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Selective depletion of marrow-T cytotoxic lymphocytes (CD8) in the prevention of graft-versus-host disease after allogeneic bone-marrow transplantation

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Abstract. In vitro depletion of mature pan-T lymphocytes has been widely and successfully used to prevent acute graft-versus-host disease (GVHD) after allogeneic bone-marrow transplantation (BMT). However, this procedure has been associated with a high incidence of graft failure and leukemic relapse. In this pilot study, we evaluated the efficiency of a selective depletion of human marrow T cytotoxic lymphocytes (CD8), a subset essential to induce GVHD in mice. Eleven patients with hematologic malignancies were included (7 HLA-matched BMT, 4 HLA-mismatched BMT). Marrow treatment with 7 anti-CD8 mAbs and rabbit complement resulted in a marked reduction of CD8+ lymphocytes from 15% (median value; range 7%-31%) to 1% (median value; range <1%-11%). Acute GVHD was not abolished by this procedure despite postgraft immunosuppression. One patient (HLA-mismatched BMT) rejected his graft and had a full autologous recovery. In conclusion, when compared to the data in the literature, CD8 depletion was shown to be less efficient than pan-T-cell depletion in the prevention of GVHD after allogeneic BMT and was still associated with a major complication associated with this procedure, i.e., graft failure.

Key words: Human allogeneic bone-marrow transplantation - Graft-versus-host disease - Cytotoxic lymphocytes - T-cell depletion.

In human bone-marrow transplantation (BMT), considerable interest has been devoted to the depletion of pan-T lymphocytes from the marrow transplant, as a result of data demonstrating the role of T-cells in experimental models of graft-versus-host disease (GVHD) [3-6, 14, 16].

It rapidly became evident that if pan-T-cell depletion was efficient in the prevention of human GVHD, a high rate of inacceptable complications, i.e., rejections and relapses, was related to this procedure [10-13, 17]. In the mouse model, it was suggested that different subsets of mature T lymphocytes were implicated in the various experimental situations that were able to induce GVHD: Lyt 1−, 2+ lymphocytes, a subset closely related to the human cytotoxic lymphocytes (CD8) [8], were responsible in most of the strain combinations of mice for GVHD elicited by minor histocompatibility antigens or MHC class I differences; L3T4 lymphocytes - a subset similar to the human helper (CD4) lymphocytes - and pan-T lymphocytes were mainly involved in experimental GVHD due to MHC class II differences [5, 16]. Furthermore, it has been shown in man that GVHD is associated with an increase in blood T-cytotoxic lymphocytes [1]. Thus, to prevent GVHD, while limiting the adverse effects of pan-T-cell depletion, in a pilot study we investigated the potential effects in human BMT of selective T-cell depletion limited to the subset of CD8-positive lymphocytes.

Patients and methods

Eleven patients with hematologic malignancies were included in the study: the first 9 patients were "high risk" for BMT because of the stage of their neoplastic disease and/or risk factors for GVHD (age > 30 years or HLA disparity with the donor); two further patients with "standard risk" were subsequently included.

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The median age was 23 years (range 11-40). Seven were recipients of HLA-matched BMT; 4 displayed HLA disparities with their donors (1 had one mismatched Ag; 3 had two mismatched Ag). All were prepared with "marrow ablative" conditioning regimens: 10 with cyclophosphamide (60 mg/kg × 2) and total body irradiation (TBI); 1 with busulfan (4 mg/kg × 4) and cyclophosphamide (50 mg/kg × 4) because of previous irradiation. TBI was done with a linear accelerator in 10 cases and with a cobalt source in 1 patient. A fractionated regimen was used in 6 patients at a cumulated dose of 12 Gy in 4 cases and 11 Gy in 2; 4 other patients received TBI in one single dose of 10 Gy. In each case, a low dose was used (< 5 Gy/min). Lung shielding was used in all patients at a maximum lung dose of 8 Gy. Patients received postgraft immunosuppression with cyclosporin A (9 patients), methotrexate (1 patient) or both (1 patient). Cyclosporin A was given IV at a starting dose of 2 mg/kg per day from day 1 to day + 15 or as soon as adequate oral intake was possible; cyclosporin A was then given PO at a dose of 10 mg/kg per day in two divided dose until day 150. Doses of cyclosporin were modified, if necessary, according to plasma dosages and values of serum creatinine. Methotrexate was used in association with cyclosporin A in patient 10 (15 mg/m^2 day + 1, 10 mg/m^2 days + 3, + 6, + 11) and alone for patient 8 in whom after day + 11, 10 mg/m^2 was administered weekly until day + 102. Protocols were approved by the scientific committees of the GEGMO and of each participating institution.

Anti-CD8 monoclonal antibodies (mAbs; B9 pool, gift from Immunotech Marseille, France) consisted in 7 mAbs directed against different epitopes of the human CD8 molecule [9]; mAbs were incubated 30 min at + 4 °C, with processed marrow cells at a final concentration of 0.8 µg/10 cells as previously described [14]. Rabbit complement was then added, undiluted (vol/vol), and incubated for another 30 min at + 37 °C. Cells were washed twice (PBS with 4% HSA) before infusion. CD8 lymphocytes and granulomonocytic progenitors (GM-CFC) were enumerated, respectively, by indirect immunofluorescence [2], and colony formation in semisolid medium using placental-conditioning medium.

Results (Table 1)

In vitro treatment of the marrow resulted in the effective removal of CD8 lymphocytes in all patients except one (patient 3): after depletion, the median of CD8-positive cells was 1% (range < 1%-11%). In absolute number, an average of 1.3×10^6 CD8-positive cells/kg were infused (range < 0.05 to 2.3); a median of 1.7×10^4 GM-CFC/kg was transplanted into the patients (range 0.4-8). One patient died early from resistant leukemia and infection. All the other patients who could be evaluated had a hematological recovery in a normal time range: median duration of granulocytopenia < 500/µl was 18 days (range 15-26) and median duration of thrombocytopenia < 50000/µl was 32 days (range 20- > 30). One HLA-mismatched patient (no. 11) rejected his marrow graft, but had a full autologous hematologic recovery, as evaluated by sex and erythrocytic markers. Of the 10 patients that could be evaluated, 4 developed acute GVHD, grade ≥ II. Two were fully HLA matched with the donor, but in 1 case a substantial amount of CD8 lymphocytes (11%) was reinfused with the graft (patient 3); 2 were HLA mismatched on two antigens. Two patients died from GVHD. Four other patients died: 3 from relapse and 1 from CMV pneumonia. Five patients are still alive with a median followup of 1460 days post-transplant (range > 1410- > 1520).

Discussion

These results show that the removal of donor CD8 lymphocytes is technically possible; in all but one of the cases studied, this was done without impairing the initial hematologic reconstitution after allogeneic BMT.

In such a small number of patients it is difficult to assess with certainty the efficiency of CD8 depletion in the prevention of GVHD; however, in several cases, consistent forms of GVHD occurred despite effective in vitro depletion and postgraft immunosuppression, even in HLA-matched situations. These observations suggest strongly that this form of selective T-cell depletion is not efficient in abolishing GVHD after human allogeneic BMT. This failure in the prevention of GVHD could be due to a small amount of CD8 lymphocytes that remained in the marrow transplant after the complement lysis: it has been effectively shown in the mouse model that the number of infused cytotoxic lymphocytes is critical in obtaining a maximal prevention of GVHD [5, 6, 16]. However, even in mice depletion of the T-cytotoxic lymphocytes cannot prevent GVHD in all cases of minor histoincompatibility. Korngold and Sprent have recently shown that there are considerable differences in the efficiency of the procedure between different strains of mice [7].

In our study, one out of ten patients that could be evaluated rejected his graft; thus, this major complication of pan-T-cell depletion is not completely prevented by selective depletion of CD8-T lymphocytes. As there was no control group in this pilot study, it is obviously impossible to assess the impact of this procedure on the relapse rate after BMT, one of the major complications of pan-T-cell depletion.

These data show that selective depletion of CD8 lymphocytes from the marrow transplant using complement lysis seems less efficient than pan-T-cell depletion in preventing GVHD [10-13, 17] and is still associated with an increased rate of graft rejection.

Table 1. Patient characteristics and outcome. *Abbreviations:* ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; CR, complete remission; CP, chronic phase; Cy, cyclophosphamide; Bu, busulfan; TBI, total body irradiation (dose in Gy); CSA, cyclosporin A; MTX, methotrexate; NUCL c, nucleated cells; GM-CFC, granulomacrocytic progenitors; Lymphoc, lymphocytes; Grc, granulocytes; Plts, platelets; a-GVHD, acute GVHD; CMV, cytomegalovirus

No.	Age (years)	Sex	Diagnosis and status	HLA disparity between donor and recipient	Conditioning regimen and immunosuppression	Collected/infused marrow			Post-BMT with		Maximum grade of a-GVHD	Outcome and survival (0/198)
						NUCL c ($kg \times 10^8$)	GM-CFC ($kg \times 10^4$)	CD8 Lymphoc (%)	Grc < 500/μl	Plts < 50,000/μl		
1	11	M	ALL relapse	None	Cy-TBI 2.4 × 5-CSA	2.9/2.3	4.1/0.6	15%/1%	22	32	0	Dead; relapse day 210
2	23	M	ALL relapse	None	Cy-TBI 2.4 × 5-CSA	3.8/2.4	0.9/0.4	7%/<1%	-	-	-	Dead day 13; persistent leukemia
3	32	F	AML CR1	None	Cy-TBI 2.2 × 5-CSA	2.2/1.3	4.4/3	15%/11%	20	<50	III	Dead day 50; GVHD
4	38	M	AML CR1	None	Cy-TBI 10 × 1-CSA	3.6/0.5	1.9/1.4	20%/<1%	15	25	I	Alive and well >1520 days
5	40	F	CML CP1	None	Cy-TBI 10 × 1-CSA	3.8/0.8	1.7/1.1	15%/2%	20	21	I	Alive and well >1500 days
6	18	F	CML CP1	None	Cy-TBI 2.2 × 5-CSA	2.7/0.9	6.7/3	8%/2%	17	41	II	Alive and well >1480 days
7	15	M	ALL CR1	None	Cy-TBI 2.2 × 5-CSA	5/3.4	9/8	19%/<1%	15	40	I	Alive and well >1410 days
8	25	M	AML CR2	A-DR-MLC	Cy-TBI 10 × 1-MTX	4.6/0.5	4/3	31%/3%	18	20	0	Dead CMV; pneumonia day 28
9	20	M	AML CR2	AB	Cy-TBI 10 × 1-CSA	2.6/0.5	-/-	13%/<1%	16	34	III	Dead day 103; GVHD
10	25	M	Hodgkin relapse	A-DR-MLC	Bu-Cy-MTX-CSA	3.8/1.9	4/2	8%/1%	18	22	II	Dead: relapse day 92
11	23	M	ALL CR1	DR-MLC	Cy-TBI 2.2 × 5-CSA	2.8/1.1	-/-	225/1%	26	44	0	Alive and well >1440 days, with full-autologous recovery

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