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Helper T-lymphocyte precursor frequency predicts the occurrence of graft-versus-host disease and disease relapse after allogeneic bone marrow transplantation from HLA-identical siblings

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Background and Objectives. Donor helper T-lymphocytes may be involved in graft-versus-host disease (GVHD) and a graft-versus-leukemia effect after bone marrow transplantation (BMT). We assayed donor helper T-lymphocyte precursor frequencies ($HTLP_f$) to see whether they could predict the severity of GVHD and disease relapse after transplantation, thereby facilitating donor selection, pre-transplant counselling and modification of GVHD prophylaxis after BMT.

Design and Methods. Thirty-six consecutive adult BMT recipients and their HLA-identical sibling donors were recruited. $HTLP_f$ was measured as a function of interleukin-2 secretion by alloreactive donor T-cells using a limiting dilution assay. Patients were followed prospectively to assess the severity of GVHD and the status of the primary disease after BMT.

Results. Eight donors had $HTLP_f$ less than or equal to 10^{-6}; no recipients of these grafts developed severe GVHD after transplantation. Twenty-eight donors had $HTLP_f$ greater than 10^{-6} and 18 recipients of these grafts developed severe GVHD (≥ grade 2) (χ^2 test, $p<0.01$). Seven donors had $HTLP_f$ greater than 10^{-5} and no recipient had disease relapse. Twenty-nine donors had $HTLP_f$ less than or equal to 10^{-5}, 11 recipients of these grafts developed disease relapse (χ^2 test, $p=0.08$).

Interpretation and Conclusions. BMT recipients from HLA-identical sibling donors with low ($<10^{-6}$) and high ($>10^{-5}$) $HTLP_f$ may have a low risk of acute GVHD and disease relapse after transplantation.

Key words: helper T-cell precursor frequency, graft-versus-host disease, relapse

Graft-versus-host disease (GVHD) is an important cause of morbidity and mortality after allogeneic bone marrow transplantation (BMT). The incidence of moderate to severe GVHD after BMT from HLA-identical sibling donors varies from 20 to 40%.[1] This is often attributed to the alloimmune reaction of donor T-cells targeted against minor histocompatibility antigens in host tissues. Donor T-cell depletion has been shown to alleviate GVHD at the expense of increased graft failure and disease relapse, suggesting that these cells may play a pivotal role in marrow engraftment and the graft-versus-leukemia (GVL) phenomenon.[2,3] Recent studies have shown that a high frequency of host-specific donor helper T-cell precursors ($HTLP_f$) predicts severe GVHD in allogeneic BMT recipients[4-6] but data on the use of this parameter in the prediction of leukemic relapse are relatively scarce.[7] We, therefore, developed a functional assay to quantify $HTLP_f$ and correlated the results with the risks of severe GVHD and disease relapse after BMT from HLA-identical sibling donors, thereby facilitating selection of transplant donors, pre-transplant counselling and modification of GVHD prophylaxis.

Design and Methods

Patients

Thirty-six consecutive unselected patients who underwent allogeneic BMT from HLA-identical siblings in Queen Mary Hospital, Hong Kong from May 1997 to January 2000 were recruited. There were no exclusion criteria. The patients' clinical characteristics are shown in Table 1.

Post-transplantation treatment

Prophylaxis against GVHD comprised methotrexate (15 mg/m^2 on day 1, 10 mg/m^2 on days 3, 6 and 11) and cyclosporin A (3 mg/kg/day intravenously or 9 mg/kg/day orally on days 1-50, tailed off at 6

months). The severity of GVHD was graded according to the criteria described by Glucksberg *et al.*[8] and was classified into mild (overall grade < 2) or severe (overall grade ≥ 2). Patients developing GVHD received additional immunosuppression according to the discretion of the attending physicians. This additional immunosuppression included intravenous methylprednisolone (2 mg/kg/day, increased to 4 mg/kg/day if no response within 48 hours) and for patients with refractory GVHD, horse anti-thymocyte globulin (ATG) (25 mg/kg/day for five days).

Measurement of HTLP$_f$ using limiting dilution analysis

Collection and storage of peripheral blood mononuclear cells. Peripheral blood (PB) was collected from donors and recipients before BMT and mononuclear cells (MNC) were separated by density gradient centrifugation on a Ficoll preparation, resuspended in 5 mL of 10% FCS-RPMI and diluted 10-fold with WBC solution. The cell density was determined and was adjusted to 2×10^7 cells/mL. A freezing medium (20% of DMSO and 80% of 20%FCS-RPMI) was added at equal volume to the cell suspension and the cells were frozen in 1 mL aliquots at –70°C using a controlled rate freezer.

HTLP$_f$ determination. Cryopreserved PBMNC were thawed and washed, counted and diluted to a concentration of 10^6 cells/mL. Recipient cells were the stimulators and donor cells the responders. Eight serial dilutions of the responder cells were made with 6 to 24 replicates at each dilution, depending on the cells available. To each well of 96 U-bottomed well plates, 50 µL of responder cell suspension were added. Recipient PBMNC were γ-irradiated (25 Gy) and 50 µL (containing 50,000 cells) were added to each well. On day 3 of culture the plates were centrifuged (800 rpm/min) and 20 µL of the supernatants were transferred to new plates and stored at -20°C until use. During HTLP$_f$ assay, the plates were thawed and the interleukin (IL-2) dependent CTLL-2 cells were starved in normal medium (deprived of IL-2) for four hours. Thereafter, the cells were diluted to 1×10^5/mL and 50 µL were added to each well and incubated for 20 hours. Calcein-AM fluorescence dye was added to each well and was incubated for 30 minutes. The fluorescence intensity of each well was measured by cytofluorimetric techniques. HTLP$_f$ were determined from the proportion of wells negative for IL-2 production at each responder (donor) cell dilution. Wells were scored positive for IL-2 production if the fluorescent emission was more than three standard deviations (SD) above the mean of the control culture containing only the stimulator cells and the CTLL-2 cells. The frequency of the responding cells was determined by maximum likelihood estimation using a computer program, as described by Fazekas de St Groth.[9]

Table 1. Clinical characteristics of the 36 patient-donor pairs.

Pt.	Age/Sex	Dx	ANC engraftment	acute GVHD (overall)	HTLP$_f$ ($\times10^{-6}$)	Relapse after BMT (months)	FU (months)
1	47/F	ALL	19	I	< 1.0	9.1	11
2	31/F	AML	15	0	< 1.0	12.2	30
3	36/M	CML	24	I	< 1.0		15
4	29/M	CML	18	0	< 1.0		34
5	45/M	CML	17	0	< 1.0		32
6	39/M	CML	15	I	< 1.0		20
7	49/M	AML	21	0	< 1.0		13
8	17/F	CML	30	I	< 1.0		13
9	23/M	NHL	21	I	2.5		29
10	37/M	ALL	15	IV	3.3	12	20
11	49/M	CML	17	III	3.8		23
12	42/M	MDS	25	III	4.6		26
13	36/M	ALL	19	II	4.8		23
14	38/F	ALL	24	0	5.1		24
15	27/M	CML	22	III	5.3		13
16	49/M	CML	17	II	5.3	14.4	17
17	28/M	CML	12	II	5.4		14
18	31/F	AML	16	III	5.4		20
19	30/M	CML	18	I	5.6	3.0	4
20	30/M	CML	27	II	6.1	3.0	8
21	43/M	CML	20	0	6.3		24
22	45/M	AML	26	III	6.9	18	24
23	35/M	CML	25	IV	8.0		6
24	31/M	AML	17	0	8.6	6.8	14
25	34/F	HD	19	I	9.1	6.5	29
26	31/F	CML	28	0	10	3.0	23
27	17/M	AML	16	III	10		27
28	40/F	AML	28	IV	10	7.7	15
29	37/M	AML	30	0	12		14
30	18/F	ALL	23	II	12		19
31	40/M	CML	23	I	16		22
32	41/M	CML	17	III	23		32
33	46/F	MDS	13	IV	23		8
34	37/M	CML	16	II	25		8
35	49/F	CML	19	IV	41		19
36	41/M	AML	15	NA	63		1

Pt.: patients; Dx: diagnosis; AML, acute myeloblastic leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; HD, Hodgkin's disease; NHL: non-Hodgkin's lymphoma; FU, duration of follow-up from the date of BMT to the date of last evaluation or death. ANC engraftment is defined as the day post-BMT when the absolute neutrophil count is equal or greater than 0.5×10^9/L. Disease relapses were defined by morphology, cytogenetics or molecular studies, whichever available.

Statistical analysis

Comparisons between groups of data were performed using the Mann-Whitney test (numerical

data) and the chi squared test (categorical data). Correlations between engraftment time and donor $HTLP_f$ were made by linear regression. *p* values less than 0.05 are considered statistically significant.

Results

Baseline characteristics

The clinical characteristics of the BMT patients are shown in Table 1. The median age of the patients was 37 years (range: 17-49) and the duration of follow-up was 20 months (range: 1-34). The median time of neutrophil engraftment was 19 days (range: 12-30 days).

Effects on GVHD

Eight donors had $HTLP_f$ less than or equal to 10^{-6}, from whom no recipient developed severe GVHD after transplantation. Twenty-eight donors had $HTLP_f$ greater than 10^{-6} and 18 recipients of these grafts developed severe GVHD (≥ grade 2) (χ^2 test, $p<0.01$) (Table 2). The median donor $HTLP_f$ in recipients with no or mild GVHD was significantly lower than that in patients who developed severe GVHD (3.8 vs 7.5×10^{-6}, $p < 0.01$) (Figure 1).

Effects on disease relapse

Seven donors had $HTLP_f$ greater than 10^{-5}, from whom no recipient had disease relapse. Twenty-nine donors had $HTLP_f$ less than or equal to 10^{-5} and 11 of the recipients of grafts from this group had disease relapse (χ^2 test, $p=0.08$) (Table 2). The probability of disease relapse after BMT was higher in patients who received BMT from donors with low $HTLP_f$, although the difference was not statistically significant (Figure 2) (log-rank test, $p=0.09$).

Effects on donor marrow engraftment and rejection

Donor marrow engraftment defined as the day after BMT on which the absolute neutrophil count was equal to or greater than 0.5×10^9/L (Table 1), was correlated with the donor $HTLP_f$. There was no correlation between engraftment time and donor $HTLP_f$ in any of the 36 patient-donor pairs tested ($p=0.259$). No patient rejected the marrow graft in this cohort.

Discussion

In this study, we demonstrated that $HTLP_f$ might predict the severity of GVHD and the risk of disease relapse after BMT from HLA-identical siblings. Similar results have been reported in unmanipulated allogeneic BMT[4,7] but not T-cell depleted allografts.[10] On the other hand, the present study shows several interesting features which may be important in the pre-transplant evaluation of GVHD and disease relapse after BMT.

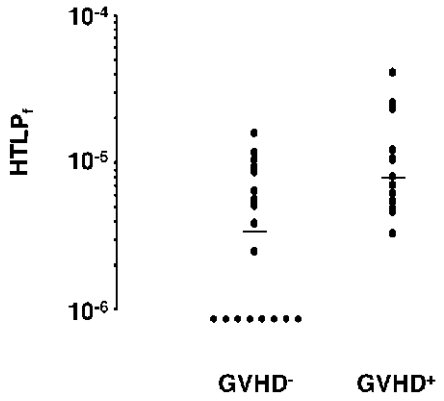

Figure 1. Association between HTLPf (Log scale) and severity of GVHD (GVHD⁺ ≥ overall grade 2; GVHD⁻ < overall grade 2). The short horizontal bars across the data represent the median $HTLP_f$ in each group. The difference in $HTLP_f$ was statistically significant ($p < 0.01$).

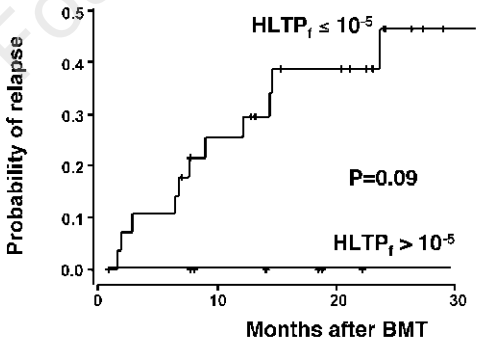

Figure 2. The probability of disease relapse in recipients of grafts with high (>10^{-5}) and low (≤ 10^{-5}) $HTLP_f$.

Table 2. The association between $HTLP_f$ and the development of GVHD and disease relapse after BMT.

	$HTLP_f \le 10^{-6}$	$HTLP_f > 10^{-6}$
GVHD⁺	0	18
GVHD⁻	8	10
	$HTLP_f \le 10^{-5}$	$HTLP_f > 10^{-5}$
Relapse⁺	11	0
Relapse⁻	18	7

GVHD⁺ ≥ overall grade 2; GVHD⁻ < overall grade 2. Disease relapses were defined by morphology, cytogenetic or molecular studies, whichever available. Patients receiving grafts from donors with low (≤ 10^{-6}) and high (>10^{-5}) $HTLP_f$ had low risk of developing severe GVHD ($p<0.01$) and disease relapse ($p=0.08$).

First, no patient who received BMT from donors with HTLP$_f$ below 10^{-6} developed severe GVHD. In these patients, the intensity of alloimmune reaction may be insufficient to trigger the cytokine cascades that mediate the development of GVHD.[11] Second, no patient who received transplants from donors with HTLP$_f$ above 10^{-5} had disease relapse after BMT. Strong expression of IL-2-secreting donor T-helper cells may reflect an enhanced alloimmune reaction targeting against tumor antigens, thereby eliciting a graft-versus-leukemia effect. On the other hand, five out of eight recipients whose donors had HTLP$_f$ above 10^{-5} developed severe GVHD, which could be explained by donor T-cells targeting against host tissues after immune reconstitution. Previous studies have shown that in transplantation from matched unrelated donors, a high HTLP$_f$ is associated with mismatch at the HLA-DPB1 locus, the latter being associated with an increased incidence of acute GVHD.[12] It remains to be seen whether donor-patient pairs with high HTLP$_f$ in this study would be related to mismatches at this and other undefined HLA loci.

It is also interesting that while high donor HTLP$_f$ is associated with low risk of disease relapse, there was no increase in relapse rate in patients who received transplants from donors with low HTLP$_f$. A subgroup of patients with low risk of GVHD and disease relapse have been demonstrated by Lachance *et al*.[7] In these patients GVHD and the GVL phenomenon may be mediated by different subsets of donor T-cells, targeting separately the patients' minor histocompatibility antigens and leukemic cells.[13] Whether this could explain the apparent disparity of the risk of GVHD and disease relapse in this cohort will have to be tested by further studies.

In conclusion, we have shown that patients who received BMT from HLA-identical siblings with low HTLP$_f$ (below 10^{-6}) had a lower risk of severe GVHD whereas those who received their graft from siblings with high HTLP$_f$ (above 10^{-5}) had a lower risk of disease relapse but a higher risk of severe GVHD. These results should be confirmed in a larger cohort of patients.

Contributions and Acknowledgments

AYHL: drafting the article and analyzing data; AKWL: formulating the conception and intellectual content of the manuscript; JK: performing part of the HTLP studies and drafting the manuscript; PC: perfoming part of the HTLP studies and analyzing data; FEC: formulating the conception and intellectual content of the manuscript; RL: formulating the design of the study and revision of the final version of the manuscript.

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Disclosures

Conflict of interest: none.

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Manuscript processing

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Potential implications for clinical practice

We have adopted the measurement of HTLP$_f$ in donors as part of the assessment of the risk of severe GvHD and disease relapse during our pre-BMT counselling.

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