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Serological Inhibition of Graft Versus Host Disease: Recent Results in 28 Patients with Leukemia

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One major obstacle to the successful application of allogeneic bone marrow transplantation is the occurrence of immunologic complications when donor and recipient are not monozygous twins and express differences in their histocompatibility properties. Even after transplantation of HLA-identical and MLC-negative marrow grafts the occurrence of graft versus host disease (GvHD) cannot be excluded due to genetic differences not detected by present histocompatibility typing techniques. In the past, several experimental approaches have been designed to eliminate the GvH-reactive cell populations in the donor marrow by incubating the graft in vitro with specific antibody preparations whose stem cell toxicity had been absorbed by various tissues, in particular non-T-lymphocytes. Pretreatment of donor spleen marrow with absorbed anti-T cell antisera suppressed GvHD in over 90% of H-2 incompatible semiallogeneic mice preirradiated with 900 R [1]. Also monoclonal Anti-Th-1 antibodies have been effective in preventing GvH reactions in incompatible murine combinations [2]. A suppressing effect of GvHD by incubation with T-cell-specific antibodies could also be shown in a canine model transferring incompatible DLA homozygous marrow to DLA heterozygous lethally irradiated littermates [3]. Since then an anti-T-cell globulin for human cells with comparable serological characteristics has been developed [4]. The present report describes the effect of an incubation treatment with anti-T-cell globulin in 28 cases of bone marrow transplantation in acute leukemias.

The production and serologic characterization of anti-human-T-cell globulin (ATCG) was described elsewhere [5, 6]. ATCG was highly active against T-lymphocytes but did not crossreact with hemopoietic progenitors in the CFU-c test and the diffusion chamber test. Twenty-eight patients with acute leukemia were transplanted between February 1978 and May 1982 with bone marrow of HLA-compatible siblings. Twelve patients were transplanted by members of the Munich Cooperative Group of Bone Marrow Transplantation. These patients received a conditioning regimen including a combined chemotherapy and 1000-rad total body irradiation (TBI) applied by two opposite ^{60}Co sources. In nine cases the chemotherapy included BCNU, cytosine arabinoside, and cyclophosphamide; in three additional cases BCNU was omitted from the protocol. Sixteen patients were transplanted by members of the Tübingen Cooperative Group of Bone Marrow Transplantation, where they received a conditioning treatment with cyclophosphamide and 1000-rad TBI applied by a linear accelerator. In these cases the lungs were shielded to a reduced dose of 800 rad.

The pretransplant status and the treatment protocols before BMT of the described 28 patients are shown in Table 1. Seven of the patients were transplanted after the second relapse, three patients after the first, one patient after the third, and one patient after the fourth relapse. Fifteen patients were transplanted in remission. Five patients had acute lymphoblastic leukemia (ALL). Fourteen had acute myelo-

Table 1. Characteristics of 28 leukemic patients receiving ATCG-incubated marrow grafts

Leukemia status	1st	2nd	3rd	4th[a]	Total[a]
Relapse	3	7	1	1	12
Remission	7	6	2		15
Chronic phase					1
Type of leukemia					
Acute myeloid leukemia (AML)					14
Acute lymphoblastic leukemia (ALL)					10
Chronic myeloid leukemia (CML)					1
Erythroleukemia					1
Acute undifferentiated leukemia (AUL)					2
Treatment before BMT					
BCNU, araC, Cy, TBI-950R[b]					9
AraC, TBI-950R[b]					3
Cy, TBI-1000R[c]					16

[a] Number of patients
[b] Total body irradiation 950 R, performed with $2 \times {}^{60}Co$ sources
[c] Total body irradiation 1000 R performed with a linear accelerator, lungs shielded to 800 R

geneous leukemia (AML). Two other patients suffered from an erythroleukemia and an acute undifferentiated leukemia (AUL), respectively. One patient was transplanted during the chronic phase of chronic myeloid leukemia (CML).

In vitro treatment of the bone marrow was performed as follows: After preparation of bone marrow from the donor, it was first separated from erythrocytes and reduced to a smaller volume, followed by incubation with ATCG. In 11 cases, the marrow was separated using a Hemonetics cell separator. In 15 cases, the marrow was reduced by these techniques to about 300 ml. Two marrow preparations were left unconcentrated. In all cases the marrow was then incubated with ATCG in a final dilution of 1:200 (0.05 mg/ml) for 30 min at 4 °C under gentle agitation. After incubation, the marrow cells were infused without further delay. An average of 6.1 (cell separator), 4.1 (buffy coat), and 3.5 (no separation) $\times 10^8$ nucleated cells/kg body wt. were transferred. Details are shown in Table 2. In all cases the incubated cells were tolerated without severe side effects. Symptoms seen in some of these cases, like frequent pulse or transient fever, have also been described for transplantations without incubation.

Documentation of marrow engraftment and incidence of GvHD after incubation with ATCG are shown in Table 3. In general, engraftment and recovery of bone marrow functions after incubation treatment seemed to be not different from that in other transplantations. Engraftment was documented by bone marrow cellularity and rise of peripheral blood cell counts. Eleven of the 28 patients were sex mismatched, five showed major ABO blood group incompatibility, two were HLA-D different from the donor, and in two others the HLA-D compatibility was unclear. Twenty-six of the 28 patients showed an engraftment between days 12 and 26 post-transplantation, indicated by a rise in the

Table 2. In vitro treatment of bone marrow with ATCG

Concentration of bone marrow	Number of patients	Volume[b]	Nucleated cells[c]		Incubation with ATCG[a]
			Total ($\times 10^9$)	Per kg body wt. ($\times 10^8$)	
Hemonetics cell separator	11	310	25.8	6.1	1 : 200 4 °C, 30 min.
Buffy coat preparation	15	321	21.4	4.1	
No. concentration	2	1210	23.6	3.5	

[a] Total of 28 patients
[b] Mean values
[c] Final incubation concentration

peripheral granulocyte counts to values over 500/mm³. This range does not markedly differ from other groups undergoing BMT without marrow incubation treatment. Two patients did not receive a sufficient engraftment. One of these patients was one-way HLA-D different and died very early on day 21 with septicemia. In the other case, the HLA-D compatibility could not be clearly documented. This patient also showed persisting leukemia at autopsy, factors that may have prevented a sustained engraftment.

In nine out of 28 cases clinically manifest GvH reactions were detected. In five cases GvHD was restricted to skin reactions grade I–II. The skin reactions occurred between day 11 and day 16, persisting for 8–14 days, except two cases with delayed regression. A sixth case showed a grade I liver reaction in addition. In two cases more severe mainly chronic GvHD of skin and liver, reaching grade II–III, was seen. In a third case the course was complicated by manifestation of GvHD on the gut (grade III). Although these patients responded well to a treatment with corticosteroids, chronic GvHD could not be completely suppressed and all three patients died later from infections. Table 4 summarizes the survival an final outcome of the transplanted patients. Two patients died without sustained engraftment on day 20 and day 34 after transplantation of lethal infections. Six patients had a leukemic relapse and died because of this complication between day 106 and 500 posttransplantation. All six patients were cases transplanted after relapse. Three patients died of infections and interstitial pneumonitis (IP). In one case the IP was caused by infection with *Pneumocytis carinii*. None of the patients with IP showed any sign of GvHD. Two patients died of other infections during persisting chronic GvHD. One patient is alive with a testicular relapse. Ten of the 28 patients are alive in complete remission between day 15 and day 26 posttransplantation.

So far our human studies have concerned almost only HLA identical MLC nonreactive leukemic siblings, a situation with a still relatively high probabilitiy for GvHD. In sex-different patients (11 out of the 28 patients reported here) bone marrow has been reported to cause GvHD more frequently [7]. Sex difference appears to influence GvHD also in dogs [8]. The formal proof that ATCG prevents GvHD in MHC-identical patients requires, of course, a greater number of patients than have been listed in this report and should be confirmed in a randomized study. So far our data indicate that ATCG did not interfere with hemopoietic engraftment at dilutions known to be toxic for T cells. The encouraging results in several MHC incompatible animal models should provide impetus for elucidating chances and limitations of such an approach in situations with some degree of MHC incompatibility. Recent mouse experiments have shown in highly H-2 incompatible combinations that an effect of ATCG on GvHD may be further duplicated by addition of complement during in vitro incubation [2, 9].

Table 3. Donor-recipient differences, engraftment, and occurrence of GvHD in 28 patients receiving ATCG-incubated marrow grafts

Donor-recipient differences (No. of patients)			
HLA-D[a] 2/28	Major blood group 5/28	Minor blood group 6/28	Sex 11/28
Engraftment			
BM recovery 26/28	No. take 2/28	Day posttransplant 17±3	Range (days) 12–26
GvHD (No. of patients)			
Clinical grade I–II 6/28[b]	Clinical grade III 3/28	Clinical grade IV 0/28	

[a] Questionable in two other cases
[b] No histological confirmation in one case

Table 4. Fatal complications and survival in 28 patients after transplantation of ATCG-incubated marrow grafts

Patients died Cause of death	No. of patients	Survival time (relapse)[a]
No engraftment	2	20, 34
Leukemic relapse	6	106(83), 126(112), 198(123), 215(181), 233(168), 500(482)
Interstitial pneumonitis and infection	5	33, 38, 39, 64, 71
Acute GvHD	–	–
Infection during chronic GvHD	3	95, 211, 420
Other	1	312

Patients surviving Leukemia type	Status	Survival time[a]	Outcome[b]
ALL	3rd relapse	>1511	CR
AML	2nd remission	> 859	Testicular relapse
AML	1st remission	> 570	CR
AML	2nd relapse	> 532	CR
ALL	4th relapse	> 511	CR
ALL	2nd remission	> 332	CR
ALL	2nd remission	> 208	CR
AML	2nd remission	> 145	CR
AML	1st remission	> 75	CR
CML	Chronic phase	> 54	CR
AML	3rd remission	> 26	CR

[a] Days posttransplantation
[b] CR, Complete remission

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