Rapid Engraftment Without Significant Graft-Versus-Host Disease After Allogeneic Transplantation of CD34+ Selected Cells From Peripheral Blood

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We have prospectively evaluated the feasibility and results of the biotin-avidin immunoadsorption method (Ceprate SC system) for a phase I/II study of T-cell depletion of granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood progenitor cells (PBPC) for allogeneic transplantation. Twenty consecutive patients, median age, 40 years (21 to 54) and diagnoses of chronic myeloid leukemia in chronic phase (n = 5), acute myeloblastic leukemia (n = 7), acute lymphoblastic leukemia (n = 2), chronic myelomonocytic leukemia (n = 1), refractory anemia with excess of blasts in transformation (n = 3), histiocytosis X (n = 1), and chronic lymphocytic leukemia (n = 1), were conditioned with cyclophosphamide (120 mg/kg) and total body irradiation (13 Gy; 4 fractions). HLA identical sibling donors received G-CSF at 10 μg/kg/d subcutaneously (SC); on days 5 and 6 (19 cases) and days 5 to 8 (1 case) donors underwent 10 L leukapheresis. PBPC were purified by positive selection of CD34+ cells using immunoadsorption biotin-avidin method (Ceprate SC) and were infused in the patients as the sole source of progenitor cells. No growth factors were administered post-transplant. The median recovery of CD34+ cells after the procedure was of 65%. The median number of CD34+ cells infused in the patients was 2.9 (range, 1.5 to 8.6) × 10^6/kg. The median number of CD3+ cells administered was 0.42 × 10^6/kg (range, 0.1 to 2). All patients engrafted. Neutrophil counts >500 and >1,000/μL were achieved at a median of 14 days (range, 10 to 18) and 15 days (range, 11 to 27), respectively. Likewise, platelet counts >20,000 and >50,000/μL were observed at a median of 10 days (range, 6 to 23) and 17 days (range, 12 to 130), respectively. Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine plus methylprednisolone. No patient developed either grade II to IV acute or extensive chronic GVHD. After a median follow-up of 7.5 months (range, 2 to 22) three patients have relapsed, and one of them is again in hematologic and cytogenetic remission after infusion of the donor lymphocytes. Two patients died in remission: one on day +109 of pulmonary aspergillosis and the other on day +251 of metastasic relapse of a previous breast cancer. Sixteen of the 20 patients are alive in remission after a median follow-up of 7.5 months (range, 2 to 22). In conclusion, despite the small number of patients and limited follow-up, it appears that this method allows a high CD34+ cell recovery from G-CSF mobilized PBPC and is associated with rapid engraftment without significant GVHD, and with low transplant related mortality.

THE SOURCE OF hematopoietic progenitor cells for both autologous and allogeneic transplants has been changing over the years. Currently, the majority of autologous transplants are performed by using peripheral blood progenitor cells (PBPC), with this source also being increasingly used in allografts.[1-5] Potential advantages of allogeneic PBPC transplants (allo-PBT) over allogeneic bone marrow transplants (allo-BMT) include a safer method for the donor and a quicker neutrophil and platelet engraftment for the recipient.[6]

Graft-versus-host disease (GVHD) remains the major cause of morbidity and mortality after allo-BMT.[7] Ex-vivo T-cell depletion (TCD) from human BM allografts is the most effective prophylaxis of GVHD.[8] Recently, a simple and rapid technique based on the positive selection of CD34+ antigen expressing cells has become available as a tool for TCD.[9] With this method, CD34+ cells from marrow or peripheral blood grafts are selected, with this resulting in a passive elimination of T lymphocytes. This approach has been successfully used to deplete T cells from granulocyte colony-stimulating factor (G-CSF) mobilized PBPC.[10]

In general, TCD in allo-BMT, effectively prevents the mortality from GVHD[11]; however, the overall survival may be compromised by increased rates of graft failure and relapse.[12] One of the reasons for graft failure in TCD transplant is the loss of CD34+ cells during graft manipulation.[13] Moreover, a factor determining TCD transplant-related mortality is the quantity of CD34+ cells transfused to the patients.[14] For these reasons, survival of patients submitted to TCD allografts might be improved by using a source with a higher number of progenitor cells, such as G-CSF– mobilized PBPC.

We have used the biotin-avidin immunoadsorption technique (Ceprate SC) for TCD of G-CSF mobilized PBPC for allogeneic transplantation (allo-PBT/CD34+) in 20 consecutive patients. Although the number of patients is small and the follow-up limited, our data suggest that the infusion of positively selected allogeneic blood CD34+ cells results in a rapid engraftment without significant GVHD and with low treatment-related mortality.

MATERIALS AND METHODS

Donors. HLA identical sibling donors (8 women and 12 men; median age, 40 years; range, 18 to 61) received filgrastim (Amgen, Thousand Oaks, CA) at 10 μg/kg/d subcutaneously for 5 (cases 1 and 3 through 20) or 7 (case 2) days. On days 5 and 6 (cases 1, 3 through 20) and days 5, 6, 7, and 8 (case 2), donors underwent 10 L (3 hours) leukapheresis with the Fenwall CS-3000 plus separator (Baxter, Deerfield, IL). Leukaphereses were initiated 12 to 18 hours following the fourth dose of G-CSF and were performed in all cases through two peripheral veins accesses.

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Immunophenotypic studies. Immunophenotyping was performed with a FACScan flow cytometer (Becton Dickinson Immunocytometry System, San Jose, CA). The antibodies used, all from Becton Dickinson, were: 8G12-PE (HPCA-2/CD34), HLe-1–FTIC (CD45), Leu-M3–PE (CD14), Leu-4–FTIC, (CD3), Leu-3a–FTIC, (CD4), Leu-2a–PE (CD8), Leu-19–PE (CD56) and PE- and FTIC-conjugated irrelevant isotype-specific antibodies. $CD34^+$ cells were quantified as follows: from the analysis of forward and 90° side scatter, a gate was established to include all lymphocytes and mononuclear cells, excluding platelets and red blood cells. Controls were set on the basis of those gated cells. A second gate was established on high $CD34^+$ immunofluorescence and low side-scatter. To analyze the number of $CD34^+$ cells, a total of 300,000 events was acquired from peripheral blood and apheresis product samples and 10,000 events from the immunoselected $CD34^+$ cell samples.

Positive selection of $CD34^+$ cells. The first leukapheresis product was stored at 4°C overnight without shaking and pooled the day after with the second apheresis product. PBPC were then purified by positive selection of $CD34^+$ cells using an immunoadsorption biotin-avidin column (Ceprate SC System; CellPro Inc, Bothell, WA). The final product was infused, without previous cryopreservation, directly to the patients as the sole source of progenitor cells. In one case (no. 2), showing a very poor mobilization, two further aphereses were processed in the same manner.

Patients. Twenty patients (8 women, 12 men; median age, 40 years; range, 21 to 54) with diagnoses of chronic myeloid leukemia (CML) in first chronic phase (CP1) (n = 5), acute myeloblastic leukemia (AML) in first complete remission (CR1) (n = 5), acute lymphoblastic leukemia (ALL) in CR1 (n = 2), AML in first relapse (n = 2), AML secondary to chronic myelomonocytic leukemia (CMML) and refractory to ICE (idarubicin 12 mg/m^2 × 10 days, cytarabine 100 mg/m^2 and etoposide 100 mg/m^2 × 10 days) therapy (n = 1), refractory anemia with excess of blasts in transformation (RAEBt) (n = 3), histiocytosis X in relapse after six cycles of CHEP (cytoxan 750 mg/m^2 × 1 day, adriamycin 45 mg/m^2 × 1 day, etoposide 5 mg/m^2 × 1 day and prednisone 100 mg/m^2 × 5 days) therapy (n = 1) and advanced chronic lymphocytic leukemia (n = 1) have been consecutively included in this prospective protocol. Thus, 15 patients were at early stage of their disease (CR1, CML CP1) at the moment of transplant and five at advanced phase (disease chemoresistant or in relapse). Patients were conditioned with cyclophosphamide (120 mg/kg administered over 2 days) and fractionated total body irradiation (total dose, 13 Gy; fractionated over 4 days in four fractions of 325 cGy each) delivered by a single linear accelerator at a dose rate of 5 to 7 cGy/min. The day of $CD34^+$ selected transfusion was considered as day 0. No growth factors were administered posttransplant. Time to neutrophil engraftment was assessed by determining the number of days after day 0 for patients to achieve 0.5×10^9/L. Time to platelet engraftment was assessed by determining the number of days after day 0 to maintain an untransfused platelet count of 20×10^9/L or greater. GVHD prophylaxis consisted of cyclosporine A, starting at 1.5 mg/kg intravenously every 12 hours from day -1 and adjusted to maintain therapeutic blood levels of 250 to 400 ng/mL and methylprednisolone 0.5 mg/kg days +7 to +14, 1 mg/kg days +15 to +28, and tapering the doses afterwards. Cyclosporine dose was reduced if renal function decreased, regardless of cyclosporine blood levels. Cyclosporine was given orally as soon as oral mucositis disappeared and was stopped by day 180 after transplantation. Pentamidine-isethionate inhalations were used as prophylaxis for *Pneumocystis carinii* pneumonia. Acyclovir, 250 mg/m^2, was given intravenously twice daily between day 1 and 28 postransplantation to patients 5 to 20. Cytomegalovirus (CMV) antigenemia was determined weekly (days 0 to +100) by immune alkaline phosphatase (APAAP) technique (monoclonal antibody pp 65) in blood samples. The diagnosis and grading of acute and chronic GVHD was established according to the Seattle criteria.[15] Chronic GVHD was defined if GVHD was present after day 90. Skin biopsies were taken from all patients with skin rashes. Regimen-related toxicity (RRT) was graded using Bearman criteria.[16] Transplant-related mortality was defined as death due to causes other than neoplastic relapse.

Documentation of engraftment. Molecular analysis of engraftment was done using polymerase chain reaction (PCR) amplification of short tandem repeats (PCR-STR) in peripheral blood samples. The STRs analyzed were: c-FES/FPS, von Willebrand factor (vWF), HumTH01, and F13A. PCR-STR studies were done in each patient-donor pair before transplantation to select the most suitable STR locus, which was studied at +28 day postransplantation and every 2 months afterwards.

Control group. A group of patients (n = 19) allotransplanted with unmanipulated apheresis product from HLA-identical donors from our institution was assembled for comparison. These patients, with a median age of 34 years (range, 18 to 41), were diagnosed with CML (n = 1), AML (n = 7), ALL (n = 8), RAEBt (n = 1), and non-Hodgkin's lymphoma (NHL) (n = 2). Five patients were at early stage of their disease (CR1) at the moment of transplant, seven at intermediate stage (CR2, CML accelerated phase), and seven at advanced phase (disease chemoresistant or in relapse). Patients were conditioned with cyclophosphamide (120 mg/kg) and total body irradiation (12 Gy; four fractions), and GVHD prophylaxis consisted of cyclosporine A and methotrexate (15 mg/m^2 intravenously at day 1 and 10 mg/m^2 on days 3 and 6). No growth factors were administered posttransplant.

Statistical methods. Student's *t*-test with two-sided *P* values, Chi-Square (Pearson) with continuity correction, and Fisher's exact test (two-tail) were used when indicated. All statistical studies were performed using the SPSS 6.1.3 for Windows (1994) statistical software (SPSS Inc).

This study was approved by the local Ethic Committee and by the Spanish Health Department. Detailed written informed consent was obtained from all donors and patients before beginning the procedure.

RESULTS

Donors. G-CSF administration was well-tolerated except for moderate bone pain occurring in all donors. The blood leukocyte counts of the donors increased from a median of 5.4×10^9/L (range, 3.8 to 6.8) before mobilization to 29×10^9/L (range, 13.2 to 48.2) at day 5 of G-CSF administration. The number of circulating $CD34^+$ cells increased from a median of 3.9×10^6/L (range, 0.8 to 6.6) to a median of 51.6×10^6/L (range, 25 to 74) at day 5 of G-CSF administration. Leukaphereses were performed without complications. No donor required a central-venous line.

Positive selection of $CD34^+$ cells. In 19 cases, two PBPC collection procedures were performed and one Ceprate immunoadsorption column was used. In one case showing a poor G-CSF mobilization of PBPC (no. 2), four collection procedures were performed and two columns were used. The number of $CD34^+$ and $CD3^+$ cells before and after immunoselection is shown in Table 1. The median number of $CD34^+$ cells × 10^6/kg before and after the procedure was of 4.9 and 2.9, respectively. The median recovery of $CD34^+$ cells after the procedure was of 65%. The PBPC preparations for transplantation contained a mean of 64% $CD34^+$ cells. The number of $CD34^+$ cells infused to the patients ranged from 1.5 to 8.6 × 10^6/kg. Four patients received a quantity of $CD34^+$ cells slightly lower than 2 million per kilogram; nine patients received between 2 and 4 million per kilogram,

Table 1. Donor, Graft, and Patient Characteristics

Patient No.	Donors	Grafts				Patients		Engraftment				GVHD		Infections				Follow-up		
		CD34		CD3				ANC		Patient				CMV						
	Age/Sex	pre	post	pre	post	Age/Sex	Diagnosis	0.5	1	20	50	Acute	Chronic	D	P	Reactivation	Bacteria	Last visit	Status	Relapse
1	51/M	6.8	4.6	363	0.45	47/M	CML 1CP	17	22	11	23	0	No	−	+	d +49		d +674	Alive	d+302 (Hematol cytog remission after DLI)
2	24/F	1.8	1.9	508	0.4	22/M	AML 1CR	14	15	12	20	0	Limited	−	−	No	S viridans	d +645	Alive	
3	32/M	7.4	7	280	0.5	37/M	AML 1CR	12	13	7	18	0	No	−	+	d +41		d +604	Alive	
4	18/F	3.6	2	476	0.3	21/M	ALL-T 1CR	12	14	14	36	0	No	+	−	d +45		d +528	Dead	d+230. Resistant to chemotherapy
5	61/M	4.5	4.4	165	0.3	46/M	RAEBt	13	15	9	19	1	No	+	+	d +44		d +442	Alive	
6	35/F	10	4.4	644	0.3	25/F	AML 1CR	12	13	10	14	I	No	+	+	d +45/+75		d +424	Alive	
7	38/M	6.6	6.6	836	0.6	41/F	CML 1CP	14	27	9	13	I	No	−	+	d +44/+105	L monocyt	d +251	Dead	of a previous breast cancer
8	42/M	3.8	2.5	396	0.3	38/F	CLL C (IV)	13	15	11	14	0	No	+	+	No		d +333	Alive	
9	39/M	—	1.5	438	0.5	37/F	CMML-AML	17	21	13	14	0	No	+	+	No		d +312	Dead	d +120. DLI
10	32/F	3.5	2.8	302	0.6	37/F	Histiocyt X	17	19	23	130	I	No	−	−	No		d +235	Alive	
11	34/M	11	8.6	366	0.5	37/M	AML 1CR	11	11	6	12	0	No	NA	−	No		d +228	Alive	
12	47/M	3.1	2	277	0.4	51/M	RAEBt	17	21	23	103	0	No	NA	+	d +57	L monocyt	d +221	Alive	
13	28/M	6.8	4.7	667	1	32/M	RAEBt	14	17	15	17	0	No	+	+	d +34	K pn/P Aer	d +186	Alive	
14	40/M	12	3.6	611	2	43/F	CML 1CP	14	16	8	14	I	No	+	+	d +55		d +109	Dead	
15	51/M	4.9	2.4	238	0.4	48/M	CML 1CP	18	19	11	19	0	No	−	+	d +44		d +151	Alive	
16	29/F	5.7	2.9	611	0.8	32/F	CML 1CP	15	16	8	14	0	No	−	−	No		d +136	Alive	
17	51/F	4.9	3.17	475	0.3	53/M	ALL 1CR	10	14	7	20	0	−	−	−	d +36		d +81	Alive	
18	52/F	4.3	2.6	550	0.3	54/F	AML 1Rel	13	13	7	13	0	−	+	−	No		d +74	Alive	
19	49/M	5.8	1.8	446	0.5	44/M	AML 1Rel	13	15	8	16	0	−	+	−	No		d +67	Alive	
20	54/F	2.4	1.46	160	0.1	53/M	ALL 1CR	12	13	10	16	I	−	−	−	No		d +60	Alive	
Median	40	4.9	2.9	442	0.42	40		14	15	10	17									

Abbreviations: CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; CLL, chronic lymphocytic leukemia; CMML, chronic myelomonocytic leukemia; RAEBt, refractory anemia with excess of blasts in transformation; Histiocyt. X, Histiocytosis X; Rel, relapse; GVHD, graft-versus-host disease; DLI, donor lymphocyte infusion; (D) donor and (P) patient seropositive. ANC, absolute neutrophil count and platelets × 10E9/L; NA, not available; values are expressed as 10E6/kg for $CD34^+/CD3^+$ cells; K.pn, Klebsiella pneumoniae; P. aer. Pseudomonas aeruginosa.

and in seven patients, this quantity was equal or superior to 4 million per kilogram. The mean number of $CD34^+$ cells infused to the patients was of 3.6×10^6/kg. This figure compares favorably with the mean number of 2.8×10^6/kg $CD34^+$ cells harvested from BM in a group of 36 donors from our institution and to the mean of 0.9×10^6/kg $CD34^+$ cells obtained from the same group when marrow was TCD by means of counterflow centrifugation (data not shown). Data for $CD3^+$ cells from collections showed approximately three logs depletion after $CD34^+$ selection. The number of $CD3^+$, $CD4^+$, $CD8^+$, and $CD56^+$ cells ($\times 10^6$/kg) infused to the patients ranged from 0.1 to 2, 0.13 to 1.18, 0.18 to 0.91, and 0.02 to 0.8, respectively. The median number of $CD3^+$ cells administered was 0.42×10^6/kg. In 17 cases the final graft contained between 0.1 and 0.6×10^6/kg T cells. In three cases graft contained 0.8, 1, and 2 million of $CD3^+$ cells, respectively. Once the leukapheresis procedure was finished, the whole process of pooling two PBPC collections, washes, and immunoselection lasted a mean of 3 hours.

Patients. $CD34^+$ selected cells engrafted in all patients, with a neutrophil recovery to 0.5×10^9/L or greater at a median of 14 days postransplantation (range, 10 to 18) and to 1×10^9/L or greater at a median of 15 days (range, 11 to 27). An untransfused platelet count of 20×10^9/L or greater was obtained at a median of 10 days postransplantation (range, 6 to 23), and higher than 50×10^9/L at a median of 17 days postransplantation (range, 12 to 130). All cases showed complete and sustained engraftment. Growth factors were not required in any of the patients posttransplant. PCR analysis of short tandem repeats has shown apparent complete donor chimerism in eight of 10 patients analyzed thus far, in six of them with a follow-up of more than 8 months. Mixed chimerism was apparent in two (nos. 1 and 4) of the 10 cases. Patient no. 1, with a diagnosis of CML, showed low level mixed chimerism at day +28 with progressive mixed chimerism at 2, 4, and 10 months. Quantitative PCR of BCR/ABL transcript showed an increase in its number after the second month postransplant. Patient no. 4, with a diagnosis of ALL, exhibited mixed chimerism at 1 and 3 months, but only donor cells were seen after 6 months (manuscript in preparation). All patients received cyclosporine and methylprednisolone as planned. In no case was acute GVHD clinical grade II to IV observed. Six patients developed acute GVHD clinical grade I (skin), five of them responded quickly to steroid treatment and one required antilymphocyte globulin therapy (case no. 7). Sixteen patients have been at risk for chronic GVHD: after a median follow up of 10 months, only one presented a localized cutaneous GVHD at 102 days posttransplant, with a good response to methylprednisolone, which was promptly stopped without GVHD reactivation. All patients were discharged from the hospital within 24 days of transplant. Eighteen of the 20 patients developed grade I or II RRT: 6 in 1 organ, 9 in 2 organs, and 3 in 3 organs. Toxicity was most common in the mouth (stomatitis), liver, bladder, and gastrointestinal tract (Table 2). No patient developed grade III to IV toxicity.

Four patients developed serious bacterial infections (sepsis by *Streptococcus viridans* in 1, *Listeria monocytogenes* in 2, and *Klebsiella pneumoniae*/*Pseudomonas aeruginosa* in 1), which were successfully treated with antimicrobials.

Table 2. Regimen-Related Toxicity According to Organ System

	Grade 0		Grade I		Grade II	
	n	%	n	%	n	%
Mucosa	5	25	3	15	12	60
Bladder	11	55	5	25	4	20
Liver	13	65	6	30	1	5
Gut	18	90	2	10	0	0
Heart	20	100	0	0	0	0
Kidneys	20	100	0	0	0	0
Lungs	20	100	0	0	0	0
CNS	20	100	0	0	0	0

Abbreviation: CNS, central nervous system.

Eleven patients received preemptive treatment with ganciclovir (5 mg/kg intravenously twice a day for 14 days) or foscarnet (60 mg/kg intravenously twice a day for 14 days) for CMV antigenemia, which was observed at a median of 44.5 days (range, 34 to 57) postransplantation. All became negative by antigen test at the end of treatment. Two patients (nos. 6 and 7) relapsed (days +75 and +105, respectively) and required further treatment. In most cases, a moderate pancytopenia was observed during CMV antigenemia, which required neither transfusions nor growth factor administration. No patient developed features of CMV disease.

After a median follow-up of 7.5 months (range, 2 to 22), three patients have relapsed. One patient (no. 4) was a young boy with ALL of T lineage and bulky disease at the time of diagnosis; at relapse the disease was resistant to further chemotherapy and the patient eventually died 14 months after transplantation. A second case (no. 1) was a CML patient who experienced a hematologic relapse 10 months after transplantation; this patient is at present in hematologic and cytogenetic remission (of 52 metaphases, 100% are Ph-) after the infusion of donor lymphocytes; quantitative PCR shows a very low number of BCR/ABL transcripts (100/μg of RNA). A third patient (no. 9) diagnosed with CMML-AML resistant to intensive chemotherapy relapsed 5 months after transplantation and she did not respond to donor lymphocyte transfusion and eventually died. Two patients died in remission: one (no. 7) died of pulmonary aspergillosis on day +109 and the other (no. 14) died 8 months after transplant of metastatic relapse of a previous breast cancer. Overall, 16 of the 20 patients are alive in remission.

Results of this allo-PBT/$CD34^+$ group with those from a control group of allo-PBT with unmanipulated apheresis are shown in Table 3. This group of allo-PBT/$CD34^+$ was older than the allo-PBT group ($P = .01$) and there were no differences either in the parity donor/recipient or in the stage of the disease between both groups. The group of allo-PBT/$CD34^+$ had significantly quicker kinetics of engraftment, probably due to the use of methotrexate for GVHD prophylaxis in the allo-PBT group, and a lower incidence of both acute ($P = .04$) and chronic ($P = .005$) GVHD.

DISCUSSION

Ex vivo TCD of the marrow harvest is the most effective prophylaxis of GVHD.[11] However, its clinical benefit is controversial because survival in recipients of TCD allografts is compromised by increased rates of graft failure and relapse.[12]

Table 3. Characteristics of Patients, Graft Content, Kinetics of Engraftment, and GVHD in Allo-PBT/CD34+ and Allo-PBT

Patients	Age	High-Risk	Female (D) to male (R)	$CD34^+$ Cells	$CD3^+$ Cells	Engraftment: Neutrophils 0.5	Engraftment: Neutrophils 1	Engraftment: Platelets 20	Engraftment: Platelets 50	GVHD: Prophylaxis	GVHD: Acute II-IV	GVHD: Chronic Extensive	Median Follow-up
Allo-PBT/ $CD34^+$ n = 20	40 (21-54)	5/20	4/20	2.9 (1.8-8.6)	0.42 (0.1-2)	14 (10-18)	15 (11-27)	10 (6-23)	17 (12-130)	CsA-Pred	0/20	0/16 at risk	7.5 mo (2-22)
Allo-PBT n = 19	34 (18-47)	7/19	3/19	4.4 (1.8-11.9)	269 (128-579)	17 (12-22)	19 (15-33)	14 (0-76)	28.5 (10-120)	CsA-MTX	4/18 at risk	5/14 at risk	7.8 mo (0.5-23)
	$P = .01$	P = NS	P = NS	$P = .048$	$P < .001$	$P = .001$	$P = .01$	$P = .04$	P = NS		$P = .04$	$P = .005$	P = NS

Abbreviations: Allo-PBT, allogeneic peripheral blood transplantation with unmanipulated apheresis; Allo-PBT/$CD34^+$, allogeneic peripheral blood transplantation with $CD34^+$ positive selection. Age of patients, engraftment kinetics, and $CD34^+$ and $CD3^+$ cells (10E6/kg recipient) are given as median and range; neutrophil count and platelets × 10E9/L, (D) donor; (R) recipient; CsA, cyclosporine A; MTX, methotrexate; Pred, prednisone; high risk, number of patients at advanced disease; NS, not significant.

Three approaches have been suggested to try to overcome these complications: (1) use of more intensive pretransplant immunosuppression,[17-19] (2) partial TCD instead of total TCD,[20] and (3) increase the number of stem cells infused.[14,21,22] Clinical application of the two former measures has shown a decrease of both graft failure and leukemic relapse rates.[17,23-26] According to this, the cases herein presented received TBI 13 Gy (325 cGy per fraction) as conditioning regimen. Moreover, apheresis products were not totally but partially T-cell depleted because, despite strong T-lymphocyte elimination, they contained a median of 0.42 $\times 10^6$/kg T lymphocytes for recipient. According to previous studies, this amount of T cells may facilitate engraftment while maintaining a certain graft-versus-leukemia effect.[17,20,23,27]

To increase the number of hematopoietic progenitor cells infused to the patients is another desirable goal when a TCD procedure is considered.[14,22] To that purpose, the number of harvested stem cells can be dramatically enhanced by using G-GSF mobilized PBPC.[28,29] In this regard, the biotin-avidin immunoadsorption method has yielded high $CD34^+$ cell recoveries from G-CSF mobilized PBPC in this and in other studies.[30] In our series, the number of $CD34^+$ cells infused to the allo-PBT/$CD34^+$ patients in comparison to an allo-BMT/TCD (elutriation) group from our institution was fourfold higher, and the mean quantity of $CD34^+$ cells infused (3.6×10^6/kg) was higher than in unmanipulated marrow grafts (2.8×10^6/kg). Infusion of this large number of hematopoietic progenitor cells might improve the quality of engraftment and survival in TCD transplants.[14] The speed of the engraftment observed in this allo-PBT/$CD34^+$ group was very rapid, similar to that observed in patients transplanted with unseparated allo-PB[2-5] and in those transplanted with positively selected autologous blood $CD34^+$ cells.[30] In addition, the engraftment was sustained, with apparently full donors' chimerism in most of the cases analyzed and there has been no late graft failure up to now. The minimum dose of $CD34^+$ cells (1×10^6/kg) required in our study was lower than the 4×10^6/kg employed by others for allo-PBT.[2-4] This quantity was chosen as we did not observe any graft failure in allo-BMT/TCD patients transplanted from HLA-identical sibling donors with a similar number of $CD34^+$ cells (data not shown). In addition, it allowed us to reduce the number of aphereses and columns required, as well as to minimize the absolute number of $CD3^+$ cells present in the final $CD34^+$ selected graft.

In this study, positive selection of $CD34^+$ cells was shown to be an efficient tool for TCD of PBPC collections. Thus, an aproximately three-log TCD was achieved after $CD34^+$ immunoselection, with a median number of T lymphocytes present in the final graft of 0.42×10^6/kg. Previous reports infusing a similar quantity of T cells for allo-BMT have been associated with a decreased, but still significant, grade II to IV acute GVHD,[17,27] despite posttransplant cyclosporin A administration. In line with this, acute GVHD clinical grade II to IV of a historical control group of allo-BMT/TCD (n = 36) from our institution was 18% (data not shown). For this reason our patients were given a more intensive posttransplant immunosuppression regimen (ie, cyclosporine A plus prednisone). Remarkably, no patient developed grade II to IV acute GVHD. The need for posttransplant immunosuppression with either cyclosporine plus prednisone or cyclosporine plus methotrexate would be further supported by cases of severe acute GVHD in allo-PBT/$CD34^+$ when cyclosporine alone was used for posttransplant GVHD prophylaxis,[31-33] although differences in the recipient age, phase of the disease, and the total number of T lymphocytes infused might also account for the higher incidence of acute GVHD in these allo-PBT/$CD34^+$ groups[31-33] (Table 4). Finke et al[34] have recently shown a low incidence of acute GVHD in an allo-PBT/$CD34^+$ series with similar characteristics to ours. Although follow-up is too short to adequately assess chronic GVHD, it must be noted that only one of the 16 patients with a follow-up more than 90 days developed chronic GVHD, which was limited and rapidly reversible with prednisone. If this is confirmed after longer follow-up, it would be of interest because whereas the incidence of acute GVHD in allo-PBT seems to be similar to that seen in allo-BMT,[2-4,35] preliminary results suggest that the incidence of chronic GVHD might be increased.[36-38] There is also the possibility that the absence of severe GVHD in this group of patients was not due to the T-cell depletion, but to the fact that only 35% of the patients were older than 45, only 25% had advanced disease, and that all of them received HLA-matched sibling donor transplants. In an attempt to further analyze this issue, a comparison of this allo-PBT/$CD34^+$ group with 19 allo-PBT patients with unmanipulated apheresis from HLA-matched sibling donors from our institution was performed. The incidence of both acute and chronic GVHD was significantly lower in the present allo-PBT/$CD34^+$ group ($P = .04$ and $P = .005$, respectively) (Table 3).

Finally, patients receiving T-cell–depleted BMT for hematologic malignancies have an increased risk of early CMV

Table 4. Results of Allo-PBT/CD34$^+$ Series

Author/Reference	No.	Age	High-Risk	Device	CD34$^+$ Cells	CD3$^+$ Cells	GVHD Prophylaxis	G-CSF Postrans.	Engraftment ANC 0.5	Engraftment Plat 20	aGVHD III-IV (%)	cGVHD Extensive (%)	Median Follow-up (mos)
Link et al[32]	5	27	20	CellPro	7.78	1.11	CsA	Yes	10	18	80	0	13
	5	37	20	CellPro	7.56	1.11	CsA-MTX	Yes	14	30	20	0	9.6
Bensinger et al[33]	5	58	80	CellPro	10.17	1.08	CsA	No	13	11	60	66	7.9
	11	48	91	CellPro	8.81	0.68	CsA-MTX	No	19	25	33.3	20	3.6
Finke et al[34]	10	40	50	CellPro	4.1	0.42	CsA n = 9 CsA-Pred n = 1	Yes	10	16	0	0	4.5
Brugger et al[42]	10	42	20	CellPro	3.6	0.53	CsA-MTX n = 10	NA	14	19	0	NA	7.8
Garcia-Conde et al[43]	7	57	57	Isolex	4.8	0.09	CsA-Pred n = 3 CsA n = 4	Yes	10	14.5	0	0	5.2
Urbano-Ispizua et al (this study)	20	40	25	CellPro	2.9	0.42	CsA-Pred n = 20	No	14	10	0	0	7.5

Abbreviations: Allo-PBT/CD34$^+$, allogeneic peripheral blood transplantation with CD34$^+$ positive selection; No. number of patients; High risk, % of patients at advanced disease. Age of patients, engraftment kinetics and CD34$^+$ and CD3$^+$ cells (10E6/kg recipient) are given as median; Absolute neutrophil count (ANC) and platelets $\times$ 10E9/L. NA, not available; aGVHD, acute GVHD, cGVHD, chronic GVHD; CsA, cyclosporine A; MTX, methotrexate; pred, prednisone.

reactivation and progression to fatal CMV disease.[39-41] Of note, although 11 of the 20 patients from our series reactivated CMV, none progressed to CMV disease. A weekly follow-up of CMV antigenemia and an early preemptive treatment with ganciclovir or foscarnet might account for this.

In conclusion, although the number of patients is small and the follow-up limited, it appears that allo-PBT/CD34$^+$ is feasible and results in a rapid and sustained engraftment without severe GVHD. The low transplant-related mortality in this series provides a background to explore this approach in older patients. Finally, the relative merits of allo-PBT/CD34$^+$ and unmanipulated allo-PBT should be further investigated in randomized clinical trials.

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