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Analysis of immune reconstitution in adults undergoing non-myeloablative allogeneic peripheral blood stem cell transplantation

The effect of non-myeloablative procedures on post-transplant immune reconstitution is unknown. We investigated the immune status of patients with leukemia following non-myeloablative allogeneic peripheral blood stem cell transplantation (NST). Ten adult were analyzed 1, 3 and 12 months after transplant. We conclude that NST may result in early immune reconstitution.

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Immune reconstitution plays a pivotal role in the long-term outcome of allogeneic hematopoietic stem cell transplantation (allo-HSCT), not only because immune defects are related to infectious morbidity post-transplant, but also because they may influence the risk of relapse and the development of secondary malignancies after HSCT.[1] Following conventional allo-HSCT, all patients experience a period of profound neutropenia and immunodeficiency that is significantly responsible for the serious infectious complications that can occur after a transplant. The entire strategy of non-myeloablative preparative regimen relies on the graft-versus-leukemia effect or the graft-versus-tumor effect as the primary therapeutic modality. Non-myeloablative stem cell transplantation (NST) has reduced conditioning-related toxicity,[2] but the effects on post-transplant immune recovery have not been studied in detail. We evaluated several immunologic parameters of patients who underwent NST at our institution.

Ten patients who had undergone NST from HLA-identical siblings were analyzed as a case group. The conditioning regimen consisted of fludarabine (Schering AG, Berlin, Germany) 30 mg/m^2/day for 5 days, busulfan 2 mg/kg/day for 4 days and anti-T lymphocyte globulin (ATG, Fresenius AG, Munich, Germany) 10 mg/kg/day for 5 days. Allogeneic hematopoietic blood stem cells were collected following mobilization with granulocyte colony-stimulating factor (G-CSF) (Kirin-Sankyo, Tokyo, Japan) 10 μg/kg/days for 5~7 days. G-CSF-mobilized blood stem cells were used with no further *in vitro* manipulation. The CD34$^+$cell count was 5×10^6/kg. Prophylaxis against GVHD included cyclosporine A 2 mg/kg/day starting on day –1 and continued at a dose to maintain therapeutic blood levels until day +100. Patients received a donor lymphocyte infusion (DLI) of 1×10^7 CD3$^+$cells/kg on day +30. DLI were given in graded incremental doses. Two- or three-color flow cytometry of CD3$^+$, CD4$^+$, CD8$^+$, CD19$^+$, CD3$^-$/CD16$^+$/CD56$^+$, CD3$^+$CD25$^+$ cell surface markers was performed 1, 3 and 12 months after NST. White blood cell counts were assessed at each point of blood collection using an automatic cell counter. T-cell proliferative responses, LAK and NK activity, and serum immunoglobulin (Ig) were examined according to the previous report.[3,4] Donor-recipient chimerism was assessed by polymerase chain reaction (PCR)-based amplification of a polymorphic short tandem repeat (STR) region.

The absolute number of CD3$^+$ and CD8$^+$ T cells, and B lymphocytes, as well as the proliferative response to T-cell mitogens, recovered with time after transplantation. CD8$^+$ T cells and B cells recovered to the normal range by 3 months. CD4$^+$ T-cell counts remained below normal up to 1 year after transplantation. Recovery of NK cell number and innate cytotoxic activity was fast. IgG and IgM levels were within normal range by 1 month post-transplant (Table 2) .

Little is known about immune reconstitution following non-myeloablative allogeneic transplantation. Immune reconstitution in this particular setting depends on three potential sources of functional lymphocytes; (i) residual host lymphocytes that survived after the conditioning regimen; (ii) naive, stem cell-derived lymphocytes of both donor and host origin; and; (iii) mature donor lymphocytes transfused as part of our transplant protocol. Our analysis is in accordance with data published previously.[4-6] The different behavior in the immune reconstitution of the CD8$^+$ subset after NST may be favored by an extrathymic origin of these cells while CD4$^+$ subset recovery, which is thymus-dependent, is impaired because of thymic involution.[7] The proliferative response of T cells to polyclonal activators (PHA) was high, which contrasts with the impaired immune reactivity observed in patients conditioned by a conventional myeloablative regimen.[8] Therefore, we conclude that, following a non-myeloablative regimen, patients may conserve an almost intact *in vitro* T cell-dependent proliferative response.

All transplanted patients investigated in the present study displayed normal or high levels of LAK and NK activity, especially during the early period post-transplantation. It is important to establish that non-myeloablative regimens do not suppress NK cell activity since NK cells may play a role in engraftment, in prevention of GVHD and in exerting graft-versus-leukemia effects.[9]

A two-step strategy has been developed to reduce the toxicity of conditioning regimens and to preserve a curative antitumoral effect corresponding to that of allo-HSCT after a non-myeloablative preparative regimen, whether followed by DLI or not, as documented by the results of both chimerism and minimal residual disease studies. The DLI contributed mostly mature T cells, which may contribute to improved T-cell function following NST. Bellucci *et al.*[10] demonstrated that DLI developed increased numbers of B cells. Further studies in animal model systems as well as in patients who receive DLI will be necessary to define the mechanism underlying this immunologic effect better.

Large cohorts of patients must be investigated to determine

Table 1. Patients' clinical characteristics.

No.	Age,y, at NST/Sex	Status at transplant	Day ANC >0.5×10⁹/L	Day platelets >20×10⁹/L	GVHD Acute	GVHD Chronic	% Donor 1 month post transplant	Donor Lymphocyte Infusion	Survival
1	18/F	CML 1stCP	+10	+11	Grade 1	–	60	d38, d75	CR+735
2	25/M	CML 1stCP	+11	+8	–	–	100	–	CR+380
3	38/M	AML 1stCR	+9	continuous	–	Limited	100	d40	CR+390
4	33/F	AML 1stCR	+12	continuous	–	Limited	100	d34	CR+654
5	34/F	ALL 1stCR	+14	continuous	Grade 2	–	100	–	Died of GVHD+192
6	42/M	AML 1stCR	+10	continuous	Grade 2	Extensive	100	–	CR+550
7	32/M	CML 1stCP	+13	+10	–	Extensive	100	d32, d70, d120	CR+710
8	39/F	AML 1stCR	+11	+10	–	–	60	–	Relapsed+50, Dead+70
9	56/M	CML AP	+12	continuous	–	Limited	100	d38, d65, d100	Died of GVHD+243
10	36/M	AML 1stCR	+16	+13	–	–	100	d35, d65	CR+470

AML: acute myelogenous leukemia; ALL: acute lymphoblastic leukemia; CML: chronic myelogenous leukemia; CP: chronic phase; AP: accelerated phase; CR: complete remission; ANC: absolute neutrophil count; GVHD: graft-versus-host disease.

the potential advantageous or disadvantageous effects of a low intensity regimen on the course of post-transplantation immune reconstitution.

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Table 2. Immunological reconstitution (mean).

	Time interval following transplantation 1 month	3 month	12 month	Normal Range
SI*	82 (42~180)	115 (60~330	86 (40~225	70~320
IgG	13 (8.2~20)	10 (9~17)	11 (8~15)	8~16
IgA	1.0 (0.6~2.5)	1.4 (0.9~2.8)	1.8 (1.0~3.0)	0.7~3.3
IgM	1.1 (0.7~1.9)	1.5 (0.8~2.0)	1.4 (0.9~1.8)	0.5~2.3
NK activity#	36 (24~45)	28 (21~38)	24 (18~30)	20~35
LAK activity#	73 (57~9)	74 (58~87)	68 (54~78)	65~85
CD4×10⁹/L	0.02 (0.01~0.08)	0.1 (0.07~0.21)	0.35 (0.3~0.65)	0.41~1.35
CD8×10⁹/L	0.05 (0.028~0.16)	0.31 (0.21~0.42)	0.49 (0.35~0.57)	0.2~0.68
CD19×10⁹/L	0.01 (0.005~0.02)	0.1 (0.75~0.15)	0.17 (0.12~0.32)	0.1~0.38
CD16/56+×10⁹/L	0.35 (0.24~0.45)	0.15 (0.1~0.37)	0.18 (0.12~0.24)	0.1~0.42
CD3/25+×10⁹/L	0.07 (0.03~0.1)	0.053 (0.04~0.09)	0.04 (0.03~0.1)	0.034~0.116

**Stimulation Index (SI) of PHA response median (range), #Results are expressed as percent of specific lysis at the effector-to-target ratio of 30:1 for NK and LAK activity.*

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Low incidence of acute graft-versus-host disease after non-myeloablative stem cell transplantation with CD8-depleted peripheral blood stem cells: an update

We examined the effect of CD8-depletion of the graft in transplant recipients conditioned with low-dose total body irradiation +/- fludarabine. Ten patients received unmanipulated peripheral blood stem cells (PBSC) (control group) and 16 CD8-depleted PBSC (CD8-depleted group). The 100-day incidence of grade II-IV acute graft-versus-host disease was 70% in the control group versus 0% in the CD8-depleted group (p=0.001).

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We prospectively analyzed the impact of CD8-depletion on the incidence of acute graft-versus-host disease (GVHD) as well as on T-cell chimerism after non-myeloablative stem cell transplantation (NMSCT). Twenty-six patients with hematologic malignancies but ineligible for a conventional myeloablative transplant or patients with metastatic renal cell carcinoma (RCC) were included in this study. Their clinical characteristics are summarized in Table 1. Written informed consent was obtained from patients and donors and our institution's Ethical Committee approved the protocol. Conditioning consisted in 2 Gy single dose total body irradiation (TBI) on day 0 (N=6). For patients not heavily pre-treated or those with an unrelated donor, TBI was combined with 30 mg/m^2/day fludarabine for 3 days (N=13). Finally, 7 patients received a combination of fludarabine and cyclophosphamide 1 g/m^2/day for 3 days (Fluda-Cy) because they had previously received 12 Gy TBI as the conditioning regimen for an autotransplant (Table 1). Post-transplant immunosuppression was carried out with oral cyclosporine (CsA, 6 mg/kg *b.i.d.* from day −1 to day 120 or longer in case of an alternative donor or chronic GVHD) and mycophenolate mofetil (MMF, 15 mg/kg *b.i.d.* from day -1 to day 28). Stem cell mobilization, collection and CD8-depletion using the Baxter Isolex 300i© were performed as previously described.[1,2] Patients 1-10 (unmanipulated PBSC) were assigned to receive unmanipulated donor lymphocyte infusions (DLI) (1×10^7 and 2×10^7 CD3$^+$ cells/kg recipient at about day 40 and day 80, respectively) whereas patients 11-26 (CD8-depleted PBSC) were assigned to receive CD8-depleted DLI (1×10^7 and 5 (2 in mismatched transplants) $\times10^7$ CD3$^+$ cells/kg recipient at about day 40 and day 80, respectively).[2] CD8-depletion of DLI was also carried out with Baxter Isolex 300i© as previously reported.[2] DLI were not to be given in the case of an antecedent grade III-IV acute GVHD or active GVHD at time of the scheduled infusions nor in recipients of unrelated transplants. Patients with mixed chimerism on day 100 received a third DLI at about day 120. Chimerism among total peripheral blood white blood cells (WBC), T cells and myeloid cells as well as in unfractionated marrow was assessed on days 28, 60, 100, 180 and 365 after HSCT using fluorescence *in situ* hybridization (FISH) to detect X and Y chromosomes for recipients of sex-mismatched transplants and polymerase chain reaction (PCR)-based analysis of polymorphic microsatellite regions for recipients of sex-matched transplants, as previously reported.[2,3] The probabilities of GVHD and graft rejection were studied by life-table analyses and Wilcoxon rank tests were used for comparisons between groups.

The 100-day actuarial incidence of grade I-IV (II-IV) acute

Table 1. Characteristics of the patients.

	Unmanipulated PBSC	*CD8-depleted PBSC*	*p value*
Number of patients	10	16	
Age [Median (range)]	58 (39-64)	51 (22-62)	NS
Sex (male/female)	7/3	14/2	NS
Disease at transplantation			
NHL beyond CR2	2	5	NS
Metastatic RCC	2	3	
Refractory multiple myeloma	1	2	
MDS	2	1	
HD in CR	0	1	
ALL in CR	0	1	
AML in CR	1	1	
Refractory AML	0	1	
CML in CP	1	1	
CML in AP	1	0	
Prior autologous HSCT (yes/no)	4/6	10/6	NS
Nonmyeloablative conditioning regimen			
2 Gy TBI alone	2	4	NS
2 Gy and fludarabine	5	8	
Fludarabine and cyclophosphamide	3	4	
Donor			NS
HLA identical sibling	5	5	
Related with 1 mismatch	2	2	
HLA identical unrelated	3	9	
Stem cell source			
PBSC	9	16	NS
BM	1	0	
Mean (+SD) cells collected ($\times10^6$)/ kg recipient			
CD34	7.7+3.4	7.7+3.6	NS
CD3	305+99	328+170	NS
CD4	189+67	192+106	NS
CD8	122+41	140+110	NS
Mean (+SD) cells grafted ($\times10^6$)/ kg recipient			
CD34	7.7+3.4	5.5+2.2	0.06
CD3	305+99	142+85	0.001
CD4	189+67	111+63	0.009
CD8	122+41	8+9	0.001

NS: not significant.