Graft versus host disease is associated with a lower relapse rate after allogeneic bone marrow transplantation for hematological malignancies. 1 Graft-derived cells (T and NK cells) or their products (IL2, IL4, IL6, TNF, IFN-γ) are considered the effector mechanism of a potent anti-leukemic effect, the so-called graft versus leukemia (GVL) effect. 2 Studies in vitro showed that immune system lymphocytes transferred from one individual to another represent a form of immunologically mediated anti-tumor therapy. 3 The possibility of obtaining GVL-mediated remission, without evidence of GVHD, and vice versa, clearly indicates the existence of different mechanisms and different effector cells for the two distinct effects. 4 The importance attributed to GVL justifies the various approaches employed recently. Infusion of lymphocytes from the original bone marrow donor without attempts at GVHD prophylaxis, for example, has been used with a high rate of complete hematological and cytogenetic remission in CML patients relapsed early after allogeneic bone marrow transplantation. 4,5

In order to increase the chances of leukemia-free survival, we treated 2 high risk patients affected by hematological malignancies (refractory T-ALL, refractory B-LBL in leukemic phase). Graft versus host disease (GVHD) prophylaxis consisted of methotrexate (MTX) alone. DLI were obtained after G-CSF 16 ug/kg/day sc. A total of 2.36 and 5.8 × 10^9/kg MNC, 5.4 and 11 × 10^9/kg CD34+ cells, 1.3 and 1.3 × 10^9/kg CD3+ lymphocytes, respectively, were infused. Hemopoietic recovery occurred promptly. Complete chimerism was detected by cytogenetic examination. One patient developed an extramedullary relapse that first involved the cranial nerves, and then the testes, soft tissue and skin; the other patient developed central nervous system disease and then bilateral paravertebral masses with progressive paraplegia. Despite complete medullary remission with normal female karyotype, both patients died from extramedullary progression of their disease. Our observation shows that, at least in high risk patients, no additional GVHD or GVL effect was evident after donor leukocyte infusion. Extramedullary relapse was not prevented despite good control of medullary disease.

**Key words**: allogeneic BMT, extramedullary leukemia relapse, donor leukocyte infusion
Case #1

A 21-year-old male affected by T-lymphoblastic leukemia in early relapse after standard chemotherapy (vincristine, daunorubicin, L-asparaginase and prednisone) was submitted to reinduction therapy (Ara-C 1 g/m² every 24 hours days 1→6, idarubicin 5 mg/m²/days 1→6, PDN 40 mg/m²/days 1→28). CNS prophylaxis with intrathecal MTX (12 mg/dose) and PDN (40 mg/dose) was maintained with no evidence of CNS leukemia. The patient reached CR and was submitted to non T-cell-depleted allogeneic BMT from his HLA-identical, MLC-compatible 24-year-old sister after a BuCy2 conditioning regimen. MTX was administered iv 15 mg/m² day +1 and 10 mg/m² on days +3, +6, +11 and then weekly as GVHD prophylaxis. On days +15 and +16 the patient received G-CSF (16 ug/kg sc days 1→3) primed buffy-coat infusions from the same donor. Leukaphereses were started on day 3, for 2 consecutive days, using a Fresenius continuous flow blood cell separator with modified monocyte protocol, kit PIY, that processes a blood volume of 8,000 mL per procedure. A total of 8.76x10⁸ MNC/kg, 5.4x10⁶ CD3⁴⁺ cells/kg, and 1.3x10⁸ CD3⁺ lymphocytes/kg were infused. Grade II skin GVHD with palm-sole rash ensued at day +31; it responded to corticosteroid and CyA. No cGVHD was observed. Hemopoietic recovery with ANC>0.5x10⁹/L and platelet count >50x10⁹/L was obtained on day +26 and +39, respectively. Bone marrow examination showed complete remission and cytogenetic analysis confirmed a normal female karyotype. On day +45 the patient was discharged. On day +53 he was readmitted because of fever, xerostomia and dysuria. Bone marrow remission was maintained. By day +80 the patient had developed monolateral cranial nerve III and VII palsy. Cerebrospinal fluid (CSF) was positive for blast cells. Intrathecal chemotherapy (MTX 12 mg/dose, Ara-C 40 mg/dose, PDN 40 mg/dose) and whole brain irradiation led to a partial response. Leukemic testicular, soft tissue and cutaneous relapse was observed soon after, and this time was sensitive to local radiotherapy. Despite no evidence of disease in the bone marrow and complete chimerism at cytogenetic analysis (30/30 XX metaphases), the patient died at day +200 from extramedullary disease progression.

Case #2

A 26-year-old male affected by B-lymphoblastic lymphoma with mediastinal and bone marrow involvement at diagnosis was first submitted to combination chemotherapy (LSA2L2 mod). Despite CNS prophylaxis with intrathecal MTX (12 mg/dose) and PDN (40 mg/dose) on days 5, 31, 34 and then weekly, monolateral cranial nerve II palsy developed during consolidation chemotherapy, thus revealing disease progression. Whole brain radiotherapy and intrathecal chemotherapy (MTX 12 mg/dose, Ara-C 40 mg/dose, PDN 40mg/dose twice per week) led to progressive normalization of CSF. The patient was therefore submitted to non T-cell-depleted allogeneic BMT, after a BuCy2 regimen, from his HLA-identical, MLC-compatible 17-year-old sister. MTX was administered 15 mg/sm on day+1 and 10 mg/sm on days +3, +6, +11 and then weekly as GVHD prophylaxis. On days +8 and +9 the patient received 2 donor G-CSF (16 ug/kg sc days 1→3) primed buffy-coat infusions (total amount: MNC 5.8x10⁸/kg, CD34 2.72x10⁶/kg, CD3⁺ lymphocytes 1.3x10⁸/kg) using the above mentioned procedure. Marrow engraftment was achieved with complete hematological reconstitution: ANC>0.5x10⁹/L and platelet count >50x10⁹/L on day +24 and +26, respectively. The patient was discharged on day +28. Bone marrow examination showed complete hematological remission and cytogenetic analysis confirmed a normal female karyotype. No evidence of active CNS disease was observed during periodical CSF sampling until day +80 when the patient relapsed with neurological involvement (left VII cranial nerve). He rapidly developed multiple cranial nerve palsy (bilateral VII, left II and IV cranial nerves). Intrathecal chemotherapy was promptly begun, leading to CSF normalization. At this time the patient developed progressive hyposthenia in both legs. Nuclear magnetic resonance showed multiple extramedullary relapse after marrow allotransplantation
epidural masses in the lumbar region. Progressive paraplegia and sphincteric incontinence appeared. Medullary continuous complete remission was maintained until the patient died at day +210 from extramedullary disease progression, in spite of local radiotherapy.

Discussion

Allogeneic bone marrow transplantation is recognized worldwide as a valuable therapeutic strategy for hematological malignancies. Its antileukemic effect is not merely related to the conditioning regimen, but there is also increasing evidence for the existence of an immune-mediated anti-tumor mechanism, the so-called graft versus leukemia effect.\(^2\) Effector cells are thought to be donor T lymphocytes and NK cells, which are at the basis of the GVH reaction.\(^2\) Direct evidence for this mechanism has been demonstrated in the murine transplantation model.\(^7\) In humans there is only indirect evidence of GVL: a higher relapse rate after allogeneic bone marrow transplantation has clearly been documented in patients who do not develop GVHD at any time after transplant;\(^1\) disease-free survival is significantly worse in syngeneic marrow recipients; T-depletion increases leukemia recurrence;\(^6\) anecdotal observations of disease remission after abrupt withdrawal of immunosuppression are reported for early relapse after BMT.

More recently, donor leukocyte infusion has been able to induce hematological and cytogenetic remission in CML patients relapsed after BMT with or without GVHD.\(^4,5\) GVL activity induced by donor leukocyte infusion has also been successfully exploited in Epstein-Barr virus-related lymphoproliferative disorders occurring after BMT.\(^9\)

Several approaches have been attempted in order to enhance the GVL effect in humans. In the late 80's, the addition of donor buffy-coat to unmanipulated bone marrow was unable to modulate the GVL effect to a clinical advantage, thereby increasing the risk of lethal GVHD. Further attempts involved the use of cytokines like IL2\(^2\) and interferons,\(^6\) or the infusion of subsets of T lymphocytes at different times after BMT, thus producing discordant clinical results.\(^11\)

In order to provoke the advantageous GVL effect in two high risk patients, we combined unmanipulated bone marrow transplantation and donor leukocyte infusion with reduced GVHD prophylaxis. Our strategy did not increase the GVHD rate, did not influence transplantation-related toxicity and was effective in protecting the bone marrow from disease recurrence. Despite the feasibility of this approach, the addition of donor buffy-coat, with an increment of one log of CD3\(^1\) lymphocytes in the inoculum, did not prevent disease recurrence in extramedullary sites. Although several unusual extramedullary relapses have been reported after allogeneic transplantation, the impact of GVHD as an epiphenomenon of the GVL effect in these cases is totally unknown.\(^12\)

While we cannot exclude that the widespread use of radiation-free conditioning regimens or the presence of occult extramedullary leukemic foci and extremely unfavorable clinical disease courses might account for poor leukemia control in classical pharmacological sanctuaries, the novel concept of immunologic sanctuaries devoid of lymphoid tissues could be a stimulating explanation for the lack of GVL effect.\(^6\)

Nevertheless, only a retrospective analysis made on a considerable number of patients will be able to clarify the correlation between GVL, GVHD and extramedullary leukemia relapse. Strict clinical surveillance is also advisable during post-transplant follow-up in high risk patients, in order to detect early extramedullary recurrence that is often still sensitive to local treatment.

References

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