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The Concept of GvHD-Suppression by In Vitro Treatment with Antisera*

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Progress in chemotherapy during the past decade resulted in considerable improvement in treatment of acute leukemias. Once the first relapse occurs, however, prognosis for prolonged survival is poor. The patient finally reaches an end stage of the disease which no longer responds to chemotherapy. In these cases the application of bone marrow transplantation opens up a second chance for a long lasting remission. Leukemic cells were eradicated by a short intensive course of chemotherapy and total body irradiation followed by a marrow infusion from a suitable donor to rescue the patient from the otherwise lethal marrow cell damage. One major obstacle to the successful application of allogeneic bone marrow transplantation is the occurence of immunologic complications when donor and recipient are not monozygous twins and express differences in their histocompatibility properties. The bidirectional immunologic barrier may cause a rejection of the marrow graft or a graft-versus-host reaction induced by immunocompetent T-lymphocytes in the donor marrow. Whereas graft rejection is supressed with increasing success by the intensive conditioning treatment of the leukemic patients, graft-versus-host disease (GvHD) is still a frequent problem of clinical bone marrow transplantation (Thomas et al. 1977). The disease is presumably attributable to a cytopathogenic reaction of immunocompetent donor T lymphocytes of the graft on host target tissues (Slavin and Santos 1973). Even after transplantation of HLA-identical and MLC-negative marrow grafts the occurence of 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 (Rodt et al. 1972; Trentin and Judd 1973; Müller-Ruchholtz et al. 1975). Pretreatment of donor spleen marrow with absorbed anti-T cell antisera which reacted with T lymphocytes but spared hemopoietic stem cells suppressed GvHD in over 90% of H-2 incompatible semiallogeneic mice preirradiated with 900 R (Rodt et al. 1972). This observation encouraged us to analyse the effect of absorbed anti-T-cell globulin (ATCG) more systematically in mice and dogs before applying it to the clinical situation.

The present report summarizes data on the prevention of a GvHD by ATCG which have been published elsewhere (Rodt et al. 1972, 1974, 1975, 1977, 1979, 1980; Thierfelder and Rodt 1978; Netzel et al. 1978a; Kolb et al. 1979a, 1980; Rodt 1979). Investigations will be reported in addition on the in vivo elimination mechanism of T cells which had been pretreated with ATCG in vitro. The survey also includes approaches to suppress GvHD by monoclonal antibodies as well as investigations on the chimerism, immunocompetence, and tolerance in the transplanted animals. Complete recovery of immune functions after transfer of ATCG-treated marrow will be

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shown in chimeras in which hemopoietic stem cells and host thymus differed by both H-2 haplotypes, including Ia specificities (Rodt et al. 1980). Finally the first results of an application of bone marrow incubation in the clinical situation will be described in which the marrow of 14 leukemic patients was preincubated with ATCG to prevent GvHD.

A. Animal Experiments

I. Studies in Mice

1. Effect of ATCG, Its Fragments, or Monoclonal ATh1.1 on GvHD

The principle and main test systems for the study of suppression of GvHD with ATCG in mice are shown in Fig. 1. The following figure (Fig. 2) measures the survival time of lethally irradiated F1-mice injected with parental spleen cells which had been pretreated with anti-T cell globulin. The latter was produced from rabbit ATG or anti-brain serum by absorption with mouse liver or kidney homogenate and B lymphoid cells from a B cell myeloma. The not incubated control group died early with GvHD. Survival of the irradiated mice signals T cell inhibition and preservation of donor-type stem cells.

The use of monoclonal antibodies in another promising modification of the described principle. Monoclonal anti-Theta (Th-1.1) was harvested in ascites form from the hybridoma cells, MRC 0×7 (a gift from S. V. Hunt and A. Williams) (Mason and Williams 1980). The hybridoma was made by fusing NS1 myeloma with speen cells from Balb/c mice immunized with purified rat Th-1. Spleen marrow of AKR/J (H-2k) incubated with monoclonal anti-Theta (Th-1.1) antibody suppressed mortality from GvHD in the majority of (C57BL/6×CBA)F1 (H-2b/H-2k) hybrids (Fig. 3).

Only incubation of the donor's spleen marrow with complete ATCG molecules prevented GvHD as is shown in Fig. 2. The Fab or $F(ab)_2$ fragments of ATCG did not increase the survival from GvHD. The importance of an intact Fc fragment in ATCG, even in the absence of complement, during incubation is also reflected by data on the fate of ^{51}chromium-labeled lymphnode cells pretreated with ATCG or its fragments and transferred to syngeneic, irradiated mice (Table 1). Opsonization of the labeled lymphocytes in the liver resulted in a high liver/spleen ratio. It occurred only if the T lymphocytes were covered with complete ATCG molecules. Fab or $F(ab)_2$ of ATCG did not cause opsonization of antibody-labeled lymphocytes. In this respect these fragments did not differ from normal rabbit globulin (NRG) (Table 1, exp. I). It is interesting that xenogeneic (rabbit) ATCG showed more pronounced opsonizing capacity than a monoclonal ATh1.1 antibody (L/S Ration ATCG 10.0, ATh1.1 6.7) as shown in Table 1, exp. II.

2. Immunocompetence, Chimerism, and Tolerance in the Recipients

The immune response of parent-to-F1 chimeras was tested using the following indicator system: C57BL/6 marrow cells were incubated with ATCG and transplanted to lethally irradiated (C57BL/6×CBA)F1 hybrid reci-

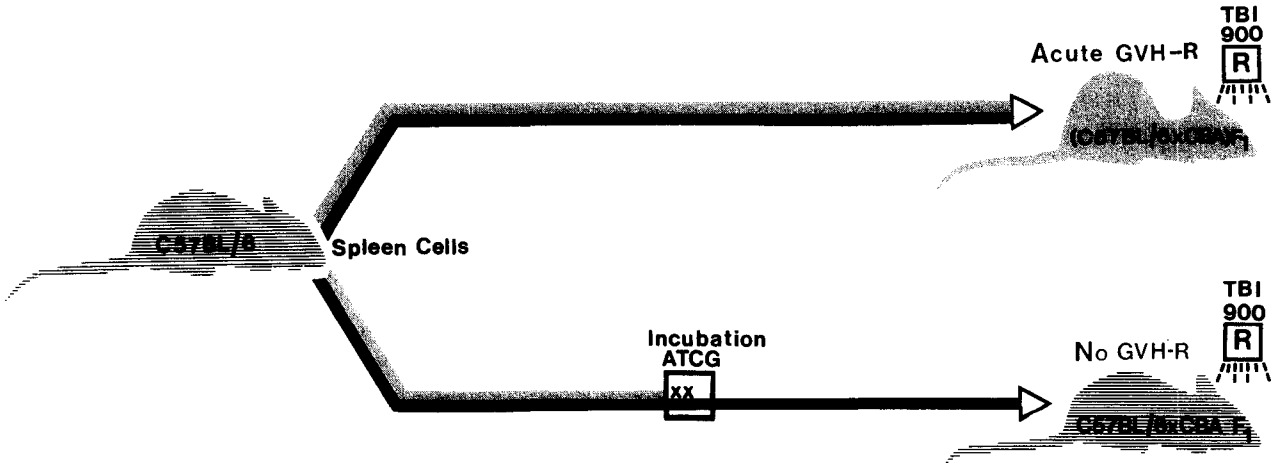

Fig. 1. Suppression of GvHD by incubation of the donor marrow with ATCG

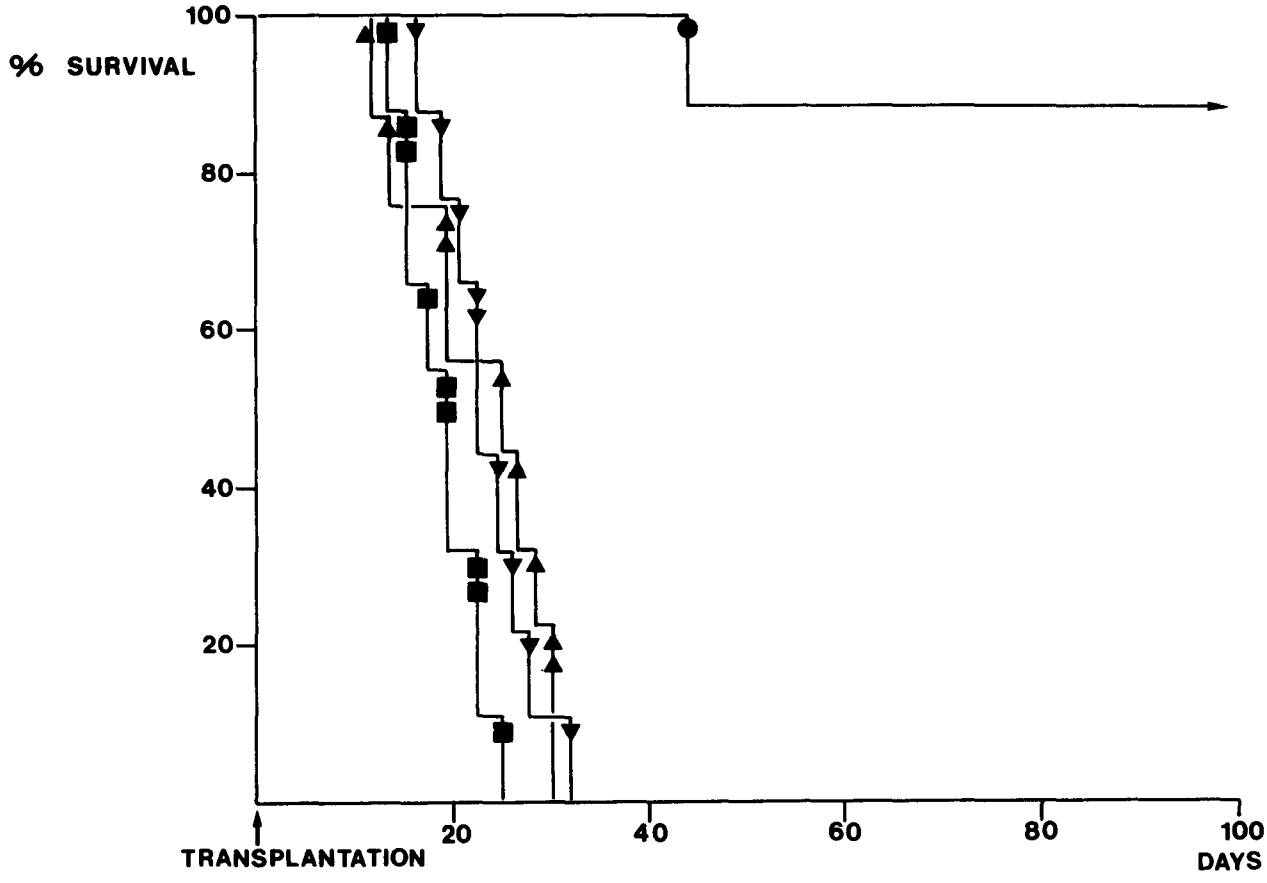

Fig. 2. Survival of lethally irradiated (C57BL/6 × CBA)F1 recipients of C57BL/6 spleen cells pretreated with ATCG (●) or its Fab (▼) or $F(ab)_2$fragments (▲). ■, no ATCG

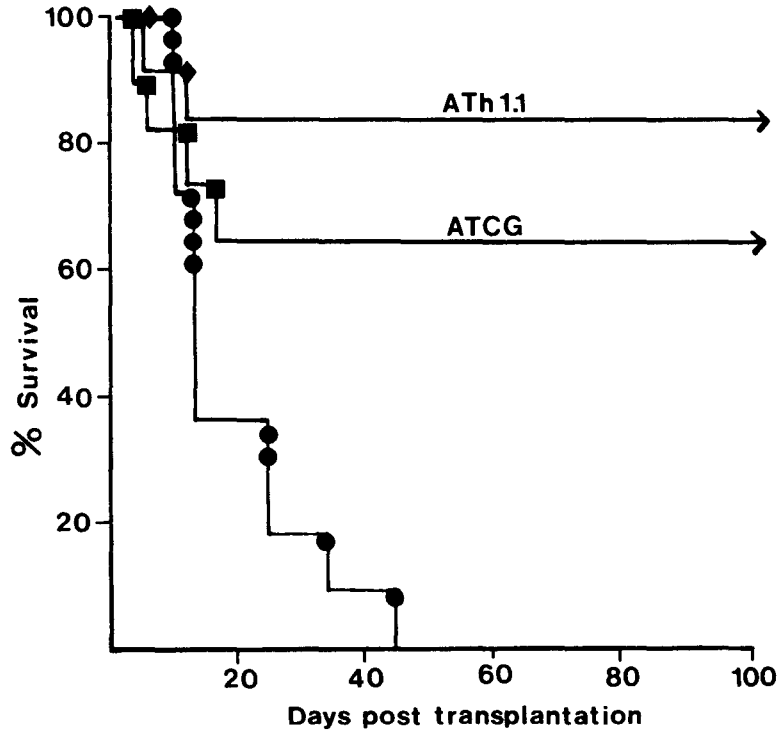

Fig. 3. Survival of lethally irradiated (C57BL/6 × CBA)F1 recipients of C57BL/6 spleen cells pretreated with ATCG (■) or monoclonal ATh1.1 (◆), ●, no ATCG

Exp. I	ATCG	ATCG F(ab)$_2$	ATCG Fab	NRG
Liver-spleen ratio[a]	8.6±1.2[b]	1.5±0.3	1.3±0.1	1.3±0.1
Exp. II	ATCG	ATh1.1(mono)	NRG	
Liver-spleen ratio[a]	10.0±1.7	6.7±0.4	1.7±0.1	

[a] calculated as follows: L/S = cpm(liver)-cpm(backgr.) / cpm(spleen)-cpm-(backgr.)
[b] mean ± standard deviation

Table 1. Lymphocyte opsonization in lethally irradiated (C57Bl/6×CBA)F$_1$ recipients of Cr^{51}-labeled lymphnode cells. Exp. I: (C57Bl/6×CBA)F$_1$ donor cells incubated with ATCG or its F(ab)$_2$ or Fab fragments or normal rabbit globulin (NRG). Exp. II: AKR donor cells incubated with ATCG, monoclonal A-Th1.1, or NRG

pients. The recipients were transplanted at 10 weeks of age (10-W) or at 70 weeks of age (70-W). The latter investigation was performed in order to determine whether the function of old involuted thymuses can be reactivated by bone marrow transplantation. Thymectomized and syngeneically transplanted recipients were used as controls. Sixty days after transplantation the survival of BALB/c skin grafts and the response against sheep red blood cells (SRBC) was analyzed. A recovered immune response against BALB/c skin and SRBC was found in the 10-W, 70-W, and syngeneic chimeras. In contrast thymectomized chimaeras rejected third party skin grafts in a delayed fashion or not at all. Not only cellular but also humoral primary or secondary immune response were low in this group.

The same restauration of immunity as in 10-W chimeras was observed in "old" (70-W) chimaeras whose thymus had already been involuted before transplantation. This suggests that involuted thymuses regain their original function in differentiating T lymphocytes no matter whether the latter derive from syngeneic or semiallogeneic precursor cells.

The recipients are almost complete donor cell chimeras. Cytogenetic studies in the bone marrow and cytotoxic testing of lymphnode and thymus cells revealed more than 90% donor-type cells in the respective tissues (Table 3). The donor cell population remains immunologically tolerant against the tissues of the recipient. When spleen cells of a C57BL/6 → (C57BL/6×CBA)F1 chimera are used for an adaptive transfer into the footpath of a second F1 host, no enlargement of the popliteal lymph node could be detected when compared with a syngeneic control. Normal C57BL/6 cells on the other hand produced a significant increase of lymphnode of this strain by a GvHR (Fig. 4).

An interesting question was whether stem cells can develop immunocompetence through a fully H-2 and Ia incompatible thymus. This was investigated with the following experi-

Table 2. Immune response of lethally irradiated (C57Bl/6×CBA)F1 recipients grafted with ATCG-incubated parental bone marrow against SRBC and BALB/c skin grafts

Donors	Recipients[a]	Rejection of BALB/c skin grafts (days)[b]	Response against SRBC[c]		
			Primary, direct	Secondary, direct	Secondary, indirect
CBL	CBL/CBA, 10 weeks of age	13	45±33[d]	62±67	236±114
CBL	CBL/CBA, 70 weeks of age	13	75±74	56±21	241± 97
CBL	CBL/CBA, thymectomized	>40	4± 3	3± 3	4± 4
CBL/CBA	CBL/CBA	11	221±56	42±37	389±210

[a] Lethally irradiated with 1000 rad, reconstituted with ATCG-incubated 20×10^{10} donor cells
[b] Mean rejection time of BALB/c(H-2^d) skin grafts, H-2 different to thymus and bone marrow cells
[c] Number of plaque-forming cells (PFC)/2×10^6 spleen cells
[d] PFC±S.D.

a) Percentage of donor cell mitoses (DCM) in the bone marrow

Recipients	%DCM[a]
Recipients transplanted at 10 weeks of age	97
Recipients transplanted at 70 weeks of age	95
Thymectomized recipients	94

b) Percentage of donor type lymphocytes (DL) in the lymphnodes (LyN) and thymus (Thy) determined with anti H-2 sera

Recipients	%DL in LyN[b]	%DL in Thy[b]
Recipients transplanted at 10 weeks of age	>90	100
Recipients transplanted at 70 weeks of age	>90	100
Thymectomized recipients	>90	–

[a] Average of 3 mice
[b] Average of 127 mice

Table 3. Hemopoietic chimerism of lethally irradiated (C57Bl/6×CBA)F1 recipients of C57BL/6 marrow cells incubated with ATCG

mental design: (C57BL/6×CBA)F1 recipients were thymectomized and transplanted with CBA-thymus tissue under the kidney capsule. After lethal irradiation these animals were reconstituted with T-cell-deprived C57BL/6 marrow cells (group 1). Thymectomized recipients without thymus transplantation (group 2) and (C57BL/6×CBA)F1 recipients with syngeneity between transplanted thymus and transferred bone marrow cells (group 3 and 4) served as controls. All different groups are indicated in Table 4. It was shown that the presence of a CBA thymus under the kidney capsule of irradiated (C57BL/6×CBA)F1 mice induced C57BL/6 precursor cells to immune reactivity which

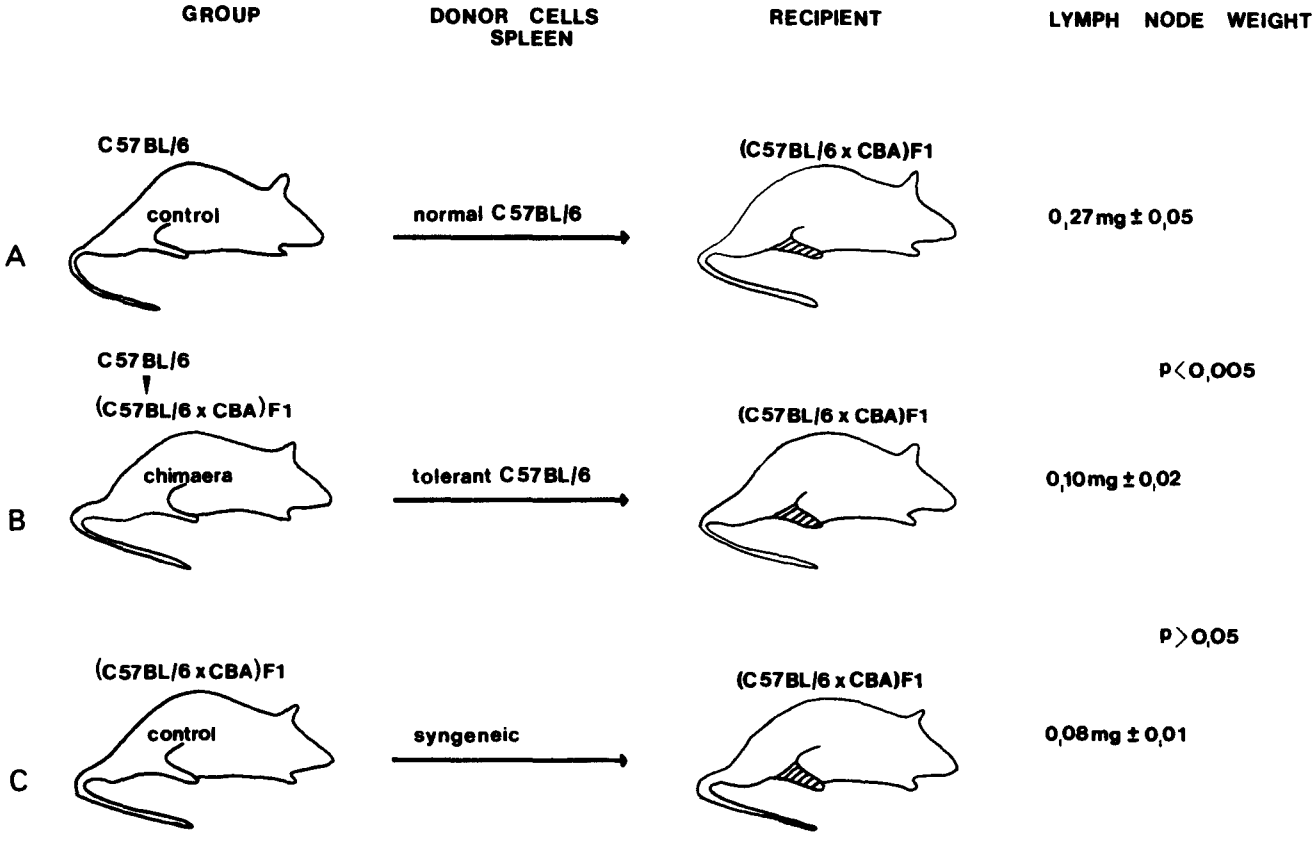

Fig. 4. Investigation of the tolerance of donor cells in the chimeras against the host C57BL/6 spleen cells of parent → F1 chimeras adaptively transferred to the footpath of a second F1 host. The enlargement of the popliteal lymphnode was determined in comparison with the transfer of normal C57BL/6 cells and syngeneic F1 cells

Table 4. Influence of a complete H-2 difference between transplanted thymus tissue and T-cell deprived bone marrow cells on the immune response of (C57BL/6×CBA)F1 recipient mice against BALB/c skin grafts and SRBC

Group	Transplanted		Rejection of BALB/c skin grafts[b] (days)	Response against SRBC[c]		
	Thymus[a] (H-2)	Bone marrow[b] (H-2)		Primary, direct	Secondary, direct	Secondary indirect
1	CBA (k)	CBL (b)	17	131± 90[d]	711±284	1110±306
2	– No –	CBL (b)	>49	9± 5	20± 18	29± 20
3	CBL (b)	CBL (b)	17	56± 24	601±304	893±403
4	CBA (k)	CBA (k)	16	413±201	389±189	1178±498

[a] Recipients = (C57BL/6×CBA)F1 hybrids
[b] Mean rejection time of BALB/c(H-2[d]) skin grafts, H-2 different to thymus and bone marrow cells
[c] Number of plaque-forming cells (PFC)/2×10[6] spleen cells
[d] PFC±S.D.

rejected BALB/c skin grafts and responded to sheep red blood cells in the same way as the syngeneic control groups.

II. Studies in Dogs

These studies were performed in order to establish the anti-GvHD effect of an incubation treatment in the dog, which is regarded a model of particular relevance for clinical bone marrow transplantation.

Removal of stem cell toxicity of crude anti-dog-thymocyte globulin was attempted by absorption with spleen cells from new-born puppies. Test systems for T cells in dogs are less well developed than those in mice and man. Also the lack of B lymphocyte cell lines complicated the production of dog ATCG. T cell specificity of dog ATCG was shown by selective immunohistologic staining of thymus-dependent T cell areas in lymphnodes. After absorption the ATCG did not reduce colonies in the CFUc test after incubation and cultivation of dog bone marrow. In vivo leucocyte and platelet recovery were followed after autologous transplantation of bone marrow preincubated or not with ATCG.

ATCG was then applied in canine allogeneic marrow transplantation. The results are shown in Table 5. In a randomly bred species such as the dog the combination of DLA homozygous donors and DLA heterozygous recipients is comparable to the murine parent-to-F1 situation with regard to the known MHC antigen. In this incompatible combination all 1000 rad irradiated recipients died within 27 days of GvHD when receiving untreated bone marrow grafts. Following in vitro treatment of the donor marrow with ATCG, two dogs died without sustained hemopoietic engraftment, in eight dogs the course of GvHD was remarkably delayed, and eight dogs had hemopoietic recovery without any sign of GvHD. These dogs showed complete chimerism, as indicated by the DLA type of lymphatic cells and by a change of the karyotype in the sex-different combinations.

B. Clinical Aspects

I. Characteristics of Anti-Human T Cell Globulin

The results of the animal experiments suggested an in vitro application for suppression of GvHD in humans, provided that a suitable ATCG for the clinical situation could be produced. Such antisera were raised in rabbits with human thymocytes. Cross reactions of this crude preparation were removed by absorption with liver-kidney homogenate, B lymphocytes from a pool of several CLL patients, and red cells. Table 6 gives complement fixation titers which are the highest against thymocytes. The lower titer against bone marrow cells is due to the low concentration of T cells in human bone marrow. No cross reactions with B lymphocytes, granulocytes, red cells, glomerulo-basal membrane and plasma proteins were found. Comparable results were obtained using the microcytotoxicity test. However, quantitative complement fixation has the advantage over microcytotoxicity that its titers

Table 5. Results of bone marrow transplantation in DLA-incompatible litter mate dogs with and without in vitro treatment of bone marrow with ATCG

No. of dogs	Donor-recipient differences	Marrow cells per kg body weight ($\times 10^8$)	ATCG-treatment of the marrow (charge No./concentration[a]	Death without sustained engraftment (No.)	Death on GvHD (No./mean survival)	Surviving without GvHD[b] (No.)
5	DLA-homozygous marrow into DLA heterozygous litter mates	4.1(1.9–6.2)	no	–	5/21 days	–
5	DLA-homozygous marrow into DLA-heterozygous litter mates	4.72.6–7.7)	No. 618/1:100	2	2/65 days	1
5	DLA-homozygous marrow into DLA-heterozygous litter mates	3.3(2.2–4.9)	No. 618/1:200	–	2/48 days	3
3	DLA-homozygous marrow into DLA-heterozygous litter mates	5.0(1.0–8.3)	No. 819/1:100	1	1/44 days	1
3	DLA-homozygous marrow into DLA-heterozygous litter mates	6.7(5.3–7.7	No. 819/1:200	–	–	3
3	DLA-homozygous marrow into DLA-heterozygous litter mates	5.7(4.8–7.0)	No. 819/ 1:400–800	–	3/32 days	–

[a] Final concentration in the incubation volume.
[b] Observation time of the surviving animals 3 months until>2 years.

are not influenced by the variable lysability of the different cell types under investigation. An important question was whether ATCG inhibited stem cell proliferation. Incubation of bone marrow with ATCG did not reduce the number of cells growing in diffusion chambers of forming colonies in agar when compared to the normal rabbit globulin control. Purification of ATCG over DEAE cellulose ion exchange chromatography and ultracentrifugation lead to a preparation lacking contaminants and immune complexes.

II. Application of ATCG to Clinical Bone Marrow Transplantation

Fourteen patients with acute leukemia were transplanted between February 1978 and May 1980 with bone marrow of HLA-compatible siblings. Ten patients were transplanted by members of The Munich Cooperative Group of BMT. These patients received a conditioning regimen including combined chemotherapy and 1000 rad of total body irradiation (TBI) applied by two opposite ^{60}Co sources. Four patients were transplanted in the Medizinische Universitätsklinik, Tübingen, where they received a conditioning treatment with cyclophosphamide and 1000 rad of TBI applied by a linear accelerator. In these cases the lungs were shielded to a total dose of 800 rad. The fractionation and concentration of bone marrow cells using a cell separator were performed as described by Netzel et al. (1978b). The technical approach of marrow incubation in vitro has already been published (Rodt et al. 1979). Six of the patients were transplanted after the second relapse, three patients after the first, and one patient after the third relapse. Four patients were transplanted in remission.

1. Reactivity on blood cell populations (Complement fixation)

Cells	Titer
Thymocytes	1:1064
Peripheral blood lymphocytes	1: 512
Bone Marrow cells	1: 32
B-lymphocytes (CLL)	neg.
Granulocytes	neg.

2. Reactivity on hemopoietic progenitors

Cells	Culture system	Effect[a]
AHTCG-incubated bone marrow cells	CFU-C diffusion chamber	No reduction of CFU No reduction of cell numbers and normal differentiation

3. Cross reactions

Antigen	Reaction
Erythrocytes	No
Glomerulo-basalmembrane	No
Plasmaproteins	No

4. Sterility, absence of pyrogenic substances, and no general toxicity

[a] Compared with normal globulin incubated controls.

Table 6. Properties of anti-human-T-cell globulin used for clinical bone marrow transplantation. For details see: Rodt et al., Experimental Hematology Today, Springer 1979

Five patients had the common type of acute lymphoblastic leukemia (cALL) and six had acute myelogeneous leukemia. Three other patients suffered from ALL of the T-cell type, an erythroleukemia, and an acute undifferentiated leukemia (AUL), respectively (Table 7).

Treatment of bone marrow with ATCG was preceeded by a concentration of the collected bone marrow to about 300 ml (Fig. 5). The recovery of CFUc was more than 80%. The use of a cell separator permitted the recovery of red cells for retransfusion into the bone marrow donor, a particular advantage when the donor is a child. Separation of red cells from the bone marrow is also advantageous in ABO-incompatible patients where hemolysis of donor red cells by preformed antibodies of the patient must be avoided. The final dilution of ATCG of 1:200 of a preparation of 10 mg/ml was derived from our studies in dogs. Injection of the ATCG-pretreated bone marrow over a period of about 20 min caused no immediate symptoms. Transient fever and chills were occasionally observed after bone marrow transfusion with or without ATCG.

In general, engraftment and recovery of bone marrow functions after incubation treatment did not differ from that of earlier transplantations without ATCG. Engraftment was documented by bone marrow cellularity and the rise of peripheral blood cell counts. Of 14 patients, 12 showed an engraftment between day 13 and day 26 after transplantation, which was indicated by a rise in the peripheral granulocyte counts to values between 500–1000/mm^3. This range does not markedly differ from other groups performing BMT without any marrow incubation treatment. Thomas et al. (1977) reported bone marrow engraftment after an average time of 16 days after transplantation in 91 cases with acute leukemia. Seven of the twelve patients were sex mismatched, three showed ABO blood group incompatibility, and in one patient sex and blood group and in another HLA-D and blood group were different. Two patients did not show substantial 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 in addition showed persisting leukemia at

Table 7. Clinical results in 14 patients with akute leukemias undergoing bone marrow transplantation. For prevention of GvHD the marrow was preincubated with ATCG

Patient	Donor-recipient-diff.			Conditioning[a]	Leukemia, Relapse	Marrow treatment			Clinic[d]				Hospital[f]
	HLA	Sex	ABO			Separat.	ATCG incub[b]	No. of cells[c]	Take	GvHD	Survival time	Outcome	
B.V.	no	+	no	BAC TBI-1000rad	cALL 3. Relapse	+	+	3,3	+	no	>872	Alive in remission	U-K-KI
K.G.	no	+	no	BAC TBI-1000rad	T-ALL 2. Relapse	no	+	4,0	+	no	38	Died on interstitial pneumonitis	M-KI-TU
B.S.	HLA-D?	no	A/B diff.	Ad AC TBI-1000rad	AML 2. Relapse	+	+	8,0	no	no	34	Died on sepsis after 2. BMT without incubation	K-P-KI
S.A.	no	+	A/O diff.	BAC TBI-1000rad	cALL 2. Relapse	+	+	6,0	+	no	190	Died on leukemic relapse	U-K-KI
A.G.	no	no	no	BAC TBI-1000rad	cALL 2. Relapse	+	+	6,0	+	skin (I–II)	215	Died on leukemic relapse	U-K-KI
B.K.	no	+	no	Ad AC TBI-1000rad	AML 1. Relapse	+	+	9,5	+	no	>424	Alive in remission	K-P-KI
T.G.	HLA-D?	+	no	Ad AC TBI-1000rad	Ery-L 2. Relapse	+	+	6,2	no	no	19	Died on pneumonitis	K-P-KI
A.E.	no	no	no	Cy TBI-1000rad[e]	AML 2. Remission	+	+	11,1	+	no	>220	Alive in remission	M-KI-UT
H.G.	no	+	no	BAC TBI-1000rad	AML 1. Relapse	+	+	4,3	+	no	49	Died on interstitial pneumonitis	M-KI-GH
D.S.	no	+	no	Cy TBI-1000rad[e]	AUL 1. Remission	+	+	3,4	+	no	64	Died on interstitial pneumonitis	M-KI-UT
Mu.H.	no	no	AB/O diff.	BAC TBI-1000rad	cALL 2. Relapse	+	+	9,1	+	no	98	Died on leukemic relapse	U-K-KI
Kr.G.	HLA-D HLA-DR ident.	no	O/B diff.	Cy TBI-1000rad[e]	AML 1. Remission	+	+	3,4	+	skin (II)	> 95	Alive in remission	M-KI-UT
H.J.	no	+	no	BAC TBI-1000rad	ALL 1. Relapse	+	+	7,7	+	no	> 53	Alive in remission	U-K-KI
M.I.	no	no	no	Cy TBI-1000rad[e]	AML 1. Remission	+	+	1,8	+	skin (II)	> 52	Alive in remission	M-KI-UT

[a] BAC=BCNU, Ara C, Cy / Ad AC=Adriblastin, Ara C, Cy
[b] Final concentration 1:200, incubation at 4° C for 30′
[c] cells $\times 10^8$ per kg body weight
[d] Methotrexate prophylaxis post BMT in all patients
[e] Lungs shielded to 800 rad
[f] U-K-KI=Universitätskinderklinik München;
K-P-KI=Kinderpoliklinik der Universität München;
M-KI-TU=I. Medizinische Klinik der TU München;
M-KI-GH=III. Medizinische Klinik, Klinikum Großhadern;
M-KI-UT=Med. Klinik, Universität Tübingen

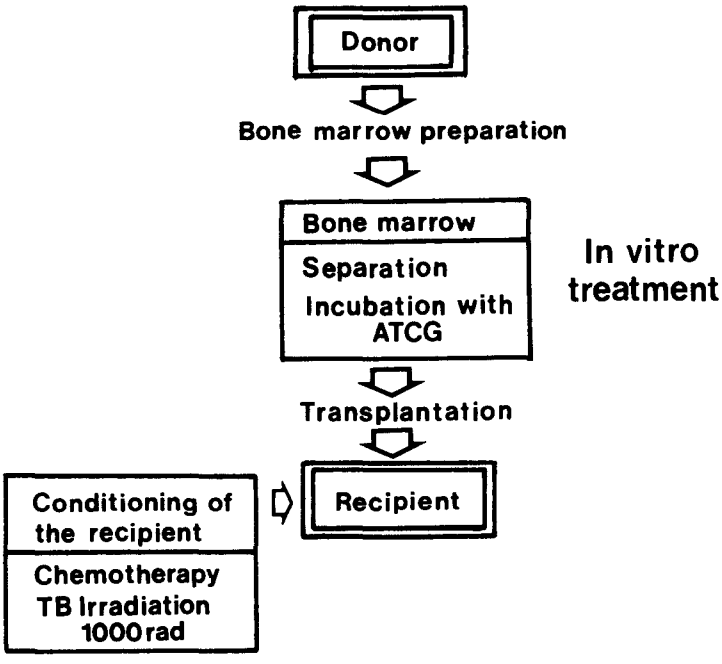

Fig. 5. Follow up of incubation treatment with ATCG in clinical marrow transplantation

autopsy, factors which may have prevented sustained engraftment. None of the patients died of severe GvHD. Three showed a transient skin rash lasting about 2 weeks consistent with a skin GvHD of grade I to II or II. The other patients showed no manifestations of acute GvHD on the skin nor on other tissues. No chronic reactions have been be detected so far, but observation time is too short for final estimations.

Table 7 summarizes the survival and final outcome of the transplanted patients. Of 14 patients ranging between day 872 and day 52 after transplantation, six are in complete remission. The three patients who showed leukemic relapse survived between 98 and 215 days. Two patients died without sustained engraftment on day 21 and day 34 after transplantation of lethal infections. Three patients had a leukemic relapse detected on days 67, 123, and 181 and died due to this complication on days 98, 197, and 215, respectively. All three patients were cases with cALL. The other three patients died of infections and interstitial pneumonitis (I.P.). In one case the I.P. was caused by infection with Pneumocytis carinii. None of the patients with I.P. showed any sign of GvHD.

C. Conclusions

Our data from mice indicate that only complete ATCG antibodies with their Fc fragments suppressed GvHD by opsonization of the donor's T lymphocytes in marrow recipients (Rodt 1979). The consequence of this T cell deprivation is not an immunologically crippled chimera. It has been shown that the marrow recipient's thymus, inspite of H-2 incompatiblity or even involution, induced the transplanted donor-type stem cells to differentiate T cells which were tolerant towards the recipient who was immunocompetent towards third party antigens. It was possible to transfer chimeric spleen cells without incubation to the footpath of secundary (C57BL/6×CBA)F1 recipients without causing local GvHD (Thierfelder et al. 1974). Immunohistologic studies (Rodt et al. 1980) showed that repopulation of T cell areas in lymphoid tissue depended likewise on the presence of the semiallogenic thymus in the host. It is of interest that recovery of immune response occurred eben in chimaeras where stem cells and thymus epithelium differed by both H-2 and Ia haplotypes. The restriction in the context of MHC of thymus and maturing T lymphocytes postulated for certain T cell functions by Zinkernagel et al. (1978) was not found to be a precondition for the recovery of immune response in our chimaeras, not even for virus-specific T cell cytotoxicity (Wagner et al. 1980). Monoclonal antibodies against T cell antigens were also effective in suppression of GvHD. Incubation with ATCG in the canine model was also found to prevent GvHD in a substantial number of DLA-D-A-B one haplotype incompatible dogs (Kolb et al. 1980). It is encouraging that no other immunosuppressive

agent had to be added and that in the surviving dogs no symptoms of GvHD were observed. There were, however, dogs which still died from GvHD inspite of an ATCG application. Whether this failure was due to the relatively high dilution of ATCG (1:100–200) remains to be shown, although the production of a good ATCG for dogs is more difficult than for mice and man as pointed out. Our studies on ATCG concerned mainly the suppression of GvHD due to major histocompatibility antigens. In mice ATCG also suppressed GvHD resulting from minor histocompatibility antigens, for instance after transfer of AKR spleen cells to irradiated CBA mice. GvHD in DLA-compatible dogs is absent or relatively weak. A considerable number of dogs would be necessary to document the effect of ATCG on GvHD in DLA identical MLC nonreactive dogs.

So far our human studies concerned only HLA identical MLC nonreactive leukemic siblings, a situation with a still relatively high probability for GvHD. In sex different patients (in 7 out of 14 patients reported here) bone marrow has been reported to cause GvHD more frequently (Storb et al. 1977). Sex difference appears to influence GvHD also in dogs (Kolb et al. 1979b; Vriesendorp et al. 1978). 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 study. So far our data only prove that ATCG did not interfere with hemopoietic engraftment at dilutions known to be toxic for T cells. The successful animal experiments, however, raise the hope that ATCG may also reduce the incidence of GvHD in clinical marrow transplantation.

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