mean volumes of 140 mL, containing 3.8×10^9 cells were collected per procedure, with the percentage of mononuclear cells ranging from 76 to 92%. Normal saline was added to the collection bag to make a volume of 300 mL, and the final hematocrit was <2% (median: 1.3%). The yielded buffy coat was transferred into a thin plastic bag and then 8-MOP was added to a final concentration of 200 ng/mL; finally, the product was exposed to UVA irradiation (365 nm,2 J/cm^2) and then reinfused into the patient. The schedules described by Rabitsch (two consecutive days every other week for two months, then two consecutive days monthly)[3] and Besnier (twice weekly for 3 weeks, once weekly for 2 weeks and then every other week)[4] were adopted for the first and second patient respectively.

Most data report that ECP is beneficial when adopted early after bone marrow transplantation;[5-10] our experience confirms that ECP is recommendable even after many years of refractory cGvHD, and that good venous access is crucial in order to complete the schedule.

Ettore Biagi,° Paolo Perseghin, Filippo Buscemi,* Maria Dassi,* Attilio Rovelli,° Adriana Balduzzi°*

*°Clinica Pediatrica dell'Università di Milano - Bicocca; *Servizio Trasfusionale, Unità di Aferesi, Ospedale S.Gerardo, Monza, Italy*

Key words

Extracorporeal photochemotherapy, chronic graft-versus-host disease, bone marrow transplantation

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Correspondence

Adriana Balduzzi, M.D., Clinica Pediatrica dell'Università di Milano, Ospedale San Gerardo, via Donizetti 106, 20052 Monza (MI), Italy. Phone: international +39-039-2333513 – Fax: international +39-039-2301646 – E-mail: ctmomonza@libero.it

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Delayed graft-versus-leukemia effect after allogeneic peripheral stem cell transplantation in a patient with chronic lymphocytic leukemia

We provide evidence of a graft-versus-leukemia (GvL) effect in a highly refractory B-chronic lymphocytic leukemia (B-CLL) treated with allo-peripheral blood stem cell transplantation (allo-PBSCT) in which a complete response was achieved coinciding with the development of acute graft-versus-host disease (GvHD). However, the patient died after extensive chronic GvHD. Allo-PBSCT is effective in generating GvL but chronic GvHD must be controlled.

Sir,

A 45-year old man was diagnosed as having B-CLL stage B (multiple lymphadenopathy and hepatosplenomegaly). His white blood cell count was 303×10^9/L (87% lymphocytes), hemoglobin 11.3 g/dL and platelets 172 $\times10^9$/L. A blood smear revealed typical CLL morphology. Bone marrow and lymph node biopsies showed a diffuse pattern of infiltration. Flow cytometry analysis was compatible with the diagnosis and monoclonal IgH rearrangement was found (Figure 1). Computed tomography of thorax, abdomen and pelvis revealed multiple lymphadenopathy on both sides of diaphragm.

The patient received two lines of chemotherapy (mitoxanthrone/fludarabine and hyperCVAD) without response.[1] Salvage chemotherapy (ESHAP)[2] was administered producing a partial response in lymphadenopathy and a significant decrease in peripheral lymphocytes (8.3×10^9/L). However, a 77% bone marrow infiltration persisted. As the patient had an HLA-identical sibling donor, an allo-PBSCT was performed. Cyclophosphamide and total body irradiation were used as the conditioning regimen. G-CSF mobilized PBSC: 3.29×10^6/kg CD34$^+$ and 3.56×10^8/kg CD3$^+$ cells. GvHD prophylaxis consisted of cyclosporin-A (CsA) and methylprednisolone.

Neutrophil ($\geq0.5\times10^9$/L) and platelet ($\geq20\times10^9$/L) engraftment was obtained on days +16 and +15, respectively. During the first month, a reduction in the number of lymphocytes was observed, with a mini-

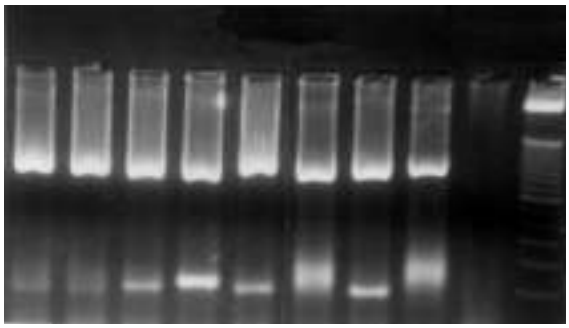

Figure 1. Monoclonal IgH/CRr III (70-120 bp) rearrangement from left to right: 1) PB at diagnosis; 2) PB after 2 cycles of E-SHAP; 3) BM after 2 cycles of E-SHAP; 4) PB prior to allo-PBSCT; 5) BM prior to allo-PBSCT; 6) PB post-transplant (day +120); 7) monoclonal control; 8) polyclonal control; 9) negative control; 10) DNA ladder (100 bp).

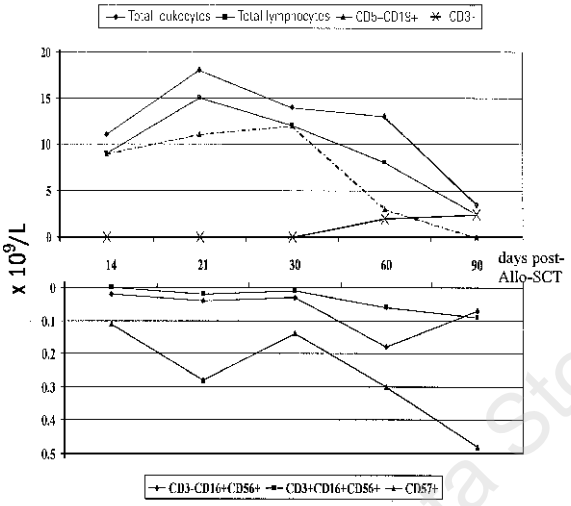

Figure 2. Post-transplant evolution of lymphoid and NK populations.

mum value of 8.4×10^9/L on day +13. From this point, total and $CD5^+/CD19^+$ lymphocyte numbers began to increase with persisting multiple lymphadenopathy. On day +50, the patient developed a cutaneous rash histopathologically documented as acute grade 2 GvHD. Coincidentally, a conspicuous decrease in the number of $CD5^+/CD19^+$ lymphocytes was observed with a total disappearance by day +90 associated with polyclonal IgH rearrangement and resolution of the polyadenopathy (Figures 1 and 2) leading to complete clinical, hematologic and molecular remissions. During this period there was a significant increase in T-cytotoxic and NK cells (Figure 2) and chimerism studies showed full-donor chimerism. Although the acute GvHD resolved with therapy, extensive chronic GvHD appeared on day +177 (skin-mucosae and liver involvement). Despite treatment with CsA and prednisone, chronic GvHD progressed and the patient died on day +205 because of infectious complications.

This report provides evidence for an association between development of acute GvHD after allo-PSCT and GvL effect eventually leading to complete disappearance of any evidence of tumor in a patient with a highly chemotherapy refractory CLL. The rationale behind allo-SCT in CLL is the potential for a GvL effect and explains the better results obtained compared with autologous transplantation. Although this is still controversial and some critics argue for the role of infused malignant cells in autologous-SCT, previous reports have demonstrated a GvL effect in CLL after allogeneic bone marrow transplantation,[3] donor lymphocyte infusion (DLI)[4] and non-myeloablative allo-PSCT followed by DLI.[5] Here, we report a case of GvL after allo-PSCT using a myeloablative conditioning regimen and without DLI.

The elderly median age of patients with CLL may justify the use of PBSC instead of bone marrow to take advantage of the former's faster hematologic recovery with a lower incidence of some complications[6,7] and a possible higher GvL effect with similar acute GvHD incidence.[8] Nevertheless, it is necessary to develop effective strategies to reduce the high incidence of chroic GvHD[9] associated with allo-PBSCT.

Antonio Gutiérrez, Carlos Solano, Felipe Prósper, Isabel Benet, Elena Sarsotti, Javier García-Conde

Department of Hematology and Medical Oncology, Hospital Clinico Universitario, University of Valencia, Spain

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Correspondence

Carlos Solano Vercet, Department of Hematology and Medical Oncology, Hospital Clinico Universitario, Av. Blasco Ibanez 17, Valencia 46010, Spain. Phone: international +34-96-3862625 – Fax: international +34-96-3622238 – E-mail: carlos.solano@uv.es

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Molecular biotyping methods for epidemiologic studies of candidemia in patients with acute leukemia

Molecular epidemiology of *Candida tropicalis* fungemia was studied in 8 isolates from patients with acute leukemia using restriction fragment analysis (RFLP) and homogeneous electric field electrophoresis (CHEF). Our data suggest that RFLP is more sensitive than CHEF and that at least two prevalent biotypes are circulating in our hospital.

Sir,

Invasive candidiasis due to *C. albicans* and recently due to other species of Candida is an important cause of morbidity and mortality in patients with acute leukemia.[1,2]

The isolation of yeasts from blood cultures is difficult and subsequently understanding of the epidemiology of Candida infections is unclear, but the potential for nosocomial transmission must be considered.[3,4]

In recent years new methodologies of molecular epidemiology, such as restriction fragment analysis (RFLP) and homogeneous electric field electrophoresis (CHEF), have been used to improve understanding of the epidemiology of these infections and appear to offer important advantages over phenotyping methods.[5,6]

To evaluate the incidence and molecular epidemiology of systemic fungemia in patients with acute leukemia, we used RFLP and CHEF to study isolates of *Candida spp.* obtained from blood cultures from patients admitted to our Department from June 1994 to June 1997.

Febrile episodes were classified according to the EORTC statement.[7]

All isolates were identified by morphology on corn meal agar with the API 20 C gallery system. Identification was independently verified by two laboratories.

For RFLP, *Candida* isolates were grown in YPD (yeast extract 10 mg/mL peptone 20 mg/mL, dextrose 20 mg/mL) for 24 h in a shaker, at 28°C. The DNA was extracted as described elsewhere.[8,9] From each strain 20 γ of DNA were restricted using 2 µL of EcoRI conc. (40 U/µL) for 3 h, at 37°C. The fragments obtained were separated on agarose gel 1.5%, TAE 1X, passing a 75V current for 2 hrs. The gel was then blotted onto nylon membrane (Amersham, Life Science). The filter was hybridized with a DIG ribosomal DNA of *Saccharomyces cerevisiae* λ DNA HINDI-II, and developed with NBT/BCIP chromogene substrate after incubation with an AP conjugate anti DIG antibody (Boehringer, Mannheim, Germany).

For CHEF, cells of *Candida* were grown in YPD medium (glucose 2%, yeast extract 1% and Bactopeptone

Table 1. Clinical characteristics of patients with *C. tropicalis* fungemia.

Pts./Year of isolation	*Age/Sex*	*Disease*	*Status*	*PMN/µL*	*CVC*	*Clinical signs*	*Therapy*	*Outcome*
BM/1994	61/F	AML	R	< 100	NO	Pneumonia	Amphotericin B	Died
GG/1994	62/M	AML	I	< 100	NO	None	Amphotericin B	Improved
CS/1994	69/M	AML	I	< 100	NO	None	Amphotericin B	Died
PL/1995	66/F	AML	I	500-1000	NO	Splenic and cutaneous lesions	Amphotericin B	Improved
RF/1995*	63/F	AML	I	< 100	NO	None	Amphotericin B	Improved
AR/1995*	53/M	AML	R	< 100	YES	None	Amphotericin B	Died
TI/1996	67/F	AML	I	< 100	NO	None	Fluconazole	Improved
GA/1996	16/M	ALL	R	< 100	NO	None	Amphotericin B	Improved
FP/1996	52/M	AML	I	< 100	NO	Pneumonia (ARDS)	Amphotericin B then fluconazole	Improved
MM/1996	54/M	AML	I	< 100	NO	None	Amphotericin B	Improved

*AML= acute myeloid leukemia; ALL= acute lymphoblastic leukemia; I= induction, R= relapse; *strains from these patients were not available for molecular biotyping methods.*