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Graft-Versus-Myeloma Effect: Proof of Principle

By Guido Tricot, David H. Vesole, Sundar Jagannath, Jennifer Hilton, Nikhil Munshi, and Bart Barlogie

The presence of a graft-versus-tumor effect has been well established in leukemia but not in multiple myeloma. A 40-year-old patient with myeloma refractory to standard chemotherapy and autologous transplantation received a matched unrelated T-cell-depleted transplant after conditioning with fractionated total-body irradiation, thiotepa, and cyclophosphamide. This procedure resulted in a transient and incomplete response with evidence of rapidly progressive disease within 2.5 months posttransplantation. The

patient then received a small number of donor peripheral blood (PB) mononuclear cells (CD3 cells $1.2 \times 10^6/\text{kg}$) without any further cytotoxic therapy. A complete remission was attained, lasting now for more than 14 months. The procedure was associated with severe acute and subsequently limited chronic graft-versus-host disease (GVHD). This report provides the first direct evidence of a graft-versus-myeloma effect after allogeneic transplantation.

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GRAFT-VERSUS-HOST disease (GVHD) is caused by recognition of host histocompatibility antigens by donor lymphocytes¹ and is probably triggered by a cytokine storm.² It was recognized early on in leukemic mice that allogeneic bone marrow grafts not only induced GVHD but also exerted antileukemic effects.³ Evidence for the existence of a graft-versus-leukemia (GVL) and a graft-versus-lymphoma effect in humans has been mainly indirect.⁴⁻⁹ Direct evidence of a GVL effect in humans was first reported by Kolb et al¹⁰ and subsequently confirmed by others¹¹⁻¹³: infusion of donor buffy coat cells in patients with chronic myeloid leukemia (CML) relapsing after transplantation resulted in durable cytogenetic and molecular genetic remissions. However, complications ensued, including GVHD (80%), cytopenias (54%), and mortality (22%).¹⁴ Although a dissociation between the GVHD and the GVL effect has been described in mice,¹⁵⁻¹⁶ these two entities have not yet been separated in humans.

Herein, we report on a patient with refractory myeloma who was rapidly progressive after a T-cell-depleted matched unrelated donor transplant. She attained a complete remission, now lasting more than 14 months, after infusion of donor peripheral blood (PB) mononuclear cells. However, the procedure was complicated by severe acute and limited chronic GVHD. This observation proves the presence of a graft-versus-myeloma effect and the powerful antitumor effect mounted by a relatively low number of donor cells.

CASE REPORT

C.S. is a 40-year-old white woman who was diagnosed with multiple myeloma IgG- κ stage IIA in November 1992. At diagnosis, M protein level was 3.3 g/dL and hemoglobin level 10 g/dL. Bone marrow aspirate and biopsy showed 70% plasma cells. No lytic bone lesions were seen on skeletal survey. The patient received one cycle

of vincristine, melphalan, cyclophosphamide, and prednisone and two cycles of high-dose dexamethasone-pulse therapy (40 mg for 4 days on days 1 to 4, 9 to 12, and 17 to 20, and every 35 days) without response. Upon evaluation at our center in March 1993, bone marrow plasmacytosis was 75% with anaplastic features. Cytogenetic analysis showed a normal diploid karyotype. The peripheral hemogram showed a hemoglobin level of 8.8 g/dL, platelet count of $185 \times 10^9/\text{L}$, and white blood cell count of $2.4 \times 10^9/\text{L}$. IgG was 8.2 g/dL, β_2 -microglobulin 2.7 mg/L, and M protein 5.8 g/dL; there was no Bence Jones proteinuria. A skeletal survey did not show lytic bone lesions. The patient progressed on interleukin-4 (5 $\mu\text{g}/\text{kg}$ for a total of 15 days) and vincristine, doxorubicin, and dexamethasone.¹⁷ In July 1993, high-dose cyclophosphamide (6 g/m²) and granulocyte/macrophage colony-stimulating factor were administered, yielding 4.3×10^8 mononuclear cells/kg. Because of persistent cytopenia (hemoglobin 9.2 g/dL, platelets $74 \times 10^9/\text{L}$, and leukocytes $10^9/\text{L}$), a repeat bone marrow biopsy was performed that demonstrated a 90% cellularity with 90% plasma cells, indicating failure to respond to high-dose cyclophosphamide. Subsequent administration of melphalan 100 mg/m² with interleukin-3, granulocyte/macrophage colony-stimulating factor, and peripheral stem-cell support resulted in a transient decline in M protein (decrease from 6.2 to 2.5 g/dL with subsequent increase to 4.3 g/dL) and reduction in marrow plasmacytosis to 40%. Because of the patient's young age, a marrow transplant from a matched unrelated donor was performed on December 16, 1993, after a conditioning regimen that consisted of hyperfractionated total-body irradiation 1,375 cGy, thiotepa 10 mg/kg, cyclophosphamide 120 mg/kg, and antithymocyte globulin 120 mg/kg. T cells were removed from the marrow graft by a two-step soybean lectin-agglutination and sheep red blood cell-rosetting procedure. The number of T cells per kilogram infused was 0.04×10^5 by a limiting dilution assay and 0.14×10^5 as measured by CD3 expression on flow cytometry. Methylprednisolone 2 mg/kg was given posttransplantation from day +5 until day +19, with rapid tapering thereafter to decrease the probability of graft rejection. The patient showed prompt engraftment with an absolute neutrophil count of 0.5, 1.0, and $1.5 \times 10^9/\text{L}$ on days +17, +18, and +22, respectively.

Cytomegalovirus (CMV) reactivation (positive CMV antigen test) was documented on days +27 and +57 posttransplantation. Serum M protein transiently decreased from 4.3 to 1.7 g/dL on day +21, with a subsequent increase to 3.4 g/dL on day +67. The bone marrow biopsy on day +35 showed a cellularity of 30% with trilineage engraftment but persistent plasmacytosis of 50%. On day +67, cellularity had increased further to 70% with 90% plasma cells and markedly decreased erythropoiesis and megakaryopoiesis. In the absence of clinical GVHD, previously cryopreserved donor peripheral buffy coat cells (original vol, 50 mL with $10^6/\text{kg}$ white blood cells [WBC]) were administered on day +69 to induce an antitumor effect. However, neither GVHD nor tumor response were observed (Fig 1).

On day +116, fresh donor PB buffy coat cells from 100 mL donor blood were obtained, and 3.74×10^8 cells were infused. Phenotypic analysis showed the following cell counts: CD3, $1.2 \times 10^6/\text{kg}$; CD4, $0.95 \times 10^6/\text{kg}$; CD8, $0.24 \times 10^6/\text{kg}$; CD56, $0.24 \times 10^6/\text{kg}$; and

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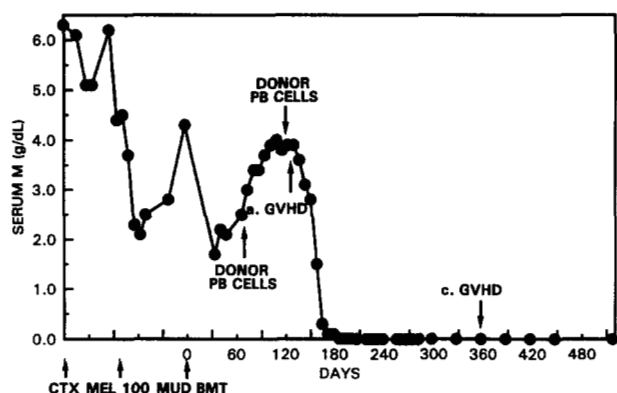


Fig 1. Evolution of serum M protein after different treatment modalities, graft versus myeloma effect. Day 0 is the day of the matched unrelated donor (MUD) BMT. CTX, high-dose cyclophosphamide (6 g/m²); MEL 100, high-dose melphalan 100 mg/m².

CD34, 0.08 × 10⁶/kg. Eight days after infusion of fresh donor cells (day +124 post-bone marrow transplant [BMT]), the patient developed bilateral retrocervical, axillar, and inguinal adenopathy, and splenomegaly (3 cm below left costal margin). Fine-needle aspirate showed reactive lymphadenopathy, and vancomycin and piperacillin were started. Four days later, a fever of 101°F and a rash developed. Antibiotics were discontinued and daily prednisone 60 mg was started for 4 days, resulting in complete disappearance of rash, splenomegaly, and adenopathy. Upon prednisone tapering, diffuse erythema recurred, prompting reinstitution of prednisone at 60 mg. A skin biopsy was diagnostic of GVHD grade IV. The patient was admitted 28 days after the second infusion of donor buffy coat cells because of progressive skin GVHD with blister formation, worsening of diarrhea (500 mL), and mild elevation of alanine aminotransferase to 78 U/L. Daily methylprednisolone at 2 mg/kg was started. Because of progressive skin desquamation, intravenous cyclosporin at a dose of 5 mg/kg was administered on alternating days with dose adjustment according to serum levels. Diarrhea increased to a maximum of 1,100 mL/d. Liver-function tests remained normal; direct bilirubin was 1.1 mg/dL, alanine aminotransferase 78 U/L, and aspartate aminotransferase and alkaline phosphatase remained normal. The skin lesions improved slowly, and the patient was discharged 56 days after infusion of fresh donor buffy coat cells.

M protein level was 3.9 g/dL at the time of fresh donor buffy coat infusion, but was no longer detectable 2 weeks after admission for acute GVHD (42 days after second buffy coat). Immunofixation analysis showed no evidence of monoclonal protein. The bone marrow biopsy was normocellular (50%) with normal trilineage hematopoiesis without evidence of residual multiple myeloma. DNA fingerprinting indicated the presence of donor cells and less than 10%, if any, recipient cells.

CMV antigenemia recurred during acute GVHD (63 days after fresh buffy coat). Persistent thrombocytopenia necessitated platelet transfusions until 18 weeks after infusion of donor cells. All immunosuppressive therapy was discontinued on day 230 posttransplantation (106 days after infusion of fresh donor cells). On day 355 posttransplantation, the patient developed chronic GVHD involving the mouth, eyes, and liver, which improved upon institution of oral prednisone at 1 mg/kg/d along with cyclosporin 5 mg/kg every other day. Prednisone has since been slowly tapered to a current dose of 5 mg/d. Abnormal laboratory tests include alkaline phosphatase elevation to 777 U/L, aspartate aminotransferase 83 U/L, alanine aminotransferase 139 U/L. The patient has not required red blood cell or platelet transfusions for 12 months, maintaining an almost normal hemogram with a hemoglobin level of 14.3 g/dL, a WBC

count of 6.5 × 10⁹/L, and a platelet count of 137 × 10⁹/L. Her Karnofsky score is more than 90%.

DISCUSSION

This patient's clinical course provides direct evidence that a graft-versus-myeloma effect exists. There was only a transient antitumor effect to total-body irradiation, thiotepa, and cyclophosphamide, and in fact progressive disease was clearly documented before day +70 posttransplantation. Cryopreserved donor PB cells had no apparent effect on myeloma parameters; fresh donor mononuclear cells obtained from 100 mL PB resulted in complete disappearance of all evidence of disease, based on morphology, flow cytometry to detect aneuploidy within the plasma cells with the same light-chain restriction as the myeloma cells,¹⁸ and M protein measurements in blood and urine on immunofixation. The lack of efficacy of cryopreserved donor cells might have been the consequence of excessive cell death during freezing and thawing or the presence of inadequate numbers of donor cells.

The antimyeloma effect in this patient was associated with severe GVHD requiring aggressive therapy with high-dose glucocorticosteroids and cyclosporin A without abrogation of the antitumor effect. The patient remains in complete remission 14 months after the fresh donor PB infusion with limited chronic GVHD and persistent mild thrombocytopenia (137 × 10⁹/L).

It is extremely unlikely that the antitumor effect in this patient resulted from corticosteroid treatment for GVHD. The patient had been resistant to two cycles of high-dose dexamethasone before transplantation and remained in complete remission during 6 months of discontinuation of all steroid therapy after the donor cell infusion.

It is a humbling experience for BMT physicians to appreciate a profound antitumor effect exerted by donor PB cells after failure of myeloablative chemoradiotherapy. Our findings and recent results with donor buffy coat infusions in patients with relapsed CML strongly argue in favor of a major contribution of a graft-versus-tumor effect in the eradication of hematologic malignancies. In CML, donor buffy coat cells effect tumor cyoreduction exceeding 6 logs.¹⁴ Our findings appear to be different from those reported in CML in at least three aspects. (1) Whereas control of CML seems to require large quantities of PB mononuclear cells,¹⁴ with no responses seen with less than 10⁷ T cells/kg,¹⁹ the complete response in our myeloma patient required 10 to 500 times fewer donor cells. It is unknown whether the same effect would have been seen if HLA-matched sibling donor cells had been infused. (2) While the best results in CML are seen in patients treated either in stable chronic phase¹³ or in early relapse (cytogenetic or molecular, before hematologic relapse),²⁰ our patient achieved a lasting complete response upon administration of donor cells in fulminant relapse. Our result is similar to observations reported by Drobyski et al,¹² who reported cytogenetic remissions in six patients with accelerated-phase CML. (3) Whereas in CML, GVL-associated cytogenetic (median, 2 to 4 months)^{11,12,20,21} and molecular (median, 6 to 10 months)^{11,13,20,21} remissions are attained slowly, the graft-versus-myeloma effect in our patient was brisk, with disappearance of myeloma

protein and resolution of bone marrow plasmacytosis in less than 1 month. Although it is unlikely, we cannot exclude the possibility that the cryopreserved donor PB cells may have undergone a rapid expansion following the infusion of fresh donor cells and thus may have contributed to this remarkable response. Occasionally, cytogenetic and molecular remissions in CML have been documented as early as 40 days postinfusion.^{11,13,21} Complete cytogenetic or molecular responses were reported by Van Rhee et al²⁰ in all five patients receiving unrelated donor cell infusions, as compared with five of nine in the sibling donor group.²⁰

Since the half-life of normal IgG is approximately 23 days,²² it can be assumed that the entire antitumor effect in our patient was accomplished within the first 2 weeks after donor cell infusion, which is in keeping with data in murine leukemia, in which the antileukemic effect of the allograft is attained within 6 to 7 days^{23,24} and precedes the onset of severe GVHD.²³⁻²⁵ Hence, a scenario can be envisioned whereby donor T cells are infused and subsequently eliminated after 14 to 21 days by exploiting the activation by ganciclovir of the previously transduced thymidine kinase suicide gene.²⁶

It is likely that future progress in cancer therapy will depend on judicious and safe manipulation of the immune system, rather than on further intensification of cytotoxic agents.

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