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Case report

Immunotherapy of relapsed resistant chronic myelogenous leukemia post allogeneic bone marrow transplantation with alloantigen pulsed donor lymphocytes

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Summary:

Allogeneic cell-mediated immunotherapy with donor lymphocyte infusion (DLI) can successfully reverse chemoradiotherapy-resistant relapse in patients with chronic myeloid leukemia treated by allogeneic bone marrow transplantation (BMT). We describe the first successful attempt in 1992 to treat DLI-resistant relapse in a patient with CML in full hematologic relapse, using immunized donor lymphocytes. Donor lymphocytes were pulsed *in vitro* with a mixture of irradiated peripheral blood lymphocytes (PBL) obtained from both parents, in order to trigger alloactivation of donor lymphocytes against host alloantigens presented by parental cells, using as stimulating cells maternal PBL expressing the shared maternal haplotype and paternal PBL expressing the shared paternal haplotype of the patient. Full hematologic, cytogenetic and molecular remission was induced for the first time, independently of GVH, and has persisted for more than 9 years. To the best of our knowledge, this report represents the first successful immunotherapy with donor lymphocytes activated against host-type antigens. We suggest that immune donor PBL may be superior to DLI, possibly effective even when all other modalities fail, perhaps even independently of GVHD. *Bone Marrow Transplantation* (2001) **28,** 795–798.

Keywords: chronic myeloid leukemia; bone marrow transplantation; immunotherapy; donor lymphocyte infusion; immune donor lymphocytes; graft-versus-leukemia

Allogeneic bone marrow transplantation (BMT) is the only effective treatment for hematologic malignancies resistant to conventional chemotherapy. In a number of patients who relapse post BMT, remission may be accomplished with adoptive allogeneic cell-mediated immunotherapy mediated by donor lymphocyte infusion (DLI).[1–4] DLI is particularly effective in patients with relapsed chronic myeloid leukemia (CML).[4] Interestingly, since the graft-versus-leukemia (GVL) effects induced with DLI are mediated primarily by alloreactive donor T lymphocytes, activation of GVL effector cells in patients resistant to DLI can be accomplished by donor lymphocytes stimulated *in vivo* and/or *in vitro* with recombinant interleukin 2 (rIL-2).[3] However, despite the remarkable therapeutic potential of DLI in patients with relapsed CML, approximately 30% of the recipients fail to respond to DLI, especially those at more advanced stages of CML.[2–4] At the present time, no cure can be offered to patients with leukemia resistant to DLI after BMT.

Adoptive transfer of donor immunity to the host in the course of BMT is well established in experimental animals[5,6] and man.[7] The concept of using a similar principle for more effective immunotherapy of resistant malignancy, by using specifically immune donor lymphocytes is supported by data in preclinical animal models.[8,9] Data from preclinical animal models suggest that allogeneic lymphocytes may be effectively activated against allogeneic tumor cells of host origin, while in parallel down-regulating their alloreactive potential against normal host somatic cells.[8,9] Such a procedure may represent one possible approach for accomplishing GVL effects independently of GVHD. The existence of GVL effects, in part independently of GVHD, was also documented in patients treated with DLI for relapse following BMT.[2–4] The following report presents the first successful attempt to treat relapsed leukemia with donor peripheral blood lymphocytes (PBL) pulsed *in vitro* against the patient's own alloantigens presented by parental lymphocytes that may have become more immunogenic in response to treatment with alpha interferon (αIFN), suggesting that improved immunotherapy against resistant relapsed leukemia may be accomplished by using specifically immune donor lymphocytes. Furthermore, using immune donor lymphocytes, effective GVL may be accomplished independently of GVHD.

Case report

NL, a female child, was diagnosed as having Philadelphia positive CML in chronic phase at the age of 7 years, in September 1990. She presented with fever, pain in her left

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leg and a spleen palpable 5 cm below the left costal margin. Her leukocyte count revealed leucocytosis in the range of 250×10^9/l, mostly mature granulocytes, with a few myelocytes and occasional blasts. Platelet counts reached 1600×10^9/l, and the level of LDH 1150 U. Bone marrow morphology was typical of CML and cytogenetic analysis of spontaneous metaphases was consistent with the typical Philadelphia chromosome translocation, 46XX t(9;22)(q34;q11) in all cells analyzed. The CSF examination was negative for malignancy. Initial cytoreduction was achieved with busulfan 2 mg/day and twice, at monthly intervals, cytosar 80 mg twice daily. Because of an insufficient response she was switched after 2 months to hydroxyurea 1 g/day, inducing a return of the blood counts to normal levels.

Allogeneic BMT from an HLA-A, B, C and DR identical 6 month old brother was performed at the Fred Hutchinson Center in Seattle on 21 February 1991, after conditioning with busulfan 4 mg/kg $\times$ 4 days, cytoxan 60 mg/kg $\times$ 2 days and one intrathecal injection of methotrexate. She received a total of 3.7×10^9 nucleated bone marrow cells (1.9×10^8 cells/kg). Anti-GVHD prophylaxis consisted of a combination of methotrexate and cyclosporin A (CsA) for nearly 6 months. She had signs of engraftment on day +11 and an ANC $>0.5 \times 10^9$/l by day +18. The post-transplant course was uneventful except for mild signs of VOD, a persistent bacteremia (*Acinetobacter anitratus*) and increased creatinine levels with mild hypertension, which were attributed to treatment with CsA. There was no evidence of clinical acute or chronic GVHD at any stage.

The patient continued to do well clinically, but on 25 November 1991, 9 months after the transplant, increases in the leukocyte and platelet counts were noticed. Bone marrow aspiration revealed typical Philadelphia chromosome translocation in 19/20 46XX cells (95%) with only one (5%) normal 46XY cell. The Ph-positive translocation was also detected by RT-PCR. The patient was treated with small doses of hydroxyurea with a good hematologic response, followed by an attempt at immunotherapy with donor lymphocyte infusion. At the time of initiation of DLI the patient was immunologically reconstituted, with CD3 43% (normal 68 $\pm$ 2%); CD4 23% (normal 46 $\pm$ 2%); CD8 15% (normal 24 $\pm$ 1%); DR 30% (normal 19 $\pm$ 3%); CD16 22% (normal 9 $\pm$ 2%); CD19 26% (normal 9 $\pm$ 2%). The proliferative response to phytohemagglutinin was normal (stimulation index of 192 as compared with 185 $\pm$ 12) with elevated spontaneous cytotoxic activity against chromium labeled K562 target cells (37 lytic units as compared with 5 $\pm$ 1 in normal controls). The patient developed avascular necrosis of the left hip, but other than that no further complications were observed.

Collection of donor lymphocytes was inadequate and problematic, because of the small size of the minor donor, and we could not give more than approximately 10^7 cells/kg isolated from 50–100 ml of whole blood. In an attempt to increase the efficacy of adoptive allogeneic cell therapy we gave small doses of cytoxan (500 mg/m^2 with forced hydration) 24 h prior to administration of the first DLI dose on 20 December 1991. No signs or symptoms of GVHD developed. Due to the lack of response to DLI and absence of GVHD, the patient was infused for the second time with 10^7 donor lymphocytes/kg activated *in vitro* with rIL-2 (allogeneic 'LAK cells') with subcutaneous rIL-2 (6×10^6 IU/m^2/day) for 3 days, starting with the cell infusion on 5 February 1992, and again for the third time on 11 March 1992, (as previously described in detail), in an attempt to activate the LAK cells *in vivo* continuously.[4] Consequently, the proportion of Ph-positive cells in the marrow decreased transiently to 67%, and to 60% after a fourth infusion of 6×10^6 'LAK cells'/kg. However, the proportion of Ph-positive cells rose gradually to 94%. All these attempts to induce GVL and/or GVHD were ineffective and did not result in a significant durable decrease in the percentage of Ph-positive cells. In an attempt to escalate the anti-host reactivity, the patient received donor lymphocytes that were sensitized to irradiated maternal and paternal blood lymphocytes, activated *in vitro* in an attempt to maximize the alloreactive responses against shared paternal and maternal MHC. Activation of donor PBL against a mixture of parental PBL was accomplished by incubating donor peripheral blood lymphocytes in a semi permeable life-cell bag (Baxter, Deerfield, IL, USA) at a responder/stimulator ratio of 10:1, at final concentrations of 1.5×10^6 nucleated cells/ml cultured in phosphate buffered saline enriched with 10% donor plasma, gentamycin 0.025% and penicillin 0.02%. Donor cells were cultured with a mixture of Ficoll-Hypaque purified maternal and paternal blood lymphocytes, inactivated by ionizing irradiation (3000 cGy). Cell cultures were incubated for 1 day in a 37°C incubator in 10% CO_2 in air, in an attempt to maximize the alloreactive responses against shared paternal and maternal MHC haplotype, presented by mismatched parental class II. A total of 9×10^7 and 2.3×10^7 viable nucleated cells/kg were infused on 7 and 15 July 1992, respectively, 1 day after conditioning with cytoxan (500 mg/m^2 with forced hydration). Together with the administration of *in vitro* alloactivated donor lymphocytes the patient received rIL-2 for 3 days (6×10^6 IU/m^2/day), starting on the day of cell infusion. Treatment was followed by administration of 1.5×10^6 units of αIFN (Roferon A) three times weekly, trying to up-regulate cell surface antigens to render Ph-positive cells more antigenic.

As documented on 21 February 1993, the level of Ph-positive cells decreased for the first time to 18% and starting on 19 October 1993 until today, Ph-positive cells were no longer detectable and RT-PCR for bcr-abl remained consistently negative. To date, 4 years off Roferon A that was continued initially to maintain remission, the patient remains in excellent health with a Karnofsky score of 100%. She is in complete molecular remission, fully reconstituted with donor male cells as confirmed by both cytogenetic analysis and amelogenin gene PCR[13] with no evidence of any residual female cells in the peripheral blood or in the bone marrow, with no clinical signs of chronic GVHD.

Discussion

Following BMT, allogeneic cell therapy induced with DLI, with or without further activation of the alloreactive potential *in vivo* or *in vitro* with rIL-2, is considered the treatment

of choice for patients with resistant hematologic malignancies, especially CML, relapsing following myeloablative doses of chemoradiotherapy in conjunction with BMT.[2–4] The present study was designed to explore a new potential modality for the treatment of patients with relapsed leukemia resistant to DLI. The case reported here documents the first successful potentiation of the GVL effects induced by allogeneic lymphocytes using donor PBL pulsed *in vitro* against a mixture of parental cells, each presenting one of the patient's haplotypes, concomitantly with administration of αIFN in an attempt to increase the antigenicity and cell surface expression of MHC and putative tumor-specific antigens on host tumor cells, in a patient with resistant CML who relapsed shortly after BMT, with a follow-up of nearly 10 years. As shown by the clinical history of the patient, donor PBL obtained from a fully matched MLR non-reactive donor could be stimulated *in vitro* against host alloantigens expressed by a mixture of parental cells that were stimulatory in MLR. Thus, despite the small number of donor lymphocytes available, limited by the low body weight of the infant donor, effective GVL effects with durable molecular remission could be induced in the patient, independently of clinically overt GVHD. It does not appear that the effects observed were due to larger number of lymphocytes rather than more alloreactive T cells since it has been shown previously that donor lymphocytes are much more alloreactive if given closer to the transplant procedure, hence, even somewhat smaller donor cell inocula given earlier should have been much more active than donor lymphocytes given late post BMT.[10] The role of interferon in enhancing the expression of cell surface antigens of the patient's tumor cells is only speculative. However, considering the well-known functions of interferon and considering the therapeutic role of interferon in patients with CML we felt that administration was fully justified. Likewise, the role of cytoxan in the procedure described is uncertain, since the rationale for administration of cytoxan was both to provide a 'niche' for maximizing the ratio of alloreactive to tolerant T cells of donor origin, as well as for elimination of putative cytoxan-sensitive suppressor cells or 'veto' cells that can down-regulate alloreactive donor T cells.[11] Furthermore, since the time interval from DLI to elimination of tumor cells of host origin may be prolonged up to several months, it cannot be formally proved that the GVL effects observed were due to the last inoculum of activated donor lymphocytes rather than the first inocula, however, in view of the course of treatment and the observed response, this appears unlikely.

Similar results were accomplished in our animal models, where we could show significantly improved GVL effects with reduced GVHD potential by immune lymphocytes as compared with DLI induced with non-immune donor lymphocytes.[9] Although formally not fully proved, since spontaneous alloreactivity of donor against host alloantigens was minimal, judging by lack of GVHD and GVL, taken together with our recent experiments in preclinical animal models,[9] our data suggest that upregulation of GVL may be accomplished in parallel with downregulation of anti-host reactivity.

Specifically immune donor lymphocytes were used previously in one case with multiple myeloma where the donor was immunized twice with myeloma idiotype coupled to KLH.[12] The patient had refractory myeloma, with monoclonal IgG, with a considerable tumor burden (M-protein 3.9 g/dl with 50% plasmacytosis) whose HLA-matched sibling donor served as the source of immune lymphocytes. Following adoptive transfer of donor immune lymphocytes, the myeloma idiotype-specific T cell response was successfully transferred to the patient as evidenced by a detectable $CD4^+$ T cell line having a unique specificity for the myeloma idiotype and by documentation of idiotype-specific donor-type cells in the host by *in situ* hybridization.[12]

Based on our observations in mice and humans, we would like to hypothesize that relapse as well as transplant-related complications due to severe GVHD may be partly prevented by using non-alloreactive immune donor lymphocytes, sensitized *in vitro* or *in vivo* by tumor cell membranes or radiation-inactivated tumor cells, while avoiding or minimizing the use of post-transplant immunosuppressive therapy. As shown here, anti-tumor responses induced by sensitized donor T cells, not excluding donor NK cells, may result in effective GVL effects independently of GVHD. The mechanism of GVL effects that may be mediated against host type minor histocompatibility antigens present on host tumor cells, yet with no clinically overt GVHD, remains unknown.

In conclusion, although patients with a variety of hematologic malignancies relapsing following BMT, especially CML, may be successfully treated with DLI or DLI with rIL-2 activation *in vivo* and/or *in vitro*, patients with tumor cells resistant to naive donor lymphocytes may still respond to donor lymphocytes activated by host alloantigens *in vitro*. In the case of fully matched siblings, where no effective response is anticipated by direct one way mixed leukocyte cultures, parental PBL, each expressing one of the patient's haplotypes, may serve as stimulators against the patient's own alloantigens, in an attempt to induce 'autoimmune-like' response against self-alloantigens represented by parental PBL, since unshared maternal and paternal haploidentical cells are expected to stimulate donor T cells in a one way mixed leukocyte culture against the unshared haplotype. When donor and recipient are fully matched at all MHC loci, such an approach may help initiate some 'anti-self' reaction against host cells mismatched at minor histocompatibility loci. Several points remain to be investigated in addition to the need to confirm the reproducibility of the basic principle. First, whether parental cells containing shared host alloantigens or nonspecific stimulation of donor PBL with unrelated allogeneic lymphocytes are required to improve the GVL capacity of donor lymphocytes. This can be addressed in the future by studying the number of anti-host reactive T cells in limiting dilution assays. Second, whether the use of parental cells containing host alloantigens as stimulators, instead of unrelated stimulating lymphocytes, have better or worse effects on GVHD. If indeed, *in vitro* sensitization of donor cells can result in improved GVL effects, especially at least in part independently of GVHD, similar benefits may be also accomplished in recipients of haploidentical mismatched allografts with no GVHD following initial transplantation with T cell-depleted stem cells, since in these recipients newly developing donor T cells are fully unresponsive to

host alloantigens, including tumor antigens, thus the patients are at risk of relapse, especially if they carry a large tumor bulk. In principle, alloimmune-mediated interactions between immunocompetent donor T cells and residual haploidentically mismatched tumor cells of host origin could be best induced *in vitro* for optimal sensitization of donor T cells against the leukemia cells while down-regulating anti-host responses, which may otherwise be lethal across MHC. The efficacy of immunotherapy with DLI, as described here for the first time, and the lack of alternative modalities for treating relapse following BMT in patients failing DLI, suggest that allogeneic cell therapy with immune donor lymphocytes may become an important tool for the treatment of CML resistant to DLI and possibly other resistant hematologic malignancies. The feasibility to induce immune mediated anti-cancer effector cells while possibly controlling for GVHD in experimental animals and man suggests that further developments in the clinical application of adoptive cell-mediated immunotherapy, may open new horizons in the treatment of patients with cancer who are otherwise resistant to all available anti-cancer modalities.

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