse the impact of HLA incompatibility on graft failure, aGVHD, chronic GVHD (cGVHD) and survival in patients with haematological malignancies who received RICT from a related donor.

Patients and methods

Patients

We retrospectively analysed data from patients with haematological malignancies who underwent RICT from a related donor at 21 transplant centres in Japan. This study was approved by Institutional Review Board of each individual centre. All patients were treated with RIC regimens before allogeneic SCT because of high-risk clinical features that made them ineligible for conventional myeloablative allogeneic SCT. The stem cell source was either bone marrow or granulocyte-colony stimulating factor (G-CSF)-mobilised peripheral blood stem cells (PBSC) from related donors. Patients who received a manipulated graft and those who received cord blood were excluded from the analyses. Patients who received a graft from an HLA-matched non-sibling donor were also excluded because the numerous secondary factors and minor histocompatibility antigens present were significantly different to those in full-sibling matches. A total of 341 patients who underwent allogeneic RICT from related donors for haematological malignancies between 1998 and 2004 were evaluated in this study.

Transplantation procedure

Serologic typing for HLA-A, -B and -DR antigens of the donor and recipient was performed with a standard two-stage complement-dependent test of microcytotoxicity. Serologically HLA-matched sibling pairs were considered to be genotypically HLA-identical based on the results of family analysis. In pairs other than serologically identical sibling pairs, alleles at the HLA-A, -B and -DRB1 loci were identified by middle-resolution DNA typing as described previously (Sasazuki *et al*, 1998). HLA-mismatch in the graft-*versus*-host (GVH) vector was defined when the recipient's antigens or alleles were not shared by the donor, while mismatch in the host *versus* donor (HVG) vector was defined as when the donor's antigens or alleles were not shared by the recipients. The conditioning regimen and GVHD prophylaxis were conducted according to the guidelines of each institution. RIC regimens were defined as reported previously (Bacigalupo, 2002, 2004; Champlin *et al*, 2000). The most frequently used RIC regimens were fludarabine-based (fludarabine 150–180 mg/m^2 with either cyclophosphamide 60 mg/kg, busulphan 8 mg/kg or melphalan 80–140 mg/m^2) with or without either total body irradiation (TBI) 2–4 Gy or antithymocyte globulin (ATG) 5–10 mg/kg. Patients conditioned with >6 Gy TBI and those conditioned with >8 mg/kg of busulphan were excluded from the study. The most frequently used prophylaxis regimens for GVHD were ciclosporin (CSP) alone or CSP plus methotrexate (MTX).

Definitions

Risk status at transplantation was categorised as either standard risk or high risk. Standard-risk diseases included acute leukaemia in first complete remission, chronic myeloid leukaemia in first chronic phase and refractory anaemia of myelodysplastic syndrome. Other diseases were categorised as high-risk disease. Graft failure was analysed in patients who survived more than 28 d post-transplant according to the criteria reported previously (Petersdorf *et al*, 2001); graft failure was defined as failure of the absolute neutrophil count (ANC) to surpass $0{\cdot}5 \times 10^9$/l before relapse, death or second

transplantation, as well as a decrease in the ANC to $<0{\cdot}1 \times 10^9$/l on at least three consecutive determinations with a finding of severe hypoplastic marrow. The aGVHD, graded according to the standard criteria (Przepiorka *et al*, 1995), was defined as moderate to severe (grade II–IV) disease. All patients who had no evidence of graft failure were considered to be evaluable for aGVHD. GVHD persisting beyond day +100 or *de novo* GVHD occurring after day +100 was classified as cGVHD. Biopsy-proven cGVHD occurring between days 80 and 100 was also included. The incidence of cGVHD was calculated in patients followed for at least 100 d and was classified as none, limited or extensive as well as none, *de novo*, quiescent or progressive (Sullivan *et al*, 1991). Overall survival (OS) was defined as the duration of survival between transplant and either death or the last follow-up.

Statistical analysis

The primary endpoint of this study was the incidence of grade II–IV aGVHD and graft failure. The secondary endpoint was the incidence of cGVHD and OS among the patients. The cumulative incidence of aGVHD was calculated using a method described by Gooley *et al* (1999) to eliminate the effect of competing risks. The competing event for aGVHD was defined as death without aGVHD II–IV. For each endpoint, a Cox proportional hazard model was used for uni- and multivariate analyses. The factors included in the analysis were HLA disparity (one-locus mismatch, two- to three-loci mismatch *versus* identical), type of graft (bone marrow *versus* PBSC), previous history of SCT (yes *versus* no), type of donor (family *versus* sibling), recipient age (age 60 years or more *versus* less than 60 years), use of TBI (yes *versus* no), use of ATG (yes *versus* no), GVHD prophylaxis (CSP with MTX, tacrolimus with MTX, and others *versus* CSP alone), and risk status (standard *versus* high). To evaluate the association between CD34 cell counts and the development of aGVHD, subjects were categorised into three groups by tertile and linearity was assessed by score test in a proportional hazard model. We defined statistical significance as a *P*-value $<0{\cdot}05$. All the statistical analyses were performed using STATA version 8 (STATA Corp., College Station, TX, USA).

Results

Patient characteristics

The numbers of patients who received a graft from an HLA-matched, one-locus-mismatched and two- to three-loci-mismatched donor were 250, 57 and 34 respectively (Table I). The respective median age of these patients were 54, 50·5 and 46·5 years. Among 341 patients, 286 received a graft from a sibling donor and 55 received a graft from a family member other than a sibling. Family donors included 22 sons, 17 daughters, three fathers, 10 mothers, one uncle and two unknown. A total of 110 patients had malignant lymphoma, 106 had acute leukaemia, 74 had mylodysplastic syndrome, 30 multiple myeloma and 21 had chronic myeloid leukaemia. A total of 323 patients received PBSC, whereas the remaining 18 were given bone marrow. The HLA-matched group included significantly higher proportions of patients who did not receive ATG ($P < 0{\cdot}001$) and those who were given CSP alone for GVHD prophylaxis ($P < 0{\cdot}001$) compared with the HLA-mismatched group. Gender, disease, risk status at transplant, previous history of SCT, stem cell source, use of a TBI-containing conditioning regimen and year of transplant were evenly distributed between the groups (Table I).

Acute GVHD

The cumulative incidence of grade II–IV aGVHD in this study population was 40% (95% CI, 35–46%) (Fig 1A). It was 39% (95% CI, 33–45%) in recipients with HLA-matched donors, 44% (95% CI, 30–57%) in those with one-locus-mismatched donors, and 50% (95% CI, 29–68%) in those with two- to three-loci-mismatched donors (Fig 1B); there was a marginally significant difference between two- to three-loci-mismatched RICT and HLA-matched RICT [hazard ratio (HR), 1·72; 95% CI, 0·94–3·14; $P = 0{\cdot}079$]. Similar results were obtained when the incidence of grade III–IV severe aGVHD was analysed (data not shown). A relationship between multiple incompatibility for HLA and a risk of aGVHD was further supported by a Cox regression model adjusted for potential confounders (Table II). Patients who received a graft from a one-locus-mismatched donor and a two- to three-loci-mismatched donor had a HR for aGVHD of 1·83 (95% CI, 1·04–3·22; $P = 0{\cdot}035$) and 2·44 (95% CI, $1{\cdot}14$–$5{\cdot}21_3$; $P = 0{\cdot}021$), respectively, when compared with those from an HLA-matched donor. A greater incidence of grade II–IV aGVHD was observed with increased HLA disparity ($P = 0{\cdot}010$). Thus, the number of mismatched HLA loci between the donor and recipient was thus a continuous variable with respect to the incidence of grade II–IV aGVHD. No other variables significantly influenced the development of aGVHD after RICT. In patients receiving PBSC grafts, there was no association between the numbers of $CD34^+$ cells and the development of aGVHD ($P = 0{\cdot}904$).

Of note, the development of aGVHD did not reach a plateau within 3 months after RICT, with a median onset on day 30 (Fig 1). The onset of aGVHD was earlier after two- to three-loci-mismatched RICT compared with HLA-matched RICT, and reached a plateau within 40 d post-transplant. The median number of days before the onset of aGVHD after HLA-matched RICT, one-locus-mismatched RICT and two- to three-loci-mismatched RICT was 39, 18 and 24 respectively.

Human leucocyte antigen-C typing is not routinely performed in haemopoietic stem cell transplantation (HSCT) from a related donor in Japan. In this study, HLA-C typing data were available in 75 donor–recipient pairs. Acute GVHD developed in 32% (95% CI, 21–44%) of patients who received a graft from an HLA-C matched donor, and in 56% (95% CI,

Table I. Characteristics of subjects according to human leucocyte antigen-matching status.

	Identical (n = 250)	One-mismatched (n = 57)	Two or more mismatched (n = 34)	P-value
Recipient age (range, median)	16–70, 54	25–61, 50·5	21–60, 46·5	0·03
Recipient sex, female:male (unknown)	109:141	43:14	20:14	0·007
Previous history of HCT, no:yes	205:45	27:9	36:19	0·64
Disease				
Acute leukaemia	73	17	16	
Chronic myeloid leukaemia	16	3	2	
Myelodysplastic syndrome	48	18	8	
Malignant lymphoma	90	15	5	
Multiple myeloma	23	4	3	0·163
Risk status				
Standard	52	8	3	
High	198	49	31	0·154
HCT type				
PBSC	236	55	32	
BM	14	2	2	0·805
Donor				
Sibling	250	28	8	
Family	0	29	26	
Year of transplant				
<2000	5	1	1	
2000+	245	56	33	0·922
TBI				
No	213	44	25	
Yes	37	13	9	0·117
ATG				
No	236	36	20	
Yes	14	21	14	<0·001
GVHD prophylaxis				
CSP alone	99	8	10	
CSP + MTX	122	30	10	
FK + MTX	5	13	12	
Other	24	6	2	<0·001

HCT, haematopoietic cell transplantation; PBSC, peripheral blood stem cells; BM, bone marrow; TBI, total body irradiation; ATG, antithymocyte globulin; CSP, ciclosporin; MTX, methotrexate; FK, tacrolimus.

21–86%) of those who received a graft from an HLA-C mismatched donor. Although this difference was not statistically significant (P = 0·261), the impact of HLA-C mismatch on the incidence of aGVHD remains to be elucidated because of limited numbers of subjects for evaluation.

CGVHD

Recipients of HLA-matched RICT (n = 156) and 42 recipients of HLA-mismatched RICT (one-locus mismatch, 32; two- to three-loci mismatch, 10) were evaluable for cGVHD. The cumulative incidence of cGVHD was 61% (95% CI, 52–67%), 69% (95% CI, 44–77%) and 67% (95% CI, 28–88%) after HLA-matched RICT, HLA one-locus-mismatched RICT and two- to three-loci-mismatched RICT respectively (Fig 2). The cumulative incidence of extensive cGVHD was 38%, 34% and 60% after HLA-matched RICT, HLA one-locus and two- to three-loci-mismatched RICT respectively. There was no significant difference between the three groups regarding the incidence of cGVHD or extensive cGVHD. Similarly, there was no significant difference between the groups in the incidence of *de novo*, quiescent and progressive cGVHD. As shown in Table III, a tendency for cGVHD was observed with increased HLA disparity in multivariate analysis (P = 0·05). In addition, use of PBSC grafts, no use of ATG, and high-risk disease were identified as independent risk factors for cGVHD. In patients receiving PBSC grafts, there was no association between the numbers of $CD34^+$ cells and the development of cGVHD (P = 0·613; HR, 0·95; 95% CI, 0·76–118).

Graft failure

The incidence of graft failure was 3·7% (95% CI, 1·7–6·9%) in recipients with an HLA-matched donor, 5·7% (95% CI,

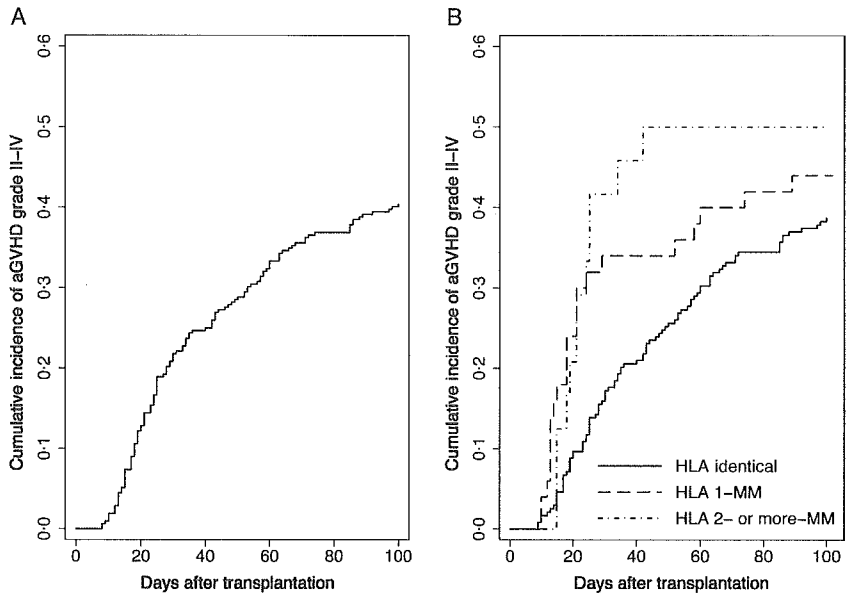

Fig 1. Incidence of grade II–IV acute graft-*versus*-host disease (aGVHD). The curves represent the cumulative incidence of grade II–IV aGVHD in patients with haematological malignancies following reduced-intensity conditioning transplant regimen from a related donor as a function of time after transplantation (A) for all available subjects ($n = 312$) and (B) in relation to the extent of human leucocyte antigen mismatch (identical, $n = 238$; one-locus mismatch, $n = 22$ and two to three-loci mismatch, $n = 7$).

1·2–15·7%) in those with a one-locus-mismatched donor, and 10·3% (95% CI, 2·2–27·4%) in those with a two- to three-loci-mismatched donor. Multivariate analysis revealed a significant increase of graft failure in patients who received a graft from a two- to three-loci-mismatched donor (HR, 8·58; 95% CI, 1·37–53·9; $P = 0·022$, Table IV), and the extent of HLA mismatch between the donor and recipient was a continuous variable with respect to the incidence of graft failure ($P = 0·033$). Use of ATG did not significantly influence the incidence of rejection after RICT ($P = 0·166$).

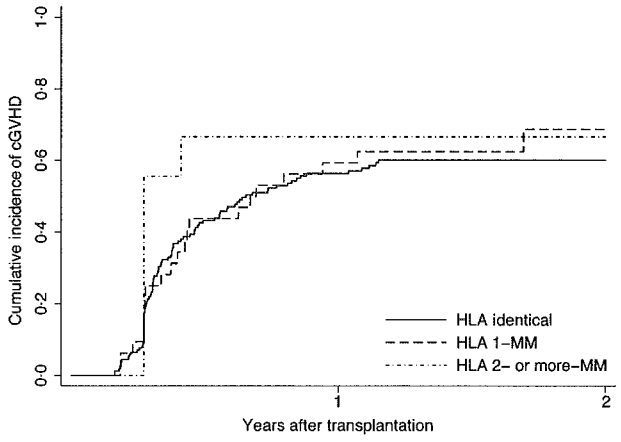

Fig 2. Incidence of chronic graft-*versus*-host disease (cGVHD). The curves represent the cumulative incidence of cGVHD in patients with haematological malignancies following reduced-intensity conditioning transplant regimen from a related donor as a function of time after transplantation in relation to the extent of human leucocyte antigen mismatch (identical, $n = 156$; one-locus mismatch, $n = 32$ and two-to three-loci mismatch, $n = 10$).

Survival

To elucidate the impact of HLA mismatch on transplant outcome, OS was analysed. With a median follow-up of 347 d, OS in patients who received a graft from an HLA-matched donor, a one-locus-mismatched donor and a two-to three-loci-mismatched donor was 48% (95% CI, 42–54%), 51% (95% CI, 39–61%) and 18% (95% CI, 7–32%), respectively, at 2 years after RICT. OS after HLA one-locus-mismatched RICT was comparable with that after HLA-matched RICT (Fig 3). However, OS after two- to three-loci-mismatched RICT was significantly worse post-transplant compared with that after HLA-matched RICT. Multivariate analysis identified two- to three-loci HLA mismatch (HR, 3·41; 95% CI, 2·03–5·73; $P < 0·001$), previous history of haematopoietic cell transplantation (HR, 1·42; 95% CI, 1·00–2·02; $P = 0·052$), and high-risk disease (HR, 2·06; 95% CI, 1·33–3·30; $P = 0·002$) as independent risk factors for shorter survival (Table V). As only three patients with standard-risk disease received RICT from a two- to three-loci-mismatched donor, the relationship between multiple HLA incompatibility and OS was further evaluated in patients with high-risk diseases and those with standard diseases separately. In high-risk patients, the 2-year OS was 15% (95% CI, 5–30%), 52% (95% CI, 40–63%) and 43% (95% CI, 36–49%) in recipients with a two- to three-loci-mismatched donor, one-locus-mismatched donor and matched donor respectively. HLA

Table II. Uni- and multivariate analyses for possible risk factors for acute GVHD of grade II or more.

	Evaluable (n = 313)					
	Univariate			Multivariate		
	HR	95% CI	P-value	HR	95% CI	P-value
HLA						
Identical	1·00			1·00		
1-MM	1·29	0·81–2·05	0·282	1·83	1·04–3·22	0·035
2 or more MM	1·72	0·94–3·14	0·079	2·44	1·14–5·21	0·021
		Trend	0·068		Trend	0·010
HCT type						
PBSC	1·00			1·00		
BM	0·65	0·26–1·59	0·342	0·70	0·28–1·73	0·441
Previous history of HCT						
No	1·00			1·00		
Yes	1·15	0·75–1·77	0·52	0·90	0·57–1·44	0·663
Recipient age (years)						
<60	1·00			1·00		
≥60	0·90	0·57–1·42	0·656	0·94	0·59–1·50	0·808
Recipient sex						
Female	1·00			1·00		
Male	1·16	0·82–1·66	0·401	1·28	0·88–1·84	0·196
TBI						
No	1·00			1·00		
Yes	0·96	0·60–1·55	0·881	0·77	0·43–1·38	0·386
ATG						
No	1·00			1·00		
Yes	0·85	0·50–1·44	0·543	0·55	0·29–1·02	0·057
GVHD prophylaxis						
CSP alone	1·00			1·00		
CSP + MTX	0·74	0·50–1·09	0·124	0·70	0·46–1·04	0·079
FK + MTX	1·07	0·57–2·02	0·826	0·70	0·32–1·50	0·355
Other	1·12	0·61–2·06	0·724	1·37	0·65–2·90	0·410
Risk						
Standard	1·00			1·00		
High	1·52	0·93–2·47	0·095	1·43	0·87–2·36	0·159

GVHD, graft-*versus*-host disease; HLA, human leucocyte antigen; MM, mismatched; HCT, haematopoietic cell transplantation; PBSC, peripheral blood stem cells; BM, bone marrow; TBI, total body irradiation; ATG, antithymocyte globulin; CSP, ciclosporin; MTX, methotrexate; FK, tacrolimus.
Multivariable adjusted for all variables listed.

two- to three-loci mismatch, but not one-locus mismatch, was again a risk factor for shorter survival ($P < 0·0001$, Fig 4A). In standard-risk patients, the 2-year OS was 73% (95% CI, 58–84%), 40% (95% CI, 12–67%) and 38% (95% CI, 1–81%) in recipients with a matched, one-locus-mismatched donor and two- to three-loci-mismatched donor respectively (Fig 4B). In contrast to high-risk disease, one-locus mismatch was a risk factor for shorter survival in patients with standard-risk disease ($P = 0·079$).

Of the 178 patients who died following RICT, 90 deaths were directly attributed to disease progression or relapse. Non-relapse mortality was 49%, including 60 deaths (34%) because of infection and/or GVHD, 22 deaths (12%) from other transplant-related toxicities and six deaths (3%) from other diseases. There was no difference in the cause of death between HLA-matched RICT and HLA-mismatched RICT (data not shown).

Discussion

In this study, we found that HLA disparity was a continuous and independent risk factor for grade II–IV aGVHD and cGVHD as well as graft failure following RICT. Furthermore, two- to three-loci HLA mismatch was a risk factor for survival, and one-locus mismatch was a risk factor for survival in patients with standard-risk disease, but not in those with high-risk disease. Our finding, that HLA disparity impacts on grade II–IV aGVHD in patients receiving RICT, was quite consistent

Table III. Uni- and multivariate analyses for possible risk factors for chronic GVHD.

	Evaluable (n = 198)					
	Univariate			Multivariate		
	HR	95% CI	P-value	HR	95% CI	P-value
HLA						
Identical	1·00			1·00		
1-MM	1·09	0·68–1·76	0·709	1·57	0·89–2·75	0·116
2 or more MM	1·25	0·55–2·85	0·6	2·20	0·84–5·75	0·108
		Trend	0·543		Trend	0·050
HCT type						
PBSC	1·00			1·00		
BM	0·31	0·10–0·97	0·044	0·28	0·88–0·90	0·032
Previous history of HCT						
No	1·00			1·00		
Yes	0·90	0·56–1·42	0·641	0·75	0·45–1·26	0·274
Recipient age (years)						
<60	1·00			1·00		
≥60	1·31	0·86–1·99	0·214	1·16	0·74–1·81	0·512
Recipient sex						
Female	1·00			1·00		
Male	1·18	0·82–1·69	0·375	1·27	0·87–1·87	0·221
TBI						
No	1·00			1·00		
Yes	0·76	0·46–1·24	0·265	0·61	0·33–1·11	0·104
ATG						
No	1·00			1·00		
Yes	0·66	0·36–1·23	0·194	0·42	0·20–0·85	0·016
GVHD prophylaxis						
CSP alone	1·00			1·00		
CSP + MTX	1·10	0·72–1·68	0·67	1·00	0·63–1·56	0·966
FK + MTX	0·76	0·37–1·55	0·444	0·53	0·23–1·24	0·143
Other	0·98	0·51–1·88	0·961	1·36	0·63–2·96	0·435
Risk						
Standard	1·00			1·00		
High	1·67	1·06–2·64	0·027	1·74	1·09–2·79	0·021

GVHD, graft-*versus*-host disease; HLA, human leucocyte antigen; MM, mismatch; HCT, haematopoietic cell transplantation; PBSC, peripheral blood stem cells; BM, bone marrow; TBI, total body irradiation; ATG, antithymocyte globulin; CSP, ciclosporin; MTX, methotrexate; FK, tacrolimus.
Multivariable adjusted for all variables listed.

Table IV. Uni- and multivariate analyses for host-*versus*-graft human leucocyte antigen mismatching on graft failure risk.

	Evaluable (n = 325)							
			Univariate			Multivariate		
	Rejected	Not-rejected	HR	95% CI	P-value	HR	95% CI	P-value
Identical	9	234	1·00	Reference		1·00	Reference	
1-MM	3	50	1·6	0·42–6·12	0·337	1·18	0·19–7·26	0·855
2 or more MM	3	26	3·01	0·77–11·8	0·114	8·58	1·37–53·9	0·022
				Trend	0·035		Trend	0·033

HR, hazard ratio; MM, mismatch.
Multivariate analysis adjusted for age, sex, donor type, previous history of haematopoietic cell transplantation, total body irradiation conditioning, antithymocyte globulin conditioning, graft-*versus*-host disease prophylaxis and risk status.

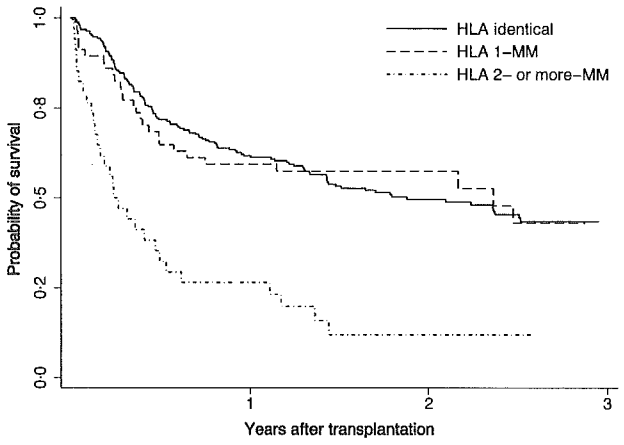

Fig 3. Overall survival (OS) based on the extent of human leucocyte antigen (HLA) mismatch. The curves represent OS in patients with haematological malignancies following reduced-intensity conditioning transplant regimen from a related donor as a function of time after transplantation in relation to the extent of HLA mismatch (identical, $n = 250$; one-locus mismatch, $n = 56$ and two to three-loci mismatch, $n = 34$).

with earlier findings in conventional myeloablative SCT (Beatty *et al*, 1985; Ringden & Nilsson, 1985; Anasetti *et al*, 1990; Anasetti & Hansen, 1994; Sasazuki *et al*, 1998; Morishima *et al*, 2002; Kanda *et al*, 2003), but has not been well described in the setting of RICT.

The cumulative incidence of grade II–IV aGVHD after HLA-matched RICT was 39% in this study population, which is similar to that in recent reports from other groups following RICT (Levine *et al*, 2003; Martino *et al*, 2003; Wong *et al*, 2003; Bacigalupo, 2004; Diaconescu *et al*, 2004; Goggins & Rizzieri, 2004), although a recent retrospective comparison of myeloablative SCT with RICT conditioned with 2 Gy TBI and fludarabine showed less aGVHD after RICT in matched unrelated donor transplants (Sorror *et al*, 2004).

It was initially assumed that the incidence and severity of aGVHD might decrease after RICT compared with conventional SCT. Pretransplant conditioning can activate host tissues to secrete inflammatory cytokines and amplify GVHD (Xun *et al*, 1994). The relationship between conditioning intensity, inflammatory cytokine and GVHD severity was further supported by animal models (Hill *et al*, 1997) and clinical observation (Gale *et al*, 1987; Clift *et al*, 1990; Deeg *et al*, 1991). In experimental models, the development of mixed donor–host chimaerism may facilitate the establishment of anti-host tolerance (Colson *et al*, 1996; Manilay *et al*, 1998). In contrast, minimally cytotoxic conditioning may enable the persistence of host antigen-presenting cells, which would enhance presentation of host alloantigens to donor T cells (Shlomchik *et al*, 1999; Teshima *et al*, 2002; Duffner *et al*, 2004).

There are several possible explanations that can account for this unexpectedly high incidence of aGVHD after RICT. First, the median age of patients who underwent RICT in the current study was 53 years, which was much higher than that among patients who received conventional SCT. A greater age has been associated with an increased risk for GVHD after conventional SCT (Ringden & Nilsson, 1985; Gale *et al*, 1987; Anasetti *et al*, 1990; Weisdorf *et al*, 1991; Nash *et al*, 1992). Secondly, CSP alone was administered for GVHD prophylaxis in one-third of the patients in this study, whereas a combination of two agents was exclusively used in conventional SCT. Thirdly, the RIC regimens used in our study were more intensive than the non-myeloablative conditioning regimens used by the Seattle group (Sorror *et al*, 2004). These differences may counterbalance the potential beneficial aspects of reducing the intensity of conditioning. Nonetheless, the onset of GVHD was delayed in RICT; 15% of aGVHD developed between days 60 and 100 in our study, as previously reported (Mielcarek *et al*, 2003). Following conventional SCT, most of aGVHD develops within 50 d post-transplant (Snover, 1984; Beatty *et al*, 1985; Sasazuki *et al*, 1998; Morishima *et al*, 2002; Kanda *et al*, 2003).

The incidence of aGVHD rose with increasing HLA mismatch, from match through multi-loci mismatch, following RICT. In addition, the onset of aGVHD was earlier with increasing HLA mismatch. These findings are consistent with data from the myeloablative setting (Beatty *et al*, 1985; Ringden & Nilsson, 1985; Anasetti *et al*, 1990; Anasetti & Hansen, 1994; Petersdorf *et al*, 1998; Sasazuki *et al*, 1998; Morishima *et al*, 2002; Kanda *et al*, 2003). Studies from the Japan Marrow Donor Programme and others have demonstrated the importance of HLA-C mismatching in rejection, GVHD and mortality in myeloablative SCT from unrelated donors (Petersdorf *et al*, 2001, 2004; Morishima *et al*, 2002; Flomenberg *et al*, 2004; Sasazuki *et al*, 1998). We did not find a significant association between HLA-C mismatch and the development of aGVHD, but this association needs to be further investigated in a larger prospective study because only 75 donor–recipient pairs were available for analysis in this study.

We found that the use of ATG was associated with a reduction in both acute and chronic GVHD without an increased risk of graft failure, as previously shown in studies using alemtuzumab or ATG (Kottaridis *et al*, 2000; Khouri *et al*, 2001; Mohty *et al*, 2003; Nakai *et al*, 2003; Faulkner *et al*, 2004). Initial clinical trials of matched unrelated RICT or haploidentical RICT appear to be encouraging with the use of T-cell depletion (Sykes *et al*, 1999; Kottaridis *et al*, 2000; Giralt *et al*, 2001; Nagler *et al*, 2001; Chakraverty *et al*, 2002; Maris *et al*, 2003; Niederwieser *et al*, 2003; Wong *et al*, 2003; Goggins & Rizzieri, 2004). Mohty *et al* (2003) reported that a high $CD34^+$ cell dose was associated with an increased incidence of chronic, but not acute, GVHD following RICT with G-CSF-mobilised PBSC. We did not find an association between acute or chronic GVHD and the number of $CD34^+$ cells infused. Interestingly, in contrast to data from a myeloablative setting (Ringden & Nilsson, 1985; Gale *et al*, 1987; Anasetti *et al*, 1990; Weisdorf *et al*, 1991; Nash *et al*, 1992), there was no

Table V. Uni- and multivariate analyses for possible risk factors for overall survival.

	Evaluable (n = 286)					
	Univariate			Multivariate		
	HR	95% CI	P-value	HR	95% CI	P-value
HLA						
Identical	1·00			1·00		
1-MM	1·01	0·67–1·53	0·966	1·03	0·64–1·66	0·899
2 or more MM	3·21	2·14–4·82	<0·001	3·41	2·03–5·73	<0·001
		Trend	<0·001		Trend	<0·001
HCT type						
PBSCT	1·00			1·00		
BMT	1·19	0·63–2·25	0·598	1·53	0·79–2·94	0·205
Previous history of HCT						
No	1·00			1·00		
Yes	1·64	1·18–2·28	0·003	1·42	1·00–2·02	0·052
Recipient age (years)						
<60	1·00			1·00		
≥60	1·05	0·73–1·51	0·808	1·09	0·75–1·60	0·65
Recipient sex						
Female	1·00			1·00		
Male	1·29	0·96–1·74	0·095	1·19	0·88–1·61	0·264
TBI						
No	1·00			1·00		
Yes	1·25	0·86–1·82	0·239	1·28	0·84–1·94	0·25
ATG						
No	1·00			1·00		
Yes	1·15	0·77–1·72	0·482	0·71	0·44–1·16	0·177
GVHD prophylaxis						
CSP alone	1·00			1·00		
CSP + MTX	0·92	0·66–1·27	0·614	0·89	0·64–1·24	0·488
FK + MTX	1·17	0·70–1·98	0·546	0·87	0·48–1·57	0·64
Other	0·89	0·52–1·51	0·66	0·69	0·38–1·25	0·223
Risk						
Standard	1·00			1·00		
High	2·21	1·42–3·43	<0·001	2·06	1·31–3·25	0·002

HLA, human leucocyte antigen; MM, mis-match; HCT, haematopoietic cell transplantation; PBSC, peripheral blood stem cells; BM, bone marrow; TBI, total body irradiation; ATG, anti-thymocyte globulin; CSP, ciclosporin; MTX, methotrexate; FK, tacrolimus.
Multivariable adjusted for all variables listed.

increase in the incidence of aGVHD in elderly patients, as previously reported (Mohty *et al*, 2002; Wong *et al*, 2003). It has been shown in experimental models that donor T-cell responses are enhanced under stimulation with antigen-presenting cells from older mice in the context of proinflammatory milieu (Ordemann *et al*, 2002). The absence of excessive inflammation in RICT may be associated with a similar incidence of GVHD in aged recipients following RICT.

The incidence of cGVHD was similar to that following myeloablative HSCT in Japan (Kanda *et al*, 2003). A greater incidence of cGVHD was observed with increased HLA disparity, although the difference was marginal. Chronic GVHD was influenced by the use of ATG, disease status and the stem cell source, with PBSC grafts having a fourfold risk of cGVHD. It has been shown that the use of PBSC grafts instead of marrow increases the frequency of cGVHD following myeloablative HSCT (Blaise *et al*, 2000; Bensinger *et al*, 2001; Cutler *et al*, 2001). Our study suggest that the use of PBSC grafts is also a risk factor for cGVHD following RICT, although there was a large difference between the number of recipients of a PBSC graft and those who received a bone marrow (BM) graft.

In a conventional allogeneic transplant setting, the incidence of graft failure has been shown to be correlated with the degree of HLA mismatch between donors and recipients (Beatty *et al*, 1985; Anasetti *et al*, 1989; Petersdorf *et al*, 1998, 2001; Petersdorf *et al*, 1997). We found that HLA mismatch was also a risk factor for graft failure following RICT. The incidence of graft failure after HLA-matched RICT was comparable with that after conventional SCT in Japan

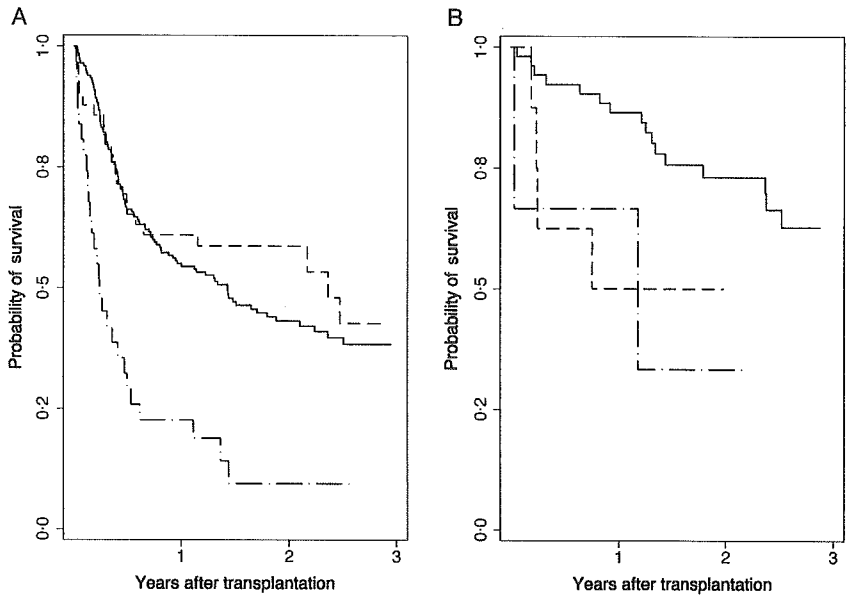

Fig 4. Overall survival (OS) according to the extent of human leucocyte antigen (HLA) mismatch and disease status. The curves represent OS as a function of time after transplantation in relation to the extent of HLA mismatch in (A) patients with a high-risk disease (total, $n = 277$; identical, $n = 198$; one-locus mismatch, $n = 48$ and two to three-loci mismatch, $n = 31$); (B) those with standard-risk disease (total, $n = 63$; identical, $n = 52$; one-locus mismatch, $n = 3$ and two- to three-loci mismatch, $n = 8$).

(Morishima *et al*, 2002; Kanda *et al*, 2003). Thus, the RIC regimens used in this study appear to have been sufficient for achieving donor cell engraftment in these patients. However, we found that the risk of rejection was extremely high (10·8%) in patients who received a graft from a two- to three-loci-mismatched donor in the HVG vector and myeloablative conditioning should be considered in this setting. Previous reports have demonstrated that the incorporation of low-dose TBI in the conditioning or the use of PBSC could reduce the incidence of graft failure (Deeg *et al*, 2001; Maris *et al*, 2003). These associations were not observed in our study probably because rate of graft failure was too low to detect a significant decrease.

The most important factor that affected OS after RICT was multiple HLA mismatch in patients with haematological malignancies. RICT from a two- to three-loci-mismatched donor resulted in a poor outcome, as has been shown in conventional SCT (Beatty *et al*, 1985; Hows *et al*, 1993; Szydlo *et al*, 1997). However, the 2-year OS after one-locus-mismatched RICT was comparable with that after HLA-matched RICT. When stratified according to disease status, one-locus mismatch was a risk factor for survival in patients with standard-risk disease, but not in those with high-risk disease. These results suggest that RICT from a one-locus-mismatched related donor may be warranted in patients with high-risk haematological malignancies when an HLA-matched sibling donor is not available. In previous studies, a younger age of patients (Faulkner *et al*, 2004) and the use of PBSC (Maris *et al*, 2003) were associated with a superior outcome after RICT, but these factors did not influence OS in the present study. However, the present study has several limitations. First, there was a large difference between the number of recipients with an HLA-matched donor and those with an HLA-mismatched donor. Secondly, since the follow-up period was short following RICT, it is too early to determine long-term outcome to these treatment regimens. Thirdly, HLA-C typing and high resolution DNA typing was not routinely performed in HSCT from a related donor.

Nonetheless, the large cohort of patients in the current study allowed us to make several important observations. First, HLA mismatch between the donor and recipient is an important risk factor for graft failure, aGVHD, cGVHD and OS after RICT. Secondly, RICT from a one-locus-mismatched related donor may represent an alternative approach in patients with high-risk haematological malignancies who lacked an HLA-matched sibling donor. These findings should be carefully confirmed in a prospective study.

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ELSEVIER

Fetal–maternal microchimerism: impact on hematopoietic stem cell transplantation

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Reciprocal cell traffic between mother and fetus during pregnancy gives rise to postpartum fetal–maternal lymphohematopoietic microchimerism, which is frequently detected in blood or tissue from healthy individuals. Although such microchimerism has been implicated in the pathogenesis of autoimmune diseases and tissue repair, recent clinical experiences have suggested the association of microchimerism with acquired immunologic hyporesponsiveness to non-inherited maternal HLA antigens (NIMAs) or inherited paternal HLA antigens (IPAs); T cell-replete HLA-haploidentical hematopoietic stem cell transplantation from a microchimeric IPA/NIMA-mismatched donor confers relatively lower incidence of severe graft-versus-host disease. The underlying mechanisms by which fetal–maternal microchimerism contributes to IPA/NIMA-specific tolerance are still elusive, although emerging experimental evidence suggests an involvement of the central deletion of IPA/NIMA-reactive T cells, the induction of peripheral regulatory T cells, and affinity-dependent modulation of NIMA-reactive B cells.

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Introduction

Although allogeneic hematopoietic stem cell transplantation (HSCT) has increasingly become a standard treatment for various hematological and non-hematological diseases, its widespread application is still limited by the lack of a histocompatible donor in a proportion of patients who have a rare or unique human leukocyte antigen (HLA) haplotype. To further extend the donor availability for allogeneic HSCT, it is beneficial to introduce the concept that some HLA mismatches are less immunogenic, or 'permissible' to the recipients, in terms of the magnitude of host-versus-graft (HVG) or graft-versus-host (GVH) reaction. In this context, important lessons have been learned from the phenomenon of fetal–maternal immunological tolerance, which is now considered to have a remote influence on the maternal or progeny's immune system after pregnancy. For instance, a substantial proportion of individuals show compromised immune responses against their non-inherited maternal HLA antigens (NIMAs) to which they had been previously exposed *in utero* [1,2]. Furthermore, although pregnancy usually induces alloreactive T cells specific for the foreign fetal histocompatibility antigens, some mothers show paradoxically protective immune responses against inherited paternal HLA antigens (IPAs) of their offspring [2,3].

Recently, several groups demonstrated that T cell-replete HLA-haploidentical HSCT from an IPA- or NIMA-mismatched family member confers high probability of durable engraftment and acceptable risks of severe graft-versus-host disease (GVHD) when the donor's peripheral blood contains the corresponding IPA- or NIMA-bearing microchimeric cells, which presumably originate from fetal–maternal two-way cell traffic during pregnancy [4]. In this review, we focus on the biological significance of such naturally occurring long-term microchimerism with special reference to its possible association with the tolerogenic 'IPA/NIMA effect', which may improve donor availability in HLA-mismatched HSCT.

The association between microchimerism and transplantation tolerance

In 1945, Owen made the remarkable discovery that most twin cattle were born with a stable mixture of each other's red cells. Billingham *et al.* [5] then showed that injection of murine fetuses with allogeneic splenocytes enabled the acceptance of later skin grafts from the same donor. This phenomenon is now referred to as neonatal tolerance and suggests that perinatal exposure to NIMA may affect the neonates developing immune system. In recipients of solid organ transplantation, a low percentage (<1%) of donor microchimerism is found due to trafficking of passenger leukocytes. Microchimerism was initially detected in long-term hepatic and renal allograft recipients requiring

minimal immunosuppressive therapy, and it was therefore assumed to have a role in tolerance induction [6]. Several studies failed to confirm this association in clinical and experimental transplantation, however [7,8]. In contrast to microchimerism, the beneficial effects of macrochimerism, or mixed chimerism, for the induction of donor-specific transplantation tolerance have been demonstrated in animal models and in some clinical observations [9,10]. A recent experimental study demonstrated that passenger leukocytes play a critical immunomodulatory effect on the induction phase of allograft acceptance, but long-lasting microchimerism is not necessary [11]. In addition, microchimerism in the thymus, but not in the blood, is associated with graft survival in a mouse model of renal transplantation [12]. These results suggest that microchimerism might play a role in allograft survival, but persistence of peripheral microchimerism is not required.

It has been shown that not only passenger leukocytes from the graft but also fetal–maternal transmission of hematopoietic cells can lead to persistent microchimerism associated with a donor-specific suppression of T cell responses [13–15]. Maternal cells and DNA were detected for a long time after parturition in peripheral blood and lymphoid organs of offspring in both humans and in mice [15–17]. Breast-feeding in the neonatal period might also contribute to building-up maternal microchimerism in the offspring because breast milk is rich in soluble maternal MHC antigens [15,18,19]. Importantly, both pregnancy and breast-feeding are required to achieve long-term survival of NIMA-mismatched grafts [15,20]. Exposure to NIMA both *in utero* and by breast-feeding appears to generate higher levels of maternal microchimerism than each exposure alone, and the degree of microchimerism was reported to show positive correlation with prolonged survival of maternal skin grafts [15]. Further studies are required, however, to determine the precise association between persistent presence of fetal–maternal microchimerism and transplantation tolerance.

Biological implications of fetal or maternal microchimeric cells

With the help of highly sensitive PCR-based techniques, long-term fetal or maternal microchimerism is easily detected from the peripheral blood or various tissues including skin, liver and thyroid gland [13]. Although many investigators have suggested the association of long-term fetal–maternal microchimerism with the development of autoimmune diseases, including systemic sclerosis, primary billary cirrhosis, juvenile inflammatory myopathies and biliary atresia [21,22], it is difficult to establish a precise etiological link, because fetal and maternal microchimerism is frequently found in healthy women with a history of uncomplicated pregnancy and more than two-thirds of immunocompetent individuals without any manifestations of autoimmune attacks (Table 1; [23]). Moreover, the passenger fetal cells might contribute to maternal tissue repair in both humans and rodents [24–26].

In a murine experiment to track maternal green-fluorescent-protein-positive cells within the offspring, maternal B, T and NK cells were persistently detected in the bone marrow and thymus of the offspring in both allogeneic and outbred crosses [27•]. Interestingly, when BCR transgenic mice that have both high-affinity H-2^k-reactive B cells and moderate-affinity H-2^b-reactive B cells were perinatally exposed to H-2^k, H-2^k-reactive B cells were partially deleted whereas H-2^b-reactive B cells were not deleted when exposed to H-2^b [27•]. Thus, maternal microchimerism in offspring might contribute to the

Table 1

The incidence of long-term fetal or maternal microchimerism among healthy subjects in relation to its postpartum duration.

	Fetal cell microchimerism in mother		Maternal cell microchimerism in offspring	
Estimated duration of chimerism (years)	Subjects examined (n)	Subjects positive for IPA-bearing cells (n; %)	Subjects examined (n)	Subjects positive for NIMA-bearing cells (n; %)
0–9	101	64; 63	29	21; 72
10–19	105	67; 64	47	35; 74
20–29	132	95; 72	158	115; 73
30–39	42	29; 69	100	72; 72
40–49	14	10; 71	64	46; 72
50–59	1	1; 100	20	14; 70
60–69	0	[a]	8	6; 75
Total	395	266; 67	426	309; 72

With the help of a sensitive IPA- or NIMA-specific nested PCR that is capable of detecting the DNA equivalent of a single cell in 105 background cells, the incidence of microchimeric cells in the peripheral blood obtained from 821 healthy donor candidates for HSCT was examined. To eliminate a false positive result, control DNA samples derived from non-hematopoietic tissues (nails) were simultaneously analyzed. The number and proportion of subjects who were positive for microchimerism in the blood but not in the nails are shown according to time (by decades) after the delivery of the corresponding offspring. Chimeric cells bearing IPA were detected in 67% of the 395 mothers tested with the longest persistence being 59 years, and chimeric NIMA-bearing cells were found in 72% of the 426 individuals tested up to the age of 66 years.

[a] Not examined. Results taken from E Maruya and H Saji, unpublished, and [51].

induction of B cell hyporesponsiveness to NIMA in a manner dependent on the degree of affinity of NIMA-allospecific B cell repertoires in the developing fetus.

Hematopoietic stem cell transplantation from a microchimeric IPA/NIMA-mismatched donor

Feasibility of T cell replete IPA/NIMA-mismatched transplantation

The possible role of long-term fetal–maternal microchimerism as an indicator of an acquired form of fetal–maternal tolerance was first proposed by Tokita *et al.* [28]. They reported the dramatic regression of thymic carcinoma in a mother who received an infusion of peripheral blood stem cells from her HLA-haploidentical daughter without any signs of GVHD. Donor microchimerism was detected both before and long after the infusion in the recipient, prompting them to speculate that the mother had been tolerant to the IPA and thus the infused donor effector lymphocytes could survive longer in face of the maternal immune system.

Soon thereafter, Ochiai *et al.* [29] reported a case of T cell-replete 'mother-to-child' HSCT from a microchimeric mother. Although the mother and son had serological mismatches at HLA-A, -B, -C and -DR in the GVH direction, acute GVHD was restricted to the skin and rapidly improved after standard therapy. This approach was soon found to be feasible by several groups [14,30,31]. Notably, although graft failure is a serious problem in HLA-haploidentical HSCT, successful engraftment has been reported even after nonmyeloablative conditioning HSCT from an IPA/NIMA-mismatched donor in patients with hematological malignancies [32,33], aplastic anemia [34] and renal cell carcinoma [35]. These results suggest that haploidentical SCT based on the fetal–maternal microchimerism without T-cell-depletion is at least feasible.

Differential immunogenicity between IPA and NIMA

We have examined the clinical outcomes of HLA-haploidentical HSCT from microchimeric IPA/NIMA-mismatched family members in a selective nationwide registry study [4]. This analysis was confined to T cell-replete HSCT from maternal donors who had IPA-bearing microchimeric cells or offspring/sibling donors who had maternal microchimeric cells bearing NIMA. Irrespective of multiple HLA disparities between the donor and recipient, 15 of 34 evaluated patients did not develop acute GVHD of grade II or more, but severe grade III or IV acute GVHD was reported in eight patients. These observations suggest that fetal or maternal microchimerism in the donor is not always a hallmark of IPA/NIMA-specific tolerance. Importantly, multivariate analysis showed that IPA mismatch in the GVH direction has a higher risk for developing severe acute GVHD when compared to mismatches for NIMA, suggesting differential immunogenicity between IPA and NIMA (Table 2; [2]).

Table 2

Differential immunogenicity of IPA and NIMA in T-cell-replete HSCT.

	van Rood *et al.* [2]		Ichinohe *et al.* [4]	
	NIMA mismatch	IPA mismatch	NIMA mismatch	IPA mismatch
Number of recipients	63	79	20	15
Disparities at HLA-A, -B, - DR antigens: n[a], ()[b]:				
1 antigen mismatched	59	26	0	0
2 antigens mismatched	4	53	7 (14)	10 (10)
3 antigens mismatched	0	0	13 (6)	5 (5)
Stem cell source	Bone marrow	Bone marrow	Mainly peripheral blood	Mainly peripheral blood
GVHD prophylaxis	Mainly cyclosporine based	Mainly cyclosporine based	Tacrolimus based	Tacrolimus based
100 day probability of grade 2–4 acute GVHD: %[d]	41 (30–53)[c]	58 (48–68)[c]	50 (27–69)	64 (34–83)
100 day probability of grade 3–4 acute GVHD: %[d]	Data not available	Data not available	10 (2–26)[c]	38 (15–60)[c]
2 year probability of chronic GVHD: %[d]	28 (17–40)[c]	31 (21–41)[c]	Data not available	Data not available

The results of two different registry studies investigating the effect of IPA and NIMA mismatches (in the GVH vector) on the development of acute and chronic GVHD [2,4]. In both studies, IPA-mismatched HSCT is defined as 'mother-to-child' transplantation. The NIMA-mismatched group [2] included only transplantation from an HLA-haploidentical NIMA-mismatched sibling, whereas our study [4] additionally included 'child-to-mother' transplantation. The first study [2] demonstrated that NIMA mismatch in the GVH vector was associated with a lower probability of acute and chronic GVHD when compared with IPA mismatch. In the second study [4], which analyzed the clinical outcomes of non-T-cell-depleted HSCT from a highly HLA-disparate microchimeric IPA/NIMA-mismatched family member, the risk for developing grade 2–4 acute GVHD was not significantly different for IPA-mismatched and NIMA-mismatched transplantation. However, severe grade 3 or 4 GVHD was less likely to occur in the NIMA-mismatched group. These results suggest that immune regulatory mechanisms associated with low immunogenicity against NIMA might be more robust than those against IPA.

[a] Recipients were classified according to the larger number of mismatched antigens either in the HVG or GVH direction.

[b] The number of patients separated by the type of mismatch in the GVH vector alone.

[c] The risk of developing GVHD was lower in the NIMA-mismatched group when compared by multivariate analysis.

[d] 95% confidence interval.

Possible application to cord blood transplantation

Cord blood is increasingly used as an alternative stem cell source, but engraftment failure is a major complication, especially following transplantation to adults. It is thus intriguing to ask whether IPA or NIMA mismatches in the HVG direction could improve the probability of engraftment in cord blood transplantation without an increased risk of GVHD. Additionally, umbilical cord blood has been shown to contain microchimeric maternal cells and thus might be less immunogenic against NIMA [36].

Possible mechanisms for IPA/NIMA effect

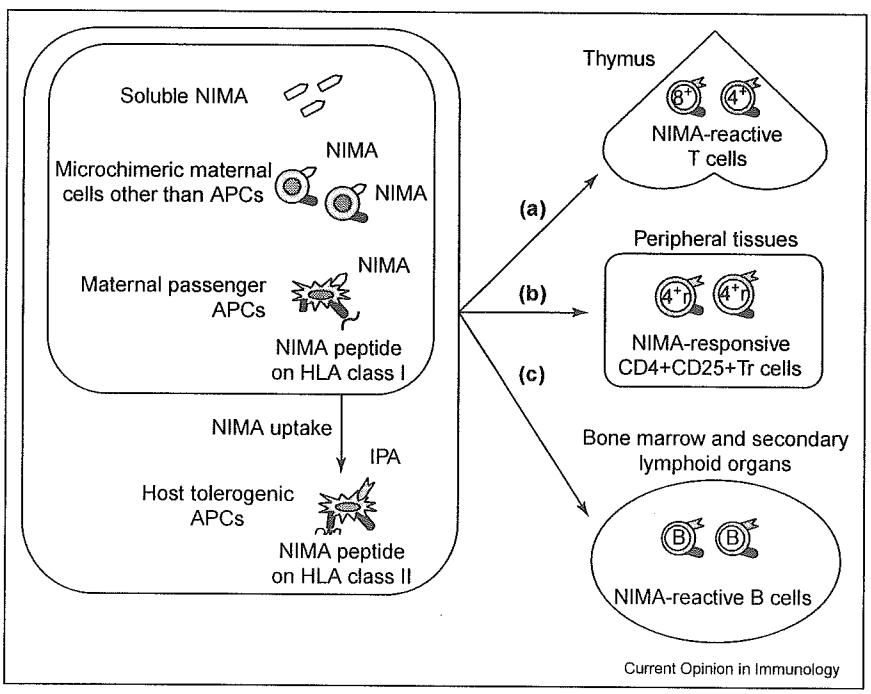
Deletional mechanisms

As described above, studies in clinical and experimental transplantation provide a body of evidence for the tolerogenic effects of IPA/NIMA; however, little is known about the mechanisms by which transmission of IPA/NIMA drives the immune system towards tolerance. T cells isolated from a NIMA-exposed child and an IPA-exposed mother show hyporesponsiveness towards the corresponding antigens [36,37]. In NIMA-exposed mice, the precursor frequency of NIMA-reactive T cells is remarkably diminished [15].

T cells recognize alloantigens via direct or indirect allorecognition pathways. As breast milk is rich in soluble NIMA and could contribute to the establishment of microchimerism in the offspring [15,19,38], an indirect allorecognition pathway might be involved in the induction of tolerogenic NIMA effects. To address this issue, pregnant female mice were injected with an MHC allopeptide [15]. This procedure induced long-term tolerization of offspring T cells towards the corresponding antigens, suggesting that alloantigen exposure to the fetus can affect developing immune systems via an indirect allorecognition pathway.

Central and/or peripheral tolerance mechanisms might contribute to tolerogenic IPA/NIMA effects. Injection of allogeneic spleen cells to neonatal mice eliminates donor-reactive T cells and prolongs skin graft survival [39].

Figure 1



Hypothetical roles for long-term maternal microchimerism in establishment and maintenance of NIMA-specific immune tolerance. Possible mechanisms include; **(a)** intrathymic deletion of NIMA-reactive T cell precursors; **(b)** peripheral induction of regulatory T cell subsets that specifically or non-specifically suppress NIMA-reactive T-cell responses; and **(c)** affinity-dependent deletion of NIMA-reactive B cells in the bone marrow and secondary lymphoid organs. Antigen presenting cells (APCs) of host or maternal origin play an important role in the tolerogenic presentation of NIMA allo-epitopes. NIMA-derived peptides can be presented on both HLA class I molecules on maternal passenger APCs and HLA class II molecules on host APCs that engulfed either apoptotic passenger maternal cells or soluble NIMA. The migration of such NIMA-bearing APCs into thymus, bone marrow and secondary lymphoid organs might result in the partial deletion of NIMA-reactive T cells and B cells. Exposure to NIMA-derived peptide or soluble NIMA might also trigger the induction of NIMA-specific and non-specific regulatory T cells in the periphery. Similar hypotheses could be generated for elucidating the roles of long-term fetal cell microchimerism in IPA-specific hyporesponsiveness.

In these mice, intrathymic deletion of donor reactive T cells is evident in their association with intrathymic donor microchimerism, suggesting the involvement of a central mechanism in allograft tolerance. In this case, however, intrathymic microchimerism is transient and thus donor-reactive T cells can be generated later in the thymus. In a patient who is tolerant to a maternal kidney allograft, little donor-specific cytotoxic T lymphocyte (CTL) activity was detected, but re-stimulation with donor stimulator cells and IL-2 restored anti-donor CTL activity, indicating that anti-donor CTL precursors were not completely deleted [40]. These results suggest that many NIMA-reactive T cells escape thymic negative selection induced by intrathymic microchimerism, and long-lasting stable tolerance requires other mechanisms.

Involvement of regulatory T cell subsets

It has been assumed that the maternal immune system ignores the fetus during pregnancy. Previous studies, however, challenged this long-held assumption and demonstrated that the maternal immune system is not only aware of fetal alloantigens but is also capable of responding to them. Recently, Aluvihare *et al.* [41•] demonstrated that the $CD4^+$ $CD25^+$ regulatory T cell pool expands during pregnancy and mediates maternal tolerance to the fetus. $CD4^+$ $CD25^+$ cells also contribute to the maintenance of neonatal tolerance by preventing the development of T cell alloreactivity [42]. Interestingly, soluble forms of class I MHC trigger $CD4^+$ $CD25^+$ responses [43], thereby implicating breast-feeding as an active regulatory mechanism. It can be speculated, therefore, that these regulatory cells are involved in the tolerogenic NIMA effects, although this hypothesis needs to be elucidated (Figure 1).

Although antigen-nonspecific suppressive effects of $CD4^+$ $CD25^+$ regulatory T cells are well recognized, several studies demonstrated that alloantigen-specific regulatory T cells could also be generated [42,44,45]. Antigen-specific expansion of regulatory T cells requires a T cell-deficient environment and stimulation with the corresponding antigens in the presence of IL-2 [44,46,47]. Therefore, the prenatal lymphopenic condition of the fetus might facilitate the expansion of antigen-specific regulatory T cells. Similarly, the lymphopenic status after myeloablative conditioning might enable the expansion of these cells following allogeneic HSCT [48]. Currently, calcineurin inhibitors, such as cyclosporine and tacrolimus, are almost exclusively used for GVHD prevention in allogeneic HSCT. These agents inhibit T cell production of IL-2, which is required for activation and expansion of $CD4^+$ $CD25^+$ regulatory T cells and thereby may suppress the effects of IPA/NIMA. Immunosuppressants that do not interfere with these regulatory cells, such as sirolimus and mycophenolate mofetil, might be of use for IPA/NIMA-associated HSCT [49,50].

Conclusions

Despite recent advances in immunological research, the biological significance of long-term fetal–maternal microchimerism still remains a great enigma. In support of the hypothesis that fetal–maternal microchimerism is associated with acquired immunologic hyporesponsiveness to non-inherited fetal or maternal antigens, HLA-haploidentical HSCT from a microchimeric IPA/NIMA-mismatched family member is proved to confer durable engraftment and an acceptable risk of acute GVHD. It is important to understand the immunological mechanisms involved in the establishment of fetal–maternal tolerance during pregnancy, and its postpartum persistence. Possible mechanisms of long-lasting fetal–maternal tolerance include neonatal exposure to soluble NIMA allopeptides by breast-feeding, the induction of peripheral regulatory T cells, and affinity-dependent modulation of IPA- or NIMA-reactive lymphocytes through the interaction with fetal or maternal microchimeric cells. Further studies are warranted to improve the outcome of allogeneic transplantation of solid organ and hematopoietic stem cells.

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- • of special interest
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