mean volumes of 140 mL, containing $3.8 \times 10^8$ 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/cm²) 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;3-10 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.

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

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References

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 $303 \times 10^9\text{L}^{-1}$ (87% lymphocytes), hemoglobin 11.3 g/dL and platelets $172 \times 10^9\text{L}^{-1}$. 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 (mitoxantrone/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 ($8.3 \times 10^9\text{L}^{-1}$). 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 PBSCT: 3.29 $\times 10^9\text{kg}^{-1}$ CD34* and 3.56 $\times 10^9\text{kg}^{-1}$ CD3* cells. GvHD prophylaxis consisted of cyclosporin-A (CSA) and methylprednisolone. Neutrophil ($\geq 0.5 \times 10^9\text{L}^{-1}$) and platelet ($\geq 20 \times 10^9\text{L}^{-1}$) 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-
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mum value of $8.4 \times 10^9$ 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, donor lymphocyte infusion (DLI) and non-myeloablative allo-PSCT followed by DLI. 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, and a possible higher GvL effect with similar acute GvHD incidence. Nevertheless, it is necessary to develop effective strategies to reduce the high incidence of chronic GvHD associated with allo-PBSCT.

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Key words
Graft-vs-host disease, graft-vs-leukemia, allo-PBSCT, CLL

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References
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 µL 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 DNA HINDIII, 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.

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

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