Adoptive immunotherapy with donor lymphocyte infusions after allogeneic HPC transplantation

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GVHD and relapse are two major causes of death after HPC transplantation.[1] The most efficient method for prevention of GVHD consists of T-cell depletion of the graft.[2-5] However, this usually leads to greatly increased risks of leukemia relapse,[3,6,7] due to the loss of the graft-versus-leukemia (GVL) effect that has been recognized as an important component of the anti-leukemic efficacy of allogeneic HPC transplantation, particularly for chronic myelogenous leukemia (CML). The demonstration of this GVL effect and of the primordial role of T-lymphocytes led investigators to transfuse donor lymphocytes to CML patients relapsing after HPC transplantation.[8-14] Indeed, donor lymphocyte infusions (DLIs) have increasingly been used to treat relapse after HPC transplantation not only for CML but also for acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), myelodysplastic syndrome (MDS), multiple myeloma (MM), and non-Hodgkin's lymphoma.[15-22]

ABBREVIATIONS: ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; BM = bone marrow; CML = chronic myelogenous leukemia; CR = complete remission; CTL(s) = cytoloxic T-lymphocyte(s); DLI(s) = donor lymphocyte infusion(s); GVL = graft-versus-leukemia; KIR(s) = killer cell inhibitory receptor(s); MDS = myelodysplastic syndrome; MHA = minor histocompatibility antigens; MM = multiple myeloma.

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DEMONSTRATION OF THE GVL EFFECT AFTER HPC TRANSPLANTATION

A GVL effect was first posited in 1956 on the basis of murine experiments: mice receiving syngeneic transplants after the injection of leukemic cell lines and total body irradiation almost uniformly relapsed, whereas mice receiving allogeneic transplants developed GVHD but had a lower incidence of relapse.[23] The first landmark reports of GVL effects in human allograft recipients were published by Weiden et al. in 1979[24] and 1981.[25] Their observations were confirmed by a large retrospective study of 2254 patients receiving HLA-identical sibling bone marrow (BM) transplants for various hematologic malignancies.[7] A lower rate of relapse was observed in recipients of non-T-cell-depleted BM transplants than in recipients of T-cell-depleted allografts or syngeneic BM transplants. In that study, the GVL effect was also evidenced by the lower rate of relapse in patients with GVHD (particularly chronic GVHD in AML and CML patients and acute GVHD in ALL patients), although the GVL reaction was not restricted to patients with GVHD.

The International Bone Marrow Transplant Registry also compared HLA-identical sibling transplants in 731 recipients of T-cell-depleted BM transplants and 2480 recipients of non-T-cell-depleted BM transplants and determined the relative risk of relapse after adjusting for the incidence and severity of acute and chronic GVHD.[3] The relative risks of relapse with and without T-cell depletion for AML and ALL in first remission and CML in chronic phase were 1.66 (NS), 1.55 (NS), and 4.87 ($p<0.0001$), respectively. Finally, the GVL effect was also demonstrated by the evolution of minimal residual disease after transplant, which often ceases to be detectable only 6 to 12 months after BM transplant,[26] and the occurrence of GVL activity with or without GVHD after cessation of GVHD prophylaxis without DLI after posttransplant relapse.[27-29]

Mechanisms of GVL

Cells involved in the GVL effect. MHC-restricted CD4+ and CD8+ T cells and NK cells are probably all involved in the process of both GVL and GVHD.[30-32] It is likely that cells implicated in the GVL effect vary as a function of the he-

matologic malignancies involved, depending upon their MHC expression[33] and the nature of antigens presented. However, the recognition by CD4+ cells of peptide antigens presented through MHC class II molecules on host cells is essential in initiating a response against leukemia.[34] In transplant recipients with CML, the most often identified are CD4+ T-cell lines or clones, which inhibit the growth of leukemic progenitors or lyse them, either through direct cytotoxicity or cytokine-mediated anti-leukemic effects.[35,36] Moreover, the efficacy of CD8+ cell depletion in reducing the risk of GVHD without impairing the GVL effect, be it by depletion of the original graft[37,38] or that of DLI after relapse,[39,40] suggests a primary role for CD4+ cells in the GVL effect (particularly in CML, but also in MM[40]) and a primary role for CD8+ cells in GVHD.

Confirming these findings, Nagler et al.[41] recently showed that selective depletion of CD4+ cells does not prevent GVHD. However, these observations do not deny the role of CD8+ cells in the GVL effect. Even after CD8+ cell depletion, about 0.6×10^6 CD3+CD8+ cells per kg are transfused,[14] and moreover, transfused CD4+ cells may recruit CD8+ cells in the host. In addition, CD8+ T-cell clones with cytotoxic activity against leukemic cells have been identified,[42] and cytoloxic T-lymphocyte(s) (CTLs) directed specifically against minor histocompatibility antigens (mHA)[43] or EBV-related antigens[44] are CD8+. To provide help to CD8+ cells (that recognize antigenic peptides complexed with MHC class I molecules), CD4+ cells require co-stimulation with molecules of the B7 family.[45,46] Many tumors, including most human leukemias, lack expression of B7, which contributes to the absence of immune recognition in these diseases; in such diseases, *B7* gene transfer could be an attractive approach.[46]

Despite the increased risk of relapse after T-cell-depleted HPC transplantation that leaves NK cells behind in the graft, NK cells may still play a role in the GVL effect in some situations. Inhibition of NK cell lysis is mediated by membrane receptors with different specificities for MHC class I alleles. In particular, killer cell inhibitory receptors (KIRs) recognize epitopes shared by HLA-C (Cw2, 4, 5, and 6 for KIR2DL2 vs. Cw1, 3, 7, and 8 for KIR2DL1) or HLA-*B*w4 (KIR3DL1).[47] Ruggeri et al.[48] recently studied the role of KIR epitope incompatibility in HLA-mismatched T-cell-depleted HPC transplantation and detected high frequencies of NK cell clones, which killed the recipient target cells in patients given cells from KIR epitope-incompatible donors. Moreover, these patients had a lower incidence of myeloid relapse than patients given cells from KIR epitope-compatible donors, which suggests a GVL effect mediated by donor-derived NK cells. In mice, adoptive immunotherapy with MHC-mismatched or donor-activated NK cells administered after BM transplant was shown to provide a strong GVL effect,[49] and depletion of NK cells from the graft enhanced the relapse rate of leukemic cell lines shown in vitro to be NK cell-sensitive.[50] These results suggest that NK cells may play a role in the GVL effect when leukemic cells are NK cell-sensitive (i.e., in myeloid malignancies) and when leukemic cells do not express MHC class I KIR ligands of the donor NK cells.

Antigens involved in the GVL effect. Several studies have shown that response to DLI is associated with conversion from a mixed chimeric state before transfusion to complete donor hematopoiesis after DLI.[11,13,16,40,51] Moreover, DLI in patients who do not have residual donor hematopoiesis induces severe marrow aplasia, which may be resolved by the transfusion of donor stem cells.[15,52] Finally, DLI can displace residual host stem cells in case of the recurrence of nonmalignant disease after allogeneic HPC transplantation.[53] Taken together, these observations suggest that the GVL reaction associated with DLI is likely directed against allospecific antigens rather than disease-specific targets.

The mHA may be a target of the GVL response,[15,40,52,54] but other, unrecognized targets may also exist. The mHA are polymorphic antigens that are inherited independently from HLA antigens and that may be recognized by alloreactive T cells.[54] One important characteristic of mHA is their tissue-restricted distribution. Some, such as the H-Y antigen, are expressed on all MHC-bearing cells, whereas the expression of others (HA-1, HA-2, HA-5, HB-1, etc.) is restricted to the hematopoietic and lymphoid tissues.[43] Mutis et al.[54] recently generated HA-1- and HA-2-specific CTLs from HA-1- and/or HA-2-negative healthy blood donors by using HA-1- and HA-2-synthetic peptide-pulsed antigen dendritic cells. These CTLs displayed specific cytotoxic activity against target cells expressing HA-1 and/or HA-2 (including leukemic cells from ALL and AML patients) but not against nonhematopoietic cells. Warren et al.[43] isolated other mHA, not only HLA-A2 or HLA-B7 but also HLA-A3, -A11, -B8, -B53, and -Cw7. Most of these mHA did not lyse dermal fibroblasts, which suggests that the transfer of these mHA-specific CTLs could have GVL activity without causing GVHD. However, some caution is necessary, as recent studies recognized a significant association of an HA-1 mismatch between donor and recipient and the occurrence of GVHD.[55,56] Moreover, data from in vitro cytolytic assays against fibroblasts could underestimate the expression of mHA in tissues in vivo and thus underestimate their potential for inducing GVHD.[43]

In treatment of malignancies in which translocations result in fusion proteins (CML and some ALL with t[9;22], AML M2 with t[8;21], AML M3 with t[15;17], and AML M4 with inv 16), these fusion proteins represent ideal targets for adoptive immunotherapy, because they are not present in normal cells. The rearrangement of the *bcr* and *abl* genes in the Philadelphia chromosome results in the expression of BCR-ABL^{b3a2} (the most frequently observed) or BCR-ABL^{b2a2} proteins. There are no convincing data that BCR-ABL^{b3a2} or BCR-ABL^{b2a2} proteins are targets in DLI-mediated GVL effects. However, peptides from the joining region of b3a2 have been identified that bind to HLA-A3, -A11, -B8, or both HLA-A3 and –A11 molecules and that elicit CTLs in vitro.[57-59]

Several studies[57,58] failed to show junctional peptide presentation by HLA-A2, the most frequent MHC class I in humans, but Yotnda et al.[60] recently identified a junctional nonapeptide that binds to HLA-A2 molecules and elicits primary CTL responses in vitro. Moreover, CTLs that are specific for this junctional peptide were found at high frequency in 5 of 21 CML patients.[60] In vitro generation of specific CTLs by donor dendritic cells pulsed with b3a2 peptide was proposed as a way to increase the GVL effect without exaggerating GVHD. The ability of normal donor peripheral blood lymphocytes stimulated with b3a2 peptide-loaded autologous dendritic cells to lyse b3a2-positive marrow cells derived from an HLA-identical sibling of a CML patient was recently reported.[59] However, HLA restriction of the b3a2-specific response may be responsible for the absence of cytotoxic activity induced by CTLs from some patients.[59]

In cases of B-cell lymphoma and MM, it has been possible to immunize the donor to the antibody idiotype of the lymphoma[61] or the MM,[62-64] after which the immunity could be transferred. However, the clinical benefits of such an approach remain to be determined.

Finally, in virus-induced malignancies, adoptive immunotherapy against virus-derived antigens may be effective. Particularly after allogeneic HPC transplantation, CTLs directed against EBV-derived antigens were effective in preventing[65] or treating[66-68] EBV-induced lymphomas. Adoptive immunotherapy against other EBV-associated malignancies (Hodgkin's disease or nasopharyngeal carcinoma) in which less immunogenic EBV antigens occur is currently investigated.[69]

DLI IN PATIENTS WITH RELAPSED MALIGNANCY AFTER ALLOGENEIC HPC TRANSPLANTATION

Efficacy of DLI

The apparent power of the GVL effect and its presumed mediation by donor lymphocytes led several groups to transfuse donor WBCs to patients with relapsed leukemia after HPC transplantation. Two large multicenter studies have reviewed the results from 27 transplant centers in Europe[15] and 25 in North America[16] (Table 1). In these studies, DLI induced a complete remission (CR) in about 64 percent of the patients with CML and in 20 to 38 percent of the patients with AML or MDS. In patients with CML, the response rate was highest when lymphocytes were transfused in early cytogeneic relapse (79%) and lowest when they were transfused during accelerated-phase CML (hematologic relapse)or blast crisis (19%). The median time to achieve a cytogenetic remission was 85 days (range, 28-241) for patients with CML (the time to achieve molecular remission can be prolonged) and 34 days (range, 16-99) for patients with AML.[16] Remissions were durable in patients treated for CML in the chronic-phase (probability of continuous remission, 87% at 3 years), but almost 100 percent of patients treated for advanced-phase CML later relapsed,[15] and less than 30 percent of them survived at the time of the analysis.[52] The median duration of remission in AML patients with complete responses was 17.9 months,[16] but the longest remissions lasted for 2 to 4 years.[52]

DLI can also induce CRs in patients with MM. In a retrospective study including 13 patients who underwent a total of 29 DLIs, 8 patients responded (4 patients achieved a partial remission and 4 achieved CR).[20] In this study, acute and chronic GVHD occurred in 66 and 56 percent of all patients and in 87 and 85 percent of the responders, respectively. However, unlike that in patients with early-stage CML, the response in patients with MM was not consistently durable. Moreover, the anti-tumor effect was essentially directed toward the BM, and development of plasmocytomas could occur while the BM and serum paraprotein showed evidence of a response.[20]

In contrast to those in myeloid forms of leukemia, responses were rare in ALL and in high-grade lymphoma (Table 1).[15,16,52] It has been speculated that the better response in chronic-phase CML may be explained by the fact that dendritic cells, the most potent antigen-presenting cells, are part of the leukemic clone in CML[52] and are capable of inducing a strong T-cell response.[70] In contrast, the malignant cells in accelerated-phase CML or in acute leukemia may be more inappropriate antigen-presenting cells

TABLE 1. Response to DLI in three studies of patients with relapsed hematologic malignancies

	Study 1[15]		Study 2[16]		Study 3[20]		Total of the three studies		
Disease	Evaluable patients	Complete responses	Evaluable patients	Complete responses	Evaluable patients	Complete responses	Evaluable patients	Complete responses	Percentage of complete responses
CML	111	73	55	33			166	106	64
Cytogenetic relapse	25	19	3	3			28	22	79
Hematologic relapse	72	53	34	25			106	78	74
Advanced-phase	14	1	18	5			32	6	19
AML	37	9	39	6			76	15	20
MDS	8	3	5	2			13	5	38
ALL	20	1	11	2			31	3	10
MM	7	1	4	2	13	4	24	7	29
Non-Hodgkin's lymphoma	2	1	6	10			8	1	13
Total	185	88	120	45	13	4	318	137	43

and may lead to the induction of anergy rather than to an anti-leukemic T-cell response.[71] The reasons for the observed differences in response rates among diseases should be further investigated. Finally, a recent report suggested that chemotherapy given after DLI did not nullify the ability of the lymphocytes to mediate GVHD as well as GVL effects.[72]

Complications of DLI

Complications of DLI include acute and chronic GVHD and transient marrow aplasia. Acute GVHD occurs in about 60 percent of the patients (grade 3 or 4 in about 20%) and is significantly correlated with CR.[16] Chronic GVHD also occurs in about 60 percent of the patients (extensive in 30%) and is also significantly correlated with response.[16] However CR may be observed in the absence of GVHD, which suggests that the GVL response may be independent of the clinical development of GVHD.[16,40,52] The risk of GVHD (but not the efficacy of DLI to induce CR) correlates with the dose of lymphocytes transfused. The MNC dose transfused varies between 0.1 to 11 × 10^8 per kg of body weight.[15,16] It is possible to reduce the risk of GVHD without impairing the GVL effect by starting with a low dose of T cells and increasing the dose in a stepwise fashion if there is no response.[13] It is interesting that the involvement of GVHD in the organs is different from that seen after HPC transplantation, and, in particular, the skin is less affected.[52]

Marrow aplasia, due to a direct cytotoxic effect of transfused lymphocytes on hematopoietic cells in the host, occurs in about 20 percent of the patients. It is significantly less frequent in patients with cytogenetic relapse (11%) than in those with hematologic relapse of CML (50%),[15] because residual hematopoiesis is still of donor origin in patients with cytogenetic relapse. Marrow aplasia resolved without treatment in about 50 percent of the patients and resolved with G–CSF in about 30 percent.[16] However, in case of severe pancytopenia, the transfusion of marrow or blood progenitor cells of the donor without further conditioning may correct myelosuppression,[15] with the possible exception of that in patients with chronic GVHD.

The primary cause of death after DLI is progressive disease, which is responsible for about 77 percent of deaths (Table 2).[15,16] Other causes include GVHD (8%), infection (5%), pancytopenia (4%), and the combination of GVHD and pancytopenia (2.5%).

Pretreatment factors influencing response of recurrent CML to DLI

Multivariate analysis of these two multicenter series[15,16] found that a pre-DLI status of chronic-phase (as opposed to more advanced disease) was favorable to a complete response to DLI. Another favorable factor was an interval between HPC transplantation and DLI of less than 2 years.[16] The role of T-cell depletion of the graft and of post-BM transplantation chronic GVHD is controversial. Finally, the T-cell dose transfused did not influence response to DLI.[15,16,52]

MANIPULATIONS OF DLI

Immunotherapy with escalating doses of donor lymphocytes

Immunotherapy with escalating doses of donor lymphocytes was evaluated in a prospective study of 22 patients with relapsed CML after allogeneic BM transplantation.[13] Patients received escalating doses of donor lymphocytes (from 1 × 10^5 to 5 × 10^8/kg) at 4- to 33-week intervals. Nineteen of the 22 patients achieved CR, and T-cell doses as low as 1 × 10^7 per kg were shown to achieve complete responses (in patients with molecular, cytogenetic, and even accelerated-phase relapse) with a low risk of GVHD (only 1/8 CR patients vs. 8/11 responders receiving >5 × 10^7/kg) (Table 3). In this study, and in accordance with previous studies,[15,52] there was no real evidence of a dose–response relationship in DLI. Low-dose therapy was primarily efficient in patients with molecular relapses.

Because there is a tendency for molecular positivity to come and go spontaneously, some caution should be observed in the interpretation of the role of DLI in correcting molecular relapses. Another recent study compared the efficacy and safety of a single transfusion of a relatively large dose of donor lymphocytes (bulk-dose regimen) and those of the transfusion of smaller doses repeated as necessary at 3-month intervals (escalating-dose regimen) in CML patients relapsing after allografting.[73] In this study, the CR rate at 2 years was higher (but not significantly so) and the risk of acute GVHD significantly lower in patients allocated to the escalating-dose regimen, even when the total number of cells administered was similar. However, a strategy in which increasing numbers of lymphocytes are transfused must be viewed cautiously, as the median time to achieve

TABLE 2. Causes of death after DLI in two studies

	Study 1[15]		Study 2[16]		Total of the two studies		
Evaluable Cause of death	Total patients	Evaluable deaths	Total patients	Evaluable deaths	Total patients	Percentage deaths	Percentage of patients who died
Progressive disease	109	47	124	70	233	117	50
GVHD	109	4	124	8	233	12	5
Pancytopenia	109	6	124	–*	233	6	3
GVHD and pancytopenia	109	4	124	–*	233	4	2
Infection	109	1	124	6	233	7	3
Hemorrhage	109	1	124	1	233	2	1
Other	109	1	124	2	233	3	1
Total	109	64	124	87	233	151	65

* In 4 patients, pancytopenia was associated with fatal GVHD or infection.

cytogenetic CR was 85 days and the time to achieve molecular CR was even greater.

Immunotherapy with DLIs depleted of CD8+ cells

Unlike T-cell depletion of donor marrow, which increases the risk of relapse (particularly in patients with CML), selective CD8+ T-cell depletion of the graft significantly reduced the risk of GVHD without losing the GVL effect.[37,38] These observations led investigators to transfuse donor lymphocytes depleted of CD8+ cells. One study included 10 patients with relapsed CML after allogeneic BM transplant (1 cytogenetic relapse, 4 in chronic phase, 2 in accelerated phase, and 3 in blast crisis).[14] Patients received DLIs depleted of CD8+ cells and containing $0.9 \pm 0.3 \times 10^8$ MNCs per kg ($0.6 \pm 0.4 \times 10^6$ CD3+CD8+ cells/kg). Six patients achieved CR (1/1 with cytogenetic relapse, 4/4 with chronic-phase CML, and 1/2 with accelerated-phase CML), whereas only three patients developed GVHD, which responded well to systemic steroid therapy. Recently, another study of 40 patients evaluated the response to 0.3 to 1.5×10^8 CD4+ cells per kg after depletion of CD8+ cells.[40] Fifteen (87%) of 19 patients with early chronic-phase CML achieved a complete cytogenetic response, 5 of 6 with MM obtained a more than 50-percent decrease in their paraprotein level, and 1 with MDS also responded. GVHD occurred in 6 (22%) of 27 patients receiving 0.3×10^8 CD4+ cells per kg and in 6 (55%) of 11 patients who received $>1.0 \times 10^8$ CD4+ cells per kg.

Transfusion of donor lymphocytes transfected with a suicide gene

Another interesting approach consisted of in vitro insertion of a suicide gene, the *HSV-tk* gene (which selectively phosphorylates gancyclovir, leading to its incorporation into DNA and causing cell death) into lymphocytes, allowing their elimination by gancyclovir if severe GVHD develops after DLI.[74-76] A clinical study using this approach was recently reported.[75] Eight patients who relapsed or developed EBV-induced malignancies after allogeneic BM transplant received genetically modified DLIs. An antitumor activity was observed in five patients, and two achieved a CR. GVHD developed in three patients, which could be efficiently controlled by the gancyclovir elimination of transfused cells. Moreover, transduced lymphocytes survived for up to 12 months. Unfortunately, the induction of a strong immune response against genetically modified cells and partial resistance to gancyclovir-mediated elimination of transduced cells in chronic GVHD was observed. This justifies the development of new nonimmunogenic and non-cell cycle-dependent suicide genes.[77]

Transfusion of IL-2-activated donor lymphocytes

Data from murine models and humans suggest that GVL effects may be increased by in vivo activation of lymphocytes with rHuIL-2. These data led Slavin et al.[17] to activate donor lymphocytes in vitro and/or in vivo by using rHuIL-2 in patients with tumor cells that are resistant to standard DLIs. Complete responses were obtained in 10 of the 16 patients (4/6 ALL patients, 0/3 AML patients, 5/6 CML patients, and 1 MDS patient). All 4 of these patients with ALL and 4 of these 5 patients with CML were still alive and free of disease 13 to 95 months (median, >2 years) after cell therapy. These results appear to be particularly interesting in patients with ALL, who classically respond poorly to DLI.

DLI to prevent relapse after allogeneic HPC transplantation

As DLIs are particularly effective when performed in early relapse,[15,16] it might be more efficient to give donor lymphocytes before relapse, a time when minimal residual disease is still present. However, DLIs given early after BM transplant are associated with a very high risk of severe acute GVHD, which precludes any improvement in the control of leukemia.[80] This dilemma between the risk of GVHD and the benefit of a GVL effect may be resolved by delaying DLI until graft-versus-host tolerance has been established.[15] This approach has been proposed after T-cell-depleted HPC transplantation.[81-83] Naparstek et al.[83] recently compared three schedules of DLI (early, starting on Day 1; intermediate, starting in Week 4; and late, starting in Week 8) for prevention of relapse in 108 patients after T-cell-depleted BM transplant. In this study, patients receiving late DLI had significantly greater survival and decreased risk of GVHD than did those given DLI earlier. Barrett et al.[82] recently demonstrated a strong GVL effect with a low risk of acute GVHD after the return of 2×10^6 T cells per kg on Day 30 and 5×10^7 T cells per kg on Day 45 after T-cell-depleted BM transplant, using cyclosporine prophylaxis. In this study, disease-free survival was comparable to that currently achieved in T-cell-replete transplants in similar patients.[82] However, the transfusion of 10^7 donor T cells per kg on Day 30 gave poorer results because of a high incidence of severe GVHD. Another recent report evaluated the feasibility of DLI 2 months after allogeneic BM transplant with selected CD34+ cells.[84] Of 16 patients included in this study, 6 underwent DLI. The first 3 received 5×10^6

TABLE 3. Results of a study[13] of immunotherapy with escalating doses of donor lymphocytes

	Response to DLI		Number of patients achieving CR at four T-cell doses per kg			
Stage of CML	Cases	CRs	1×10^7	5×10^7	1×10^8	5×10^8
Molecular relapse	2	2	2	2	0	0
Cytogenetic relapse	6	6	5	1	0	0
Chronic phase	10	9	0	3	2	4
Accelerated phase	4	2	1	0	1	0
Total	22	19	8	4	3	4

CD3+ cells per kg and all developed serious complications (grade 3 GVHD [n = 3] and severe aplasia [n = 1]). The other 3 received 1×10^5 CD3+ cells per kg, and only 1 developed GVHD. Taken together, these results suggest that DLI as a method of preventing relapse after allogeneic HPC transplantation is feasible, but its benefits remain to be demonstrated in large clinical trials.

Transfusion of tumor-specific CTLs

Several investigators have reported that DLI can cure EBV-induced lymphoproliferative disease after allogeneic BM transplant in more than 50 percent of patients.[66,68] However, several patients developed moderate to severe GVHD. To diminish the risk of GVHD, researchers at one institution[44,65,67,85,86] transfused specific anti-EBV donor T cells induced ex vivo with EBV-transformed B-lymphoblastoid cell lines. Donor-derived EBV-specific CTLs were transfused 45 days after allogeneic BM transplant in 50 patients at high risk of developing EBV lymphoma after T-cell-depleted transplantation from a matched unrelated donor or a mismatched related donor. None of the patients developed EBV-induced lymphoproliferative disease, whereas there was a cumulative risk of 11 percent in patients who did not receive this treatment. Moreover, two patients who were treated for clinically evident EBV-induced lymphopro-liferative disease achieved a prolonged remission after CTL transfusion.[44] Remarkably, no significant toxicity or GVHD was observed with this early posttransplant cell immunotherapy. The development of donor-derived CTLs against other antigens such mHA[43,54] or the BCR-ABL fusion product[42,59] is currently being investigated, and recently Falkenburg et al.[71] reported the achievement of CR in a patient with accelerated-phase CML by treatment with leukemia-reactive CTLs.

Cell immunotherapy after nonmyeloablative preparative regimens

There is considerable evidence that the usual high-dose preparative regimen frequently does not eradicate malignancy. Moreover, its high-level toxicity restricts the use of allogeneic HPC transplantation to young patients without other medical illnesses. On the other hand, the high risk of relapse after T-cell-depleted HPC transplantation, the even greater risk after syngeneic HPC transplantation, and the effectiveness of DLI in inducing CR in case of relapse after allogeneic HPC transplantation indicate that the main therapeutic component of allogeneic HPC transplantation may sometimes be due to the GVL effect rather than to the elimination of tumor cells through high doses of cytoreductive agents. These observations led several centers to perform allogeneic HPC transplantation after nonmyeloablative (but sufficiently immunosuppressive to allow engraftment), fludarabine-based preparative regimens to induce a GVL effect. In this approach, the posttransplant transient mixed chimerism may be successfully completed by DLI.[87,88]

More recently, Storb[89] and McSweeney[90] and their coworkers tried allogeneic HPC transplantation after a nonmyeloablative but highly immunosuppressive (200 cGy total body irradiation and cyclosporine plus mycophenolate mofetil) preparative regimen to prevent host-versus-graft reactions as well as GVHD. This approach permitted the achievement of stable mixed chimerism in patients with either nonmalignant or malignant hematologic disease. Secondary DLI in malignant disorders allowed the achievement of complete donor chimerism. The potential advantage of this technique would be a reduced risk of conditioning regimen-related toxicity, infection, or hemorrhage.

Although the preliminary results of "mini-transplants" (transplant after a nonmyeloablative conditioning regimen) are encouraging, long-term results in regard to leukemia-free survival as well as the incidence of chronic GVHD remain to be achieved in appropriate studies before this approach can be considered effective and not merely anecdotal.

CONCLUSION

DLIs have been used increasingly to treat leukemic relapse after allogeneic HPC transplantation, inducing CR in about 65 percent of the patients with CML and 25 percent of those with AML or MDS. The risks of DLIs include transient marrow aplasia and acute and/or chronic GVHD. DLIs are not associated with severe aplasia when given in early cytogenetic relapse, because residual hematopoiesis is still of donor origin. It is possible to maintain a GVL effect without GVHD by decreasing the number of T cells transfused to 1×10^7 per kg or by depleting donor lymphocytes of CD8+ cells. Preliminary observations have been generated for a number of newer approaches to DLI. In vitro transduction of donor WBCs with a suicide gene to eliminate donor lymphocytes in case of severe GVHD, transfusion of donor lymphocytes for the prevention of relapse, development of CTLs specifically recognizing tumor antigens, activation of lymphocytes by cytokines, and DLI after allogeneic HPC transplantation with a nonmyeloablative preparative regimen are promising new approaches that are currently being investigated.

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