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REVIEW ARTICLE

Preemptive Cellular Immunotherapy after T-Cell–Depleted Allogeneic Hematopoietic Stem Cell Transplantation

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ABSTRACT

GVHD is a life-threatening complication of allogeneic hematopoietic stem cell transplantation (HSCT). GVHD is due to donor lymphocytes that are cotransplanted with donor stem cells. These donor lymphocytes are primed by histocompatibility differences between donors and recipients and activated by a cytokine storm caused by the conditioning regimen. The most efficient method for prevention of GVHD consists of T-cell depletion (TCD) of the graft. However, TCD usually leads to an increased risk of leukemia relapse because of the loss of the graft-versus-leukemia (GVL) effect. Several groups have studied the feasibility of preemptive donor lymphocyte infusion (DLI) to lessen the impact of TCD on leukemia relapse. Preemptive DLI is given several weeks to months after the transplantation, ie, after the cytokine storm and after the patient has recovered from conditioning-regimen–related toxicities. After briefly discussing various techniques of TCD of the graft and the efficacy of DLI, this article reviews the first clinical studies evaluating a strategy of TCD of the graft followed by preemptive DLI.

KEY WORDS

Graft-versus-host disease • T-cell depletion • Graft-versus-leukemia effect • Preemptive donor lymphocyte infusion

INTRODUCTION

Graft-versus-host disease (GVHD) is the main complication of allogeneic hematopoietic stem cell transplantation (allo-HSCT) [1-5]. The pathophysiology of acute GVHD includes 3 sequential phases [6,7]. In the first phase, the conditioning regimen damages and activates host tissues. Activated host cells secrete several cytokines and growth factors, leading to increased expression of adhesion and cell surface recognition molecules by host cells and thereby enhancing the recognition of host minor or major histocompatibility antigens by mature donor T-cells. Antigen presentation, as well as activation, proliferation, and differentiation of donor T-cells, occurs in the second phase. Finally, in the third phase, activated T-cells, but also natural killer cells and tumor necrosis factor α, induce organ damage and the clinical manifestations of GVHD [6,7]. The most efficient method for prevention of GVHD is T-cell depletion (TCD) of the graft [4,8-10]. However, this process usually leads to an increased risk of relapse due to the loss of the donor T-cell–mediated graft-versus-leukemia (GVL) effect [4,8-11]. More recently, donor alloreactivity against host tumor cells has been recognized as a major factor of success in allo-HSCT [11-13]. This GVL effect is so potent that some relapses after allo-HSCT can be efficiently (70% long-term complete remissions [CRs] in chronic myeloid leukemia [CML]) treated with donor lymphocyte infusions (DLIs) [14-22]. However, although DLI permits the achievement of CR in the majority of CML patients, results in treatment of acute leukemia or high-grade lymphoma patients are more disappointing, probably because of the high proliferative capacity of these tumor cells [14-16,21]. Because DLIs are particularly effective in inducing CRs if the infusions are performed in early relapse, it may be more efficient to give DLI before relapse at a time when minimal residual disease is still present [14,15,23,24]. Several investigators have studied a strategy combining TCD of the graft followed by preemptive DLI [25-32]. The aim of these approaches is to administer donor lymphocytes after the cytokine storm [7] and after the patient has recovered from conditioning-regimen–related toxicities, thus diminishing the risk of acute GVHD while preserving the GVL effect.

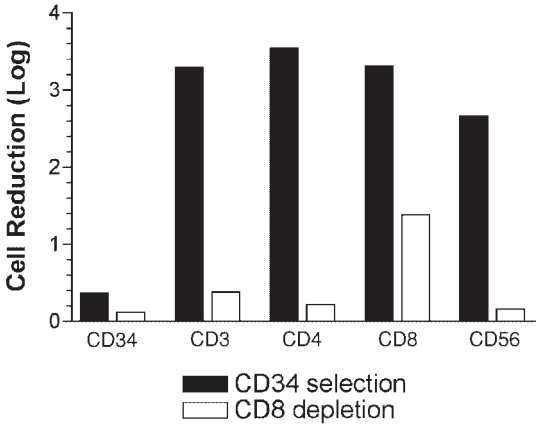

Figure 1. Comparison of 2 methods of T-cell depletion of the graft: CD34 selection (black bar) or CD8 depletion (white bar) [84].

TCD OF THE GRAFT

Since 1981, hundreds of T-cell–depleted transplantations have been performed using various methods of TCD [4]. These trials resulted in low incidence of acute and chronic GVHD and reduced transplantation-related mortality but also increased incidence of graft rejection, delayed immune reconstitution, and posttransplantation lymphoproliferative disorders and increased rates of leukemic relapse, particularly for CML patients [4,8,10,33].

The initial methods of TCD were based on negative selection techniques using physical separation (soybean lectin agglutination, counterflow elutriation, and albumin-gradient fractionation) or antibody-based purging (complement-mediated lysis, immunotoxins, and immunomagnetic beads) [4]. In the 1990s, $CD34^+$ cell selection techniques were developed [34-36]. These techniques permitted the positive selection of hematopoietic stem cells and progenitors, thereby reducing the number of T-cells infused by 3 to 4 logs (Figure 1) [37].

Although both GVHD and GVL reactions are initiated by donor T-cells from the graft, there is evidence that different subsets of T-lymphocytes may be involved in these 2 processes and that it may be possible to separate the GVL effect from GVHD (Table 1 and Figure 2). The Houston group first showed that CD8 depletion combined with cyclosporine prophylaxis could reduce the incidence and severity of acute GVHD without compromising GVL activity [38,39]. This group reported results of a double-blind randomized trial showing that patients included in the CD8-depletion arm experienced significantly less grades II through IV acute GVHD than patients included in the control arm (20% versus 80%, $P < .004$), with a relapse rate (10.5%) similar in the 2 groups [39].

Instead of eliminating all T-cells, some investigators developed techniques in which only alloreactive T-cells are removed from the graft or in which donor T-cells are anergized prior to transplantation. Using this approach, Guinan et al. recently showed that anergization of donor cells by cytotoxic T-lymphocyte (CTL)-associated molecule 4 (CTLA-4) immunoglobulin may reduce GVHD after HLA-mismatched bone marrow transplantation (BMT) [40].

DONOR LYMPHOCYTE INFUSION

From the first evidence in 1990 that leukemic relapse after HSCT may be efficiently treated with DLI [20,41], DLI has become standard therapy to treat relapses after allo-HSCT [14-17,19,20,22,42-44]. Results of the 2 largest multicenter studies showed that DLI can induce CR in 60% to 65% of CML cases and 15% to 38% of acute lymphocytic leukemia (ALL) and myelodysplasic syndrome cases [14,15].

In patients with CML, the response rate is highest when lymphocytes are infused in early cytogenetic relapse (79%) and lowest in accelerated phase or blast crisis (19%) (Table 2) [14,15,45]. It has been speculated that the better response of CML may be explained by its low evolutivity (because the time to response after DLI is often prolonged, the GVL reaction may not have sufficient time to develop in patients with more rapidly progressive disease) and by the fact that dendritic cells, the most potent antigen-presenting cells, are part of the leukemic clone in CML and are capable of inducing a strong T-cell response [18,45]. Moreover, several observations suggest that BCR/ABL expression may increase the susceptibility of leukemic cells to immune cytolysis [46-48].

By contrast, the malignant cells in acute leukemia may be less appropriate antigen-presenting cells and may lead to the induction of anergy rather than antileukemic T-cell response [18]. Some patients with ALL, chronic lymphocytic leukemia, Hodgkin's disease, or lymphoma [49] as well as multiple myeloma [50] have also responded to DLI or discontinuation of immunosuppressive therapy [19,51-53]. Finally, the GVL effect mediated by DLI needs time: the median time to achieve a cytogenetic remission was 85 days (range, 28-241 days) for patients with CML (the time to achieve molecular remission can be prolonged) and 34 days (range, 16-99 days) for patients with AML.

The main complication of DLI is GVHD [14,15]. Acute GVHD occurs in approximately 60% of patients (grade III or IV in approximately 20%) and is significantly correlated with CR [14,15]. Chronic GVHD also occurs in approximately 60% of patients (extensive in 30%) and also correlates with response [14,15,54]. However, CR may be observed in the absence of GVHD, indicating that the GVL response may be independent of the clinical development of GVHD [15,18,55,56]. It is possible to reduce the risk of GVHD without impairing the GVL effect by using CD8 depletion of DLI (Table 2) [55,57,58] or by starting with a low dose of T-cells and increasing the dose in a stepwise fashion in case of no response [18,55,59-61].

Table 1. *Observations Demonstrating that GVL Effects Can Occur in the Absence of GVHD*

1. **AML patients who received identical-twin transplants have an increased probability of relapse (relative risk, 2.58; $P = .008$) compared with patients who received an HLA-identical sibling allograft and did not develop GVHD [98].**
2. **Complete responses after DLI have been observed in the absence of GVHD [14].**
3. **Complete responses after nonmyeloablative stem cell transplantation have been observed in the absence of GVHD [84].**

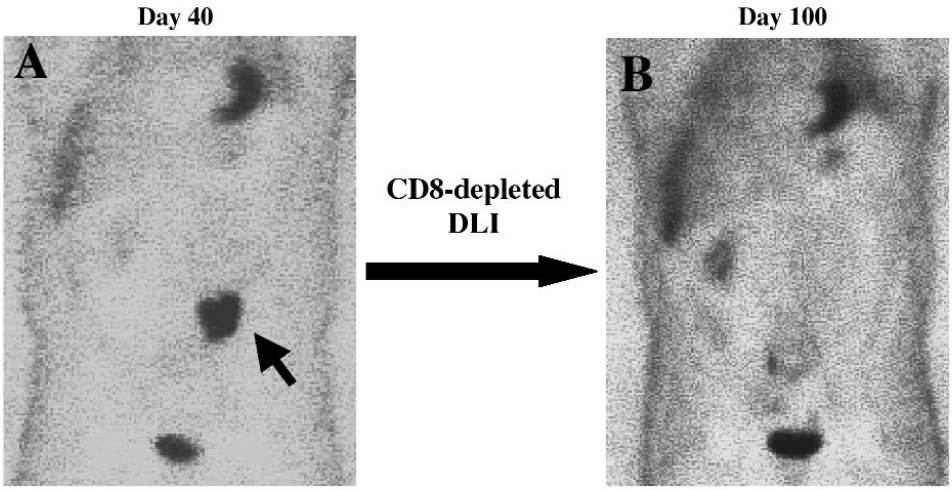

Figure 2. GVL effect in the absence of GVHD after NMSCT in a patient with non-Hodgkin's lymphoma who relapsed after a prior autograft. A, Positron emission tomographic (PET) scan with ^{18}F-fluorodeoxyglucose on day 40 after NMSCT evidenced the persistence of an abdominal adenopathy (arrow). The patient received CD8-depleted DLI on days 40 and 80 after NMSCT and did not experienced any GVHD. B, New PET scan on day 100 demonstrated the achievement of CR.

The other complication of DLI is marrow aplasia, which occurs in about 20% of the patients and more frequently if residual hematopoiesis is at least partially of recipient origin [14,15]. Marrow aplasia resolves spontaneously in 50% of patients and can be reverted in the majority of the other patients by granulocyte colony-stimulating factor (G-CSF) and/or donor hematopoietic stem cells [14,15]. The Seattle group has compared results of G-CSF–mobilized and non-mobilized DLI [62]. They observed no difference between the 2 groups in the incidence of response, GVHD, or aplasia [62], showing that the pathogenesis of aplasia after DLI is not restricted to the destruction of recipient hematopoietic cells but also involves failure of donor hematopoiesis by undefined mechanisms [62].

RATIONALE FOR TCD OF THE GRAFT FOLLOWED BY PREEMPTIVE DLI

The pathophysiology of acute GVHD includes 3 sequential phases [6,7,63,64]. In the first phase, the conditioning regimen damages host tissues. Activated host cells then secrete inflammatory cytokines, leading to increased expression of adhesion and cell surface recognition molecules by host cells and thereby enhancing the recognition of host histocompatibility antigens by donor T-cells [6,7]. Interestingly, the more intensive the conditioning regimen, the more important is the cytokine storm and the higher the risk of GVHD [65]. Thus, after TCD of the graft, delaying the administration of donor lymphocytes after resolution of tissue damage and the cytokine storm may not only delay but circumvent acute GVHD.

A second factor that may confer resistance to GVHD in the TCD setting may be the state of mixed chimerism [66-70]. In rodents, the initial persistence of host hematopoiesis has been demonstrated to decrease the severity of GVHD [71]. Kolb et al. demonstrated in a dog model that DLI given 60 days after the transplantation converted mixed into complete chimerism without any significant acute GVHD, whereas dogs transfused on day 1 or on day 21 after the transplantation developed lethal GVHD [72]. Similar results were recently reported by Pelot et al. in a rodent model [67]. Nonspecific suppressor cells, which recover first from host marrow in mixed chimeras, probably play an important role in this phenomenon [67,72].

PREEMPTIVE DLI

The feasibility of TCD of the graft followed by preemptive DLI was first demonstrated by the Jerusalem group [25]. In a first series of 38 patients whose marrow

Table 2. *Response to DLI in CML patients*

	Kolb et al. [14] Unmanipulated DLI		Collins et al. [15] Unmanipulated DLI		Shimoni et al. [58] CD8-Depleted DLI	
	Evaluable Patients, n	Complete Responses, %	Evaluable Patients, n	Complete Responses, %	Evaluable Patients, n	Complete Responses, %
Cytogenetic relapse	17	14 (82)	3	3 (100)	15	13 (87)
Hematologic relapse	50	39 (78)	34	25 (74)		
Advanced phase	8	1 (13)	18	5 (28)	11	1 (9)

was T-cell depleted with Campath 1M, patients received weekly incremental doses of up to 10^7 T-cells/kg before day 28, and the incidence of acute GVHD was 42% but there was little chronic GVHD. In another group whose marrow was T-cell depleted with varying doses of Campath 1G, 43 patients received 3 incremental infusions of up to 10^7 T-cells/kg either before day 28 (n = 7) or between days 28 and 84 (n = 36). In this heterogeneous group, the crude incidence rates of acute and chronic GVHD were 53% and 40%, respectively. Since these data were reported, several other studies have also investigated the infusion of T-cells a few weeks to a few months after T-cell–depleted transplantation (Table 3) [25-32].

Confirming the results of studies in dogs [72], a study by Barrett et al. suggested the importance of the delay between the transplantation and preemptive DLI [26]. In the Barrett et al. study, 26 patients received 2×10^6 donor T-cells/kg on day 30 and 5×10^7 cells/kg on day 45 (schedule 1) and 12 other patients received 1×10^7 donor T-cells/kg on day 30 (schedule 2). Thus, the total dose of lymphocytes was higher but more delayed in schedule 1. The authors observed a significantly higher incidence of grades II through IV acute GVHD in schedule 2 patients than in schedule 1 patients (100% versus 31.5%, respectively, $P = .02$) [26] (Table 3). However, the study was not randomized and no definitive conclusion can be drawn.

Schaap et al. compared 2 schedules of preemptive DLI given at a median of 150 days after a partially T-cell–depleted BMT [30]. Thirty-five patients without significant GVHD were scheduled to receive DLI (DLI group) and 47 patients who developed grade >1 acute GVHD or chronic GVHD and did not receive DLI served as controls (control group). In the DLI group, the first 6 patients received 7×10^7 $CD3^+$ cells/kg and 5 of these 6 patients experienced acute GVHD (grade I, n = 2; grade III, n = 2; grade IV, n = 4). The next 25 patients received 1×10^7 $CD3^+$ cells/kg and only 8 of them developed acute GVHD (grade I, n = 4; grade II, n = 4), suggesting that the dose of T-cells in preemptive DLI influences the incidence and severity of acute GVHD. However, because this was a single-arm sequential study in which DLIs were given at widely varying time points after transplantation, these results must be confirmed by a prospective randomized trial.

In the Schaap et al. study [30], the 3-year disease-free survival rate was higher in the DLI group (77%) than in the control group (45%) ($P = .024$) because of a lower 3-year probability of relapse (18% versus 46%, $P = .022$), suggesting that preemptive DLI may induce GVL effects. Confirming this finding, Alyea et al. achieved a higher 2-year progression-free survival rate in a cohort of 14 myeloma patients receiving CD8-depleted DLI after a CD6-depleted BMT (65%) compared with a historical cohort of myeloma patients who underwent CD6-depleted BMT without preemptive DLI (41%) [29].

We have recently reported the results of a phase I-II study evaluating the feasibility and toxicity of CD34-selected allogeneic peripheral blood stem cell (PBSC) transplantation followed by preemptive CD8-depleted DLI given in incremental doses (2×10^6, 1×10^7, and 5×10^7 $CD3^+$ cells/kg on days 60, 100, and 140, respectively, [patients 1-13] or 1×10^7 and 5×10^7 $CD3^+$ cells/kg on days 60 and 100, respectively, [patients 14-24]). The 180-day incidence of grades II through IV acute GVHD was 13% for HLA-identical sibling transplantations and 38% for mismatched transplantations [32].

Finally, the Boston group reported at the 2001 American Society of Hematology meeting the results of a randomized trial comparing the outcome of patients receiving unmanipulated (n = 9) or CD8-depleted (n = 9) preemptive DLI 5 to 6 months after they had undergone a T-cell–depleted HSCT. Six of 9 patients receiving unmanipulated DLI developed acute GVHD compared to 0 of 9 recipients of CD8-depleted DLI ($P = .009$). The study also suggested that CD8 depletion did not compromise antitumor activity or conversion from mixed to complete donor chimerism [73].

PERSPECTIVES

T-Cell–Depleted Nonmyeloablative Stem Cell Transplantation Followed by Preemptive DLI

Several studies have now shown that PBSC transplantation after a reduced-intensity nonmyeloablative conditioning regimen resulted in diminished toxicity compared to conventional transplantations, sustained engraftment, and long-term disease-free survival in many patients [68,69,74-82]. However, the transplantation-related mortality ranged from 10% to 20%, mainly because of GVHD and its consequences [80,82,83]. To find a way to decrease the incidence of GVHD after nonmyeloablative stem cell transplantation (NMSCT), we recently investigated the feasibility of NMSCT with CD8-depleted or CD34-selected PBSC followed by preemptive CD8-depleted DLI [84]. Twenty-one patients (median age, 51 years) with high-risk malignancies and an HLA-identical sibling (n = 10) or alternative donor (n = 11) who were ineligible for a conventional transplantation were included. The nonmyeloablative conditioning regimen consisted of 2 Gy total body irradiation (TBI) alone (n = 7), 2 Gy TBI and 90 mg/m^2 fludarabine (n = 9, previously untreated patients), or 3 g/m^2 cyclophosphamide and 90 mg/m^2 fludarabine (n = 5, patients who had previously received ≥12 Gy TBI). Patients 1 through 5 (controls) received unmanipulated PBSC and DLI; patients 6 through 18, CD8-depleted PBSC and DLI; and patients 19 through 21, CD34-selected PBSC followed by CD8-depleted DLI. Post-transplantation immunosuppression was carried out with cyclosporine A and mycophenolate mofetil. Initial engraftment was seen in all patients, but 2 CML patients (13%) later had graft rejection. The actuarial 180-day incidence of grades II through IV acute GVHD was 80% for patients 1 through 5 versus 18% for patients 6 through 21 ($P = .0005$) (Figure 3A). The evolution of white blood cell chimerism is shown in Figure 3B.

REPLACING DLI BY SPECIFIC CTLS

Another method for separating the GVL effect from GVHD could consist of the infusion of specific CTLs instead of DLI. Donor-derived CTLs have been successfully used to restore immunity against cytomegalovirus [85] and Epstein-Barr virus [86-88] after allo-HSCT. Remarkably, neither significant toxicity nor GVHD were observed with

Table 3. *Studies of T-Cell–Depleted Allogeneic Transplantation Followed by Preemptive DLI**

Reference	No. of Patients	Median Age, y	% Alternative Donor	Conditioning Regimen	% High-Risk Patients	Source of Stem Cells	TCD Method	Other GVHD Prophylaxis	Day of CyA Discontinuation	% Patients with Grade II-IV Acute GVHD before DLI	% Patients Receiving I (All) Scheduled DLI
Barrett [26]	26	40	0	Myeloablative	46	PBSC	Elutriation	CyA	180	16	88 (73)
Barrett [26]	12	42	0	Myeloablative	66	PBSC	Elutriation	CyA	180	16	83 (83)
Martino [28]	10	51	0	Myeloablative	70	PBSC	CD34 selection	CyA	60-75	40	40
Alyea [29]	24	46	0	Myeloablative	83	BM	CD6 depletion	None	—	21	58
Schaap [30]	35	43	0	Myeloablative	37	BM	Counterflow centrifugation	CyA	80	0†	{100}†
Nakamura [31]	51	37	0	Myeloablative	53	PBSC	CD34 selection	CyA	130	12	86 (63)
Baron [32]	24	46	33	Myeloablative	50	PBSC	CD34 selection	CyA	180	17	79 (67)

	Manipulation of DLI	DLI No. 1		DLI No. 2		DLI No. 3		Overall % Patients with Grade II-IV Acute GVHD after DLI	%Patients with Chronic GVHD (Extensive Chronic GVHD)	% Overall Survival (Follow-up, mo)
		Day	Dose‡	Day	Dose‡	Day	Dose‡			
Barrett [26]	None	30	2×10^6	45	5×10^7	—	—	32	46 (8)	46 (24)
Barrett [26]	None	30	1×10^7	—	—	—	—	100		50 (24)
Martino [28]	None	104	1×10^7	—	—	—	—	25	100 (50)	NR
Alyea [29]	CD8-depletion	180-270	3×10^7	—	—	—	—	NR (§)	NR (§)	57 (24)
Schaap [30]	None	150	7×10^7	—	—	—	—	{66}†	{40 (NR)}†	{80 (30)}†
			1×10^7	—	—	—	—	{16}†	{12 (NR)}†	
Nakamura [31]	None	45	1×10^7	100	5×10^7	—	—	39	54 (18)	51 (18)
Baron [32]	CD8 depletion	60	2×10^6	100	1×10^7	140	5×10^7	28	37 (13)	45 (24)

*Data in { } are not fully reported. CyA indicates cyclosporine A; NR, not reported.

†Fifty-seven percent of the patients were excluded from analysis because they had experienced grades II through IV acute GVHD before scheduled DLI.

‡$CD3^+$ cells/kg.

§Fifty percent of the patients who received CD8-depleted DLI experienced grades II through IV acute or extensive chronic GVHD.

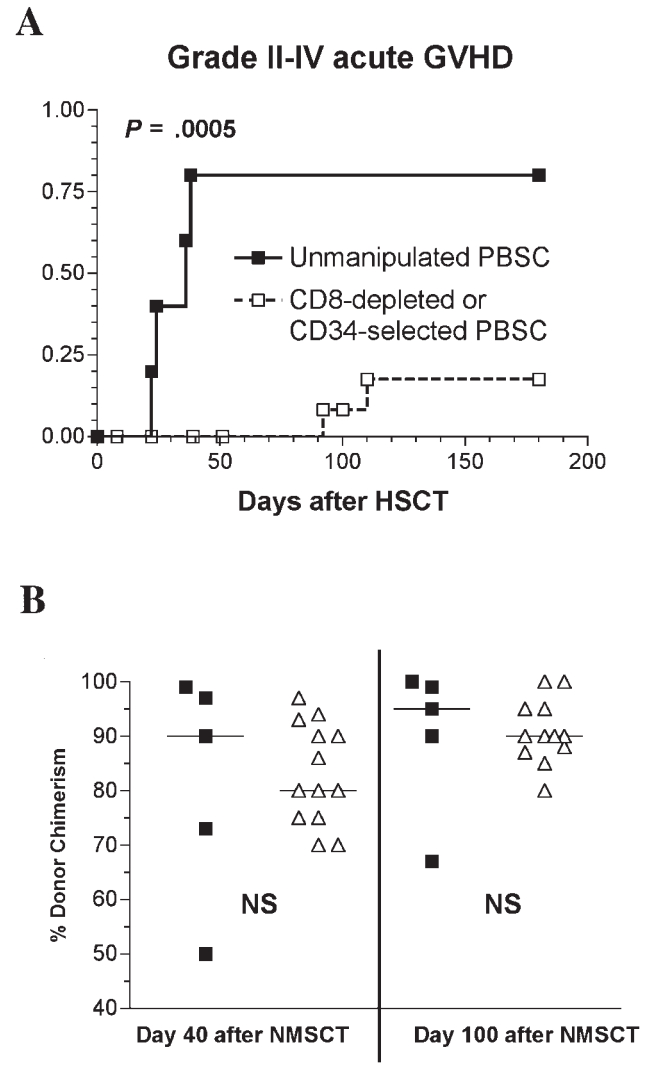

Figure 3. A, Actuarial risk of grades II through IV acute GVHD in patients undergoing an NMSCT with unmanipulated (n = 5) or CD8-depleted (n = 13) or CD34-selected (n = 3) PBSC. The relative risk was 10.8 (95% confidence interval, 5.7-500). B, Donor cell chimerism on days 40 and 100 after NMSCT with unmanipulated (closed squares) or CD8-depleted (open triangles) PBSC.

this early posttransplantation cell immunotherapy. The infusion of donor-derived specific CTLs against specific antigens such as minor histocompatibility antigens (mHA) preferentially expressed in the hematopoietic system [89,90], tumor-specific antigens [91], or antigens overexpressed in tumor cells (such as proteinase 3 [92,93] or WT-1) [94] all represent promising methods of immune cell therapy. In the littermate dog model, the Seattle team has shown that mHA-sensitized DLI (contrary to unmanipulated DLI) can reverse mixed to complete donor chimerism with a low incidence of GVHD [95]. In humans, the Leiden group reported the achievement of CR in a patient with accelerated-phase CML by treatment with leukemia-reactive CTLs [96,97]. Other studies assessing the adoptive infusion of mHA-specific donor-derived CTLs to patients with posttransplantation leukemic relapse are currently in progress [80].

CONCLUSION

Although several authors have demonstrated the feasibility of TCD of the graft followed by preemptive DLI, 2 important questions remain unresolved: (1) Does this approach decrease the incidence of GVHD? (2) Does this approach improve disease-free survival? Prospective randomized trials comparing transplantation of unmanipulated PBSC to that of T-cell–depleted PBSC followed by preemptive DLI are needed to answer these 2 important questions.

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