Antilymphocytic Antibodies and Marrow Transplantation. XII. Suppression of Graft-Versus-Host Disease by T-Cell–Modulating and Depleting Antimouse CD3 Antibody Is Most Effective When Preinjected in the Marrow Recipient

By Josef Mysliwietz and Stefan Thierfelder

A hamster antimouse CD3 monoclonal antibody (MoAb) opened the way to experimental studies on the suppression of allograft rejection and cytokine-related morbidity after treatment with antibodies modulating the CD3/T-cell receptor complex (CD3/TCR). Because earlier attempts to suppress graft-versus-host disease (GVHD) in patients by in vitro treatment of donor marrow with anti-CD3 MoAb had remained inconclusive, we used a rat IgG2b antimouse CD3 MoAb (17A2) with fewer side effects to analyze suppression of GVHD in the mouse model. Detailed phenotyping of blood, spleen, and lymphnode T cells after the injection of 400 μg 17A2 in C57BL/6 mice showed 60% CD3 downmodulation and 50% T-cell depletion for spleen cells. Injection of these spleen cells, together with bone marrow cells, in fully mismatched preirradiated CBA mice delayed GVHD by only 6 days. Ex vivo treatment of donor cells with 17A2 was not effective. In contrast, conditioning of marrow recipients with a single injection of 17A2 delayed 50% GVHD mortality by 100 days and prevented GVHD altogether after prolonged treatment, with survivors showing complete chimerism and specific transplantation tolerance. This difference in antibody effect contrasts with earlier experiences with nonmodulating but more strongly T-cell–depleting MoAbs of the same isotype, which prevent GVHD no matter whether applied in vitro or injected into donor or recipient mice. Our data indicate that CD3/TCR reexpression in marrow recipients with no circulating 17A2 is the reason why ex vivo donor cell treatment with anti-CD3 MoAb is comparatively ineffective. Our data, which allow separate evaluation of cell-depleting and cell-modulating antibody activity, help to explain previous clinical failure to suppress GVHD and provide evidence in favor of conditioning the marrow recipient with anti-CD3 MoAb as a therapeutic alternative.

THE ANTI-CD3 monoclonal antibody (MoAb) OKT3[1-3] is the immunosuppressive anti–T-cell MoAb that has been studied in patients most extensively.[4,5] CD3 on T cells is in close contact with the T-cell receptor (TCR) heterodimer. The CD3/TCR complex plays an important role in signal transduction after binding to appropriately presented antigen. However, binding of anti-CD3 MoAb induces TCR signaling in an antigen-independent way.

In the field of clinical bone marrow transplantation, early studies reported reduction of graft-versus-host disease (GVHD) after in vitro treatment of the donor marrow with anti-CD3 MoAb.[6,7] Nevertheless, cases with severely acute GVHD were observed even when heterologous complement had been added to the antibody.[8] This seemed surprizing because OKT3 depleted circulating T lymphocytes when injected in patients with GVHD.[9] On the other hand, OKT3-induced reversal of kidney graft rejection was also seen in patients without clearly depressed T-lymphocyte counts, suggesting immunosuppression after dysfunction, sequestration of T cells, or modulation of the CD3/TCR complex[10,11] rather than pure T-cell elimination. The generation of a hamster antimouse-CD3 MoAb (145-2C11) was, therefore, an important step towards analyzing the in vivo consequences of antibody binding to CD3.[12-16]

We have long been interested in analyzing antibody-induced prevention of GVHD in mice, having studied mouse and rat isotypes of T-cell–depleting MoAb. Only strongly T-cell–depleting (>95%) rat IgG2b or mouse IgG2a anti–Thy-1 MoAb prevented GVHD in fully mismatched mice, no matter whether used in vitro[17] or applied to the marrow donor[18] or recipient.[19-21] Therefore, we were interested in studying the suppression of GVHD with modulating anti-CD3 MoAb whose suppression of skin graft rejection was accompanied by a lower degree of T-cell depletion.[13] We did not use the hamster anti-CD3 145-2C11 MoAb because of its well-documented cytokine-related morbidity.[16] It caused significant mortality even in our control mice grafted with syngeneic bone marrow. Fortunately, the rat antimouse-CD3 17A2, which cross-competes with the hamster 145-2C11 anti-CD3 MoAb on the CD3ε chain and similarly stimulates interleukin-2 (IL-2) production[22] and proliferation of T cells, was better tolerated. We measured an approximately 15% weight loss 3 days postinjection compared with 30% weight loss with the hamster antibody. However, delay of skin graft rejection was comparable. 17A2 is an IgG2b antibody of the isotype, which was shown to be the most immunosuppressive of the T-cell–depleting rat MoAb.[17,23,24] This allowed us to more directly compare 17A2 with primarily T-cell–depleting anti–Thy-1 MoAb. We found that anti-CD3 MoAb 17A2 in fact suppresses GVHD, even in fully allogeneic H2 mismatched mice, but only when injected and circulating in the marrow recipient at the time of bone marrow transplantation. Treatment of marrow donors or marrow donor cells was clearly less effective, probably because mature T cells in the marrow inocculum recovered their CD3/TCR and GVHD-inducing potential in marrow recipients when no 17A2 was circulating. In connection with earlier clinical failures, our conclusions help to explain why maximal suppression of GVHD by CD3 modulating MoAb cannot be expected when the latter are used for ex vivo T-cell treatment rather than for conditioning of the marrow recipient.

From the Institut für Immunologie der GSF—Forschungszentrum für Umwelt und Gesundheit, GmbH, Munich, Germany.

Submitted April 1, 1992; accepted July 22, 1992.

Supported by Sonderforschungsbereich 217 der Ludwig-Maximilians-Universität, München.

Address reprint requests to Josef Mysliwietz, MD, Institut für Immunologie, GSF, Marchioninistr. 25, D-8000 Munich 70, Germany.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" *in accordance with 18 U.S.C. section 1734 solely to indicate this fact.*

0006-4971/92/8010-0012$3.00/0

MATERIALS AND METHODS

Animals. C57BL/6 mice originally obtained from the Jackson Laboratory (Bar Harbor, ME) were raised and maintained in our breeding facilities. CBA and (C57BL/6 × CBA)F1 mice were bred from stock in our own laboratory. Three-month-old female animals were used as bone marrow recipients.

Antibodies and flow cytometry. Flow cytometry was performed as described previously,[21,25] using fluorescein (FITC)- or phycoerythrin (PE)-conjugated anti-L3T4 (Becton Dickinson, Heidelberg, Germany), anti-Lyt2 (Serva, Heidelberg, Germany), anti-B220 (Medac, Hamburg, Germany), anti–Thy-1.2 (Medac), MoAb, or polyclonal mouse $F(ab)_2$-antirat IgG (Dianova, Hamburg, Germany). 17A2 (rat2b-antimouse-CD3) was donated by Dr R. MacDonald (Ludwig Institute for Cancer Research, Lausanne, Switzerland),[22] anti–IL-2R (TIB 222; ATCC) and anti–NK-1.1 (HB-191; ATCC) were purified from culture supernatant using protein G chromatography. Ascitic fluids of anti-$H2K^k$ and anti-$H2D^b$ MoAb (Camon, Wiesbaden, Germany) were purified as described elsewhere.[26] MoAb 17A2, anti-$H2K^k$, anti–IL-2R, and anti–NK-1.1 were FITC-labeled as described elsewhere[27]; MoAb anti-$H2D^b$ was biotinilated as described previously.[28]

In vivo modulation of CD3 and T-cell depletion after 17A2 treatment. C57BL/6 mice were injected intravenously (IV) with a single 400 (20 mg/kg), 100, or 25 μg dose of purified anti-CD3 (17A2) MoAb. Heparinized blood, spleen, and lymphnodes (axillary, inguinal, mesenteric) from three mice were pooled 1, 3, 7, and 10 days after injection, a cell suspension was prepared, and leukocytes were counted. One million leukocytes were double-labeled using saturating concentrations of anti-L3T4(PE)/anti-17A2(FITC), anti-Lyt2(PE)/anti-17A2(FITC), mouse $F(ab)_2$-antirat IgG(FITC)/anti–Thy-1.2(PE), or anti–IL-2R(FITC)/anti-B220(PE) and, after standard NH_4Cl lysis of erythrocytes, were processed on FACScan (Becton Dickinson). The quantitative fluorescence measurements of CD3 expression were performed directly using FITC-labeled 17A2, or indirectly using purified 17A2 and mouse $F(ab')_2$ antirat IgG (FITC), as described previously.[25] FCSC Microbead Standards (Becton Dickinson) were used as fluorescence standards.

Serum concentration of 17A2 (anti-CD3) MoAb and anti-17A2 antibodies after in vivo treatment. Concentration of 17A2 (rat IgG2b) was determined in enzyme-linked immunosorbent assay (ELISA), using mouse-antirat IgG (Dianova) as the capture and the same peroxidase-labeled antibodies as the indicator. Sera from treated mice or purified 17A2 as the concentration standard were titrated twofold and the concentration of rat Ig in serum was calculated. To determine anti-17A2 antibodies, ELISA microtiter plates (Greiner, Nürtingen, Germany) were coated with purified 17A2. Sera from 17A2-treated mice or affinity-purified polyclonal mouse-antirat IgG (Dianova) as the concentration standard were titrated twofold and indicated with peroxidase-labeled rat-antimouse IgG (Dianova). The concentration of anti-17A2 antibodies was calculated from mouse-antirat IgG standard curves.

Bone marrow transplantation and X-radiation were performed as described in detail previously.[19] Briefly, in vivo treatment was as follows: groups of six irradiated (8.5 Gy) CBA ($H\text{-}2^k$) or (C57BL/6 × CBA)F1 recipient mice were injected IV with a given concentration of purified anti-CD3 (17A2) MoAb. A mixture of 5×10^7 spleen and 2×10^7 bone marrow cells from C57BL/6 ($H\text{-}2^b$) donors was injected 4 hours later. Alternatively, prospective C57BL/6 donors were injected with 17A2 or control antibody of the same rat IgG2b isotype, but irrelevant specificity (anti-DNP); bone marrow and spleen cells were removed 3 days later and injected into recipient mice at the same proportion as above. For in vitro treatment, a mixture of 35×10^7 spleen and 14×10^7 bone marrow cells from C57BL/6 mice was incubated in 1 mL minimal essential medium (MEM; Biochrom, Berlin, Germany) with 5× saturating concentration of purified 17A2 MoAb for 30 minutes at room temperature. Afterwards 150 μL of a mixture of 2×10^7 bone marrow and 5×10^7 spleen cells per mouse was injected into lethally irradiated CBA or (C57BL/6 × CBA)F1 recipient mice. Statistical evaluation of survival curves was performed using the logrank method.[29]

Test for chimerism and specific transplantation tolerance. Heparinized blood was collected from the tails of mice who received

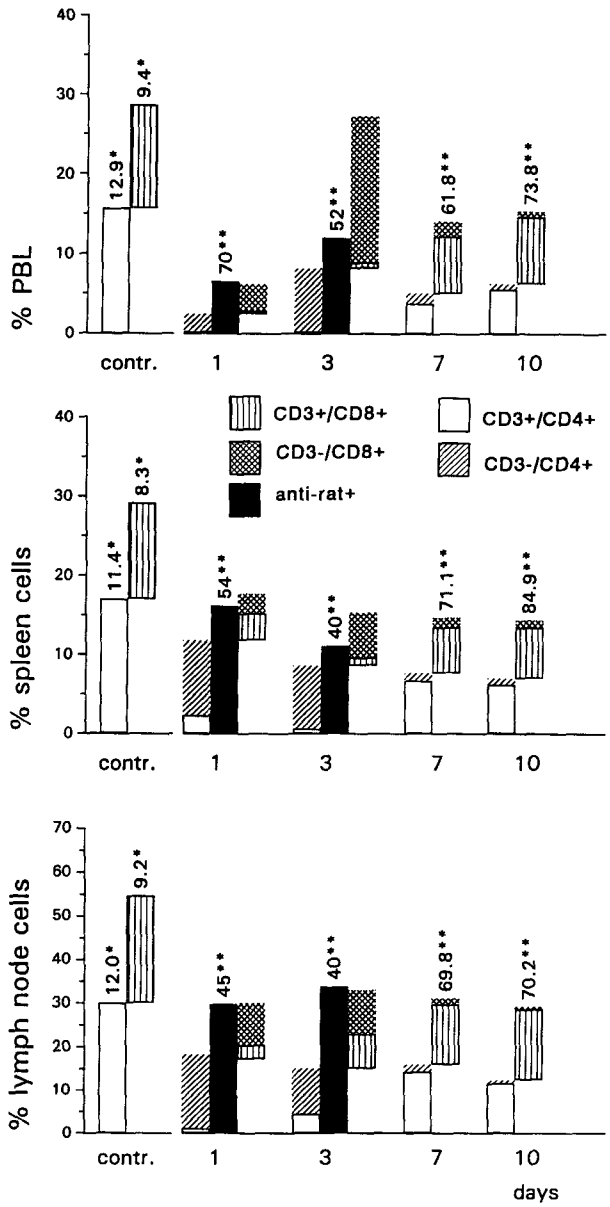

Fig 1. T-cell depletion and CD3 antigen modulation in lymph node, spleen, and blood of C57BL/6 mice 1, 3, 7, and 10 days after a single IV injection of 400 μg of 17A2 (anti-CD3 MoAb). Double-color FACS analysis and quantitative fluorescence measurements were performed as described in Materials and Methods. (*) CD3 antigen expressed as number of directly (FITC) labeled 17A2 molecules bound per cell ×10³. (**) CD3 antigen downmodulation: diminution of fluorescence intensity of T cells (mean of $CD4^+$ and $CD8^+$) from 17A2-injected mice expressed as a percentage of untreated control.

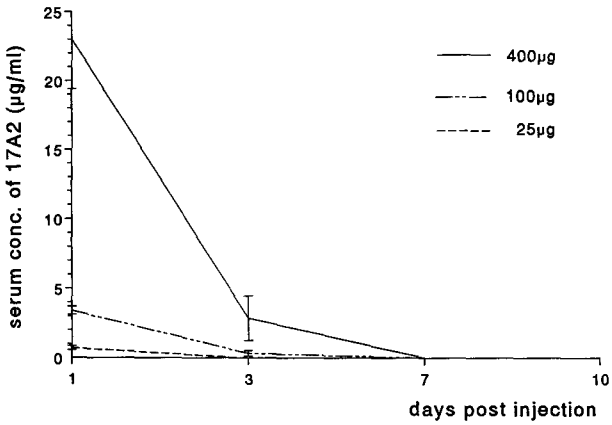

Fig 2. Serum concentration of 17A2 (anti-CD3) 1, 3, 7, and 10 days after IV injection of C57BL/6 mice with 400 µg, 100 µg, or 25 µg of 17A2 was measured in ELISA as described in Materials and Methods.

bone marrow transplants and the cells were double-labeled with anti-$H2^k$ FITC and biotinylated anti-$H2^b$ MoAb. After probing with avidin-PE and FACScan processing, live lymphoid cells were evaluated and compared with cells of untreated control mice. Bone marrow-reconstituted mice were tested for specific tolerance 50 days after transplantation. They were grafted with marrow donor-type (C57BL/6) and third-party (Balb/c) tail skin grafts, both placed on the lateral thoracic wall. Dressings were removed on day 12 and grafts were inspected every day for signs of rejection.

RESULTS

Suppression of GVHD by anti-CD3 MoAb (17A2) was tested after antibody treatment of marrow donors or recipients or of donor cells in vitro. Changes in T-cell depletion and modulation after treatment of C57BL/6 donor mice were measured by detailed immunophenotyping.

T-cell depletion with anti-CD3 MoAb. Single injections of 400 µg 17A2 in C57BL/6 mice produced a reduction of T cells, which were measured on day 1, 3, 7, and 10 postinjection in blood, spleen, and lymphnode cells (Fig 1). Cell depletion was most pronounced in blood (80%) on day 1 and approximately 50% T-cell reduction was measured in spleen and lymphnode cell suspension pooled from axillary, inguinal, and mesenteric lymphnodes. Splenic T cells had reached a 50% reduction by day 3, at which time peripheral blood lymphocytes (PBL) T-cell counts had already increased, although CD3 was still modulated (see below). T-cell counts on days 7 and 10 had stabilized at about half the normal values. Virtually the same pattern was observed after a single injection of 100 µg 17A2. Markedly diminished T-cell depletion and modulation were noted in blood, lymph nodes, and spleen after the injection of 25 µg 17A2 (data not shown).

Fifty days after a single injection of 400 µg of 17A2, $CD4^+$ cells had reached normal values; $CD8^+$ cells recovered slowly, reaching some 65% to 75% of untreated controls (data not shown).

T-cell modulation after anti-CD3 MoAb. Figure 1 shows expression of CD3 on $CD4^+$ and $CD8^+$ T cells. Reduction of CD3 expression on T cells was measured by staining 17A2 after incubation with FITC-labeled mouse $F(ab')_2$-antirat Ig secondary antibody and/or by using FITC-labeled 17A2 directly. Fluorescence was related to FCSC Microbead Standards. The use of sensitive double staining with mouse $(Fab')_2$-antirat IgG(FITC) and mouse anti–Thy-1(PE) was crucial for detection of in vivo 17A2-coated cells, showing strongly diminished CD3 expression. The sensitivity threshold using other reagents, such as complete goat-IgG antirat Ig or even IgG mouse-antirat Ig, was not sufficient. On day 3 postinjection of 400 µg, the day when a mixture of spleen cells and bone marrow cells was used for

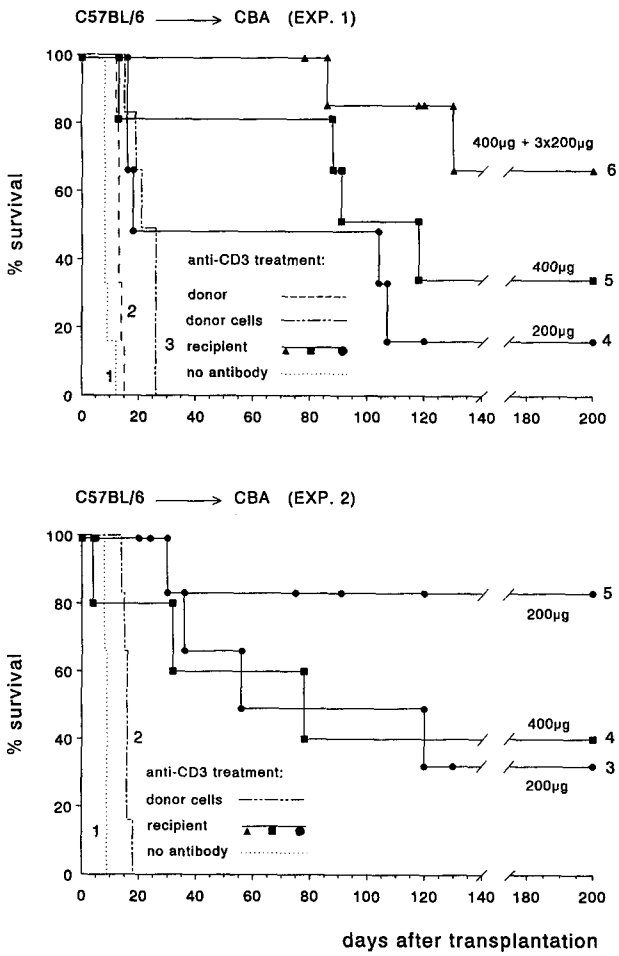

Fig 3. Survival of lethally irradiated CBA recipients (groups of 6 mice) of 5 × 10^7 spleen cells together with 2 × 10^7 bone marrow cells from C57BL/6 donors. Recipient: CBA recipients (400 µg + 3 × 200 µg) received 400 µg 17A2 IV 4 hours before transplantation of BM/spleen from C57BL/6 mice and additionally 200 µg 17A2 IV 3, 6, and 9 days after transplantation; the other recipients received single injections of 400 µg or 200 µg 17A2. Donor: C57BL/6 donor mice received 400 µg IV 17A2; spleen and bone marrow cells were prepared 3 days later and transplanted into CBA mice. Donor cells: bone marrow and spleen cells from C57BL/6 donors were incubated in vitro with 17A2 and transplanted into CBA recipients. No antibody: survival of control mice grafted with untreated bone marrow and spleen cells. Analysis of the survival curves by the logrank method gave the following *P* values of statistical significance: experiment 1: 1 *v* 2, *P* = .008; 1 *v* 3, *P* = .010; 1 *v* 4, *P* = .012; 1 *v* 5, *P* = .011; 1 *v* 6, *P* = .005; 4 *v* 6, *P* = .057; 5 *v* 6, *P* = .202; experiment 2: 1 *v* 2, *P* = .010; 1 *v* 3, *P* = .014; 1 *v* 4, *P* = .076; 1 *v* 5, *P* = .003; 3 *v* 5, *P* = .122; 4 *v* 5, *P* = .179.

Table 1. Blood Cell Phenotype, Antibody, and Antiantibody Concentration in Serum After Prolonged Treatment of Bone Marrow Transplanted Mice With Anti-CD3 (17A2) MoAb

	% of Positive PBL*						Serum Concentration (μg/mL)	
Day†	$CD3^+$ $CD4^+$	$CD3^-$ $CD4^+$	$CD3^+$ $CD8^+$	$CD3^-$ $CD8^+$	$H2b^+$	$NK1.1^+$	17A2	a-17A2
4	0.0	3.9	0.0	9.6	97.7	17.9	6.5	0.13
8	0.7	0.5	5.1	2.5	ND	16.9	0.7	0.09
15	0.9	0.1	1.4	0.4	ND	14.0	0.0	0.0
50	5.3	0.0	1.4	0.0	97.9	21.8	ND	ND
200	7.7	0.0	6.3	0.0	98.7	12.9	ND	ND
Untreated	14.1		11.9		99.1	6.9	ND	ND

CBA recipients were injected with 400 μg 17A2 IV 4 hours before transplantation of bone marrow/spleen cells from C57BL/6 mice and additionally with 200 μg IV on days 3, 6, and 9 after transplantation.

Abbreviation: ND, not determined.

*Heparinized tail blood from four to six animals was pooled, double-stained, and processed in FACScan as described in Materials and Methods.

†Days after last injection of 17A2.

transplantation, all splenic $CD4^+$ and $CD8^+$ cells were saturated with circulating 17A2; CD3 downmodulation can be assumed because the concentration of bound 17A2 was 60% lower than for untreated controls. The diminished antigen density was still seen, even after 17A2 had disappeared from serum on days 7 and 10 after treatment (Figs 1 and 2). Complete modulation must have occurred on PBL because approximately 40% to 50% of PBL on day 3 post injection of 100 or 400 μg 17A2 did not stain with directly labeled 17A2 nor with FITC-labeled antirat Ig secondary antibodies. They had lost their CD3 antigen completely, at least below the threshold of sensitivity of the FACS. During in vivo treatment with 17A2, we did not find increased expression of IL-2R on T cells as reported for 145-2C11.[14]

Effect of anti-CD3 MoAb on GVHD. Transplantation of 5×10^7 spleen cells together with 2×10^7 bone marrow cells to irradiated, minor and major MHC class I and II-mismatched CBA mice induced acute mortality, which was only slightly delayed if donor or donor cells were pretreated with anti-CD3 MoAb (Fig 3). In contrast, the injection of 400 or 200 μg antibody in marrow recipients 20 hours after irradiation and 4 hours before transplantation suppressed GVHD mortality by about 50% on day 100 posttransplantation and 20% to 40% on day 200. In one experiment, permanent survival of 80% of chimeras was observed after single injection of 200 μg 17A2 (Fig 3, experiment 2). Prolonging antibody treatment by further injections of 200 μg on days 3, 6, and 9 after transplantation led to long-term survivors with 90% to 97% chimerism on days 50 and 100. Compared with untreated controls, GVHD suppression in 17A2-treated recipients was statistically significant ($P < .05$) for all above described treatments. The differences in survival resulting from dose of 17A2 or prolonging of treatment were not statistically significant (Fig 3). Chimeras showed long-lasting changes in blood T-cell frequency (Table 1). Even 50 days after the last injection of 17A2, the frequency of $CD4^+$ cells was reduced to about 50% and $CD8^+$ cells were reduced to 10% of untreated controls. On day 200 posttransplantation, recovery of blood $CD8^+$ cells improved to 50% of untreated controls; percentage of splenic $CD4^+$ cells had reached control values and splenic $CD8^+$ cells were reduced by 65%. C57BL/6-into-CBA chimeras (chimerism was proved on days 30, 50, and 100 posttransplantation and regularly found to be greater than 90%), grafted 50 days after bone marrow transplantation with donor C57BL/6 or third party (Balb/c) skin, accepted donor skin and promptly rejected third-party skin. Besides changes in the frequency of $CD4^+$ and $CD8^+$ lymphocytes, we regularly observed an increase in donor NK 1.1 cells (Table 1); however, this was not predictive for GVHD in our mice. The formation of antiantibodies against 17A2 was negligible in these experiments (Table 1).

We repeated the GVHD experiments with C57BL/6 donors in semiallogeneic (CBL/6 × CBA)F1 recipient mice. This donor-recipient incompatibility is notoriously easy to overcome. Even the injection of normal rabbit Ig causes some delay of GVHD. Again, conditioning of recipient mice with 17A2 was most effective. Pretreatment of donors or donor cells ex vivo showed a clearly delayed, but 100% mortality (Table 2). Taking advantage of the sensitivity of this mouse model, we titrated the spleen cells from donors pretreated with an MoAb of irrelevant specificity in the

Table 2. Effect of Anti-CD3 MoAb on Suppression of GVHD in the C57BL/6-to-(C57BL/6 × CBA)F1 Combination

Anti-CD3 Treatment	Survival (%)*		
	Day 30	Day 50	Day 100
Donor cells†	66	50	0
Donors‡			
400 μg	100	66	0
Recipients§			
400 μg	100	100	83
200 μg	83	83	50
100 μg	100	83	66
25 μg	83	50	33
Untreated control	0	0	0

*Groups of 6 (C57BL/6 × CBA)F1 mice were irradiated with 9 Gy 24 hours before transplantation of 5×10^7 spleen cells and 2×10^7 bone marrow cells of C57BL/6 donors.

†Bone marrow and spleen cells from C57BL/6 donors were incubated in vitro with 17A2 and transplanted.

‡Donors were injected IV with 400 μg 17A2; bone marrow/spleen cells were prepared 3 days later and transplanted.

§17A2 injected IV at indicated dose 4 hours before transplantation.

semiallogeneic GVHD model. The cell-dose–dependent delay of GVHD was correlated to a delay of GVHD after the transplantation of 5×10^7 spleen cells from donors who had been pretreated with 17A2 (Fig 4). Because 17A2 induced 50% T-cell depletion in the spleen (see above), we expected a delay of GVHD mortality corresponding to the delay after the transfer of 2.5×10^7 spleen cells of our control mice. However, anti-CD3 MoAb delayed GVHD significantly more ($P = .009$, Fig 4). These results support the conclusion that immunosuppression by 17A2 cannot be explained by its T-cell depletion alone. The CD3/TCR modulation must at least have played a role.

DISCUSSION

The present observations concern an interrelationship between antibody action and type of conditioning for suppression of GVHD. Extending experimental studies on antimouse CD3 MoAb to the suppression of GVHD, we show in the mouse GVHD model that this modulating antibody is relatively ineffective in ex vivo T-cell treatment, but successful when used for conditioning bone marrow recipients. This difference in immunosuppressive effect contrasts with that of primarily T-cell–depleting MoAbs, which are equally effective in both types of treatment.[17,19] Whereas purging with anti–T-cell antibodies depends on their Fc-parts,[30] an important anti-CD3 antibody effector function extends to its variable T-cell–binding part that causes downmodulation of the CD3/TCR complex. $F(ab')_2$ fragments of anti-CD3 MoAb have thus been shown to conserve much of its immunosuppressive activity by prolonging survival of skin allografts.[15]

In the present study, pretreating of C57BL/6 donor mice with 17A2 resulted in dose-dependent T-cell elimination and downmodulation of the CD3/TCR receptor complex, which were most pronounced in blood and spleen cells. We transplanted spleen and bone marrow cells of mice injected with up to 400 μg 17A2 3 days earlier. At this time, splenic T cells had decreased to about 50%. They also showed a reduction in CD3 expression of about 60%, as measured by binding 17A2 (Fig 1). The spleen T cells, despite their reduced number and TCR downmodulation, hardly delayed acute GVHD mortality at all (Fig 3). In vitro tests for T-cell function, in which spleen cells from mice injected with anti-CD3 MoAb 145-2C11 and tested for generation of cytotoxic T-lymphocyte–mediated lysis (CTL) of complete MHC-disparate target cells, showed no CTL activity for at least 5 weeks posttreatment.[13] Clearly, in vivo conditions for GVHD-inducing T cells must be quite different. Other factors, eg, preirradiation of marrow recipients, cytokines, or upregulation of target antigens, may have contributed to in vivo T-cell stimulation and early recovery of graft-versus-host response. On the other hand, successful marrow-recipient conditioning—not only in the notoriously 'easier' parent-to-F1 system, but also in fully mismatched CBA mice—proved the potential anti-GVHD effectiveness of 17A2. It underscores the importance of circulating anti-CD3 MoAb for maintaining inhibition and dysfunction of donor cell TCR in the early stages after transplantation. It is conceivable that T-cell–depleting antibodies like anti–Thy-1 MoAb (and we found the same for antimouse CD4/CD8 antibody pairs [data not shown]) are equally effective in donor, donor-cell, or recipient treatment as long

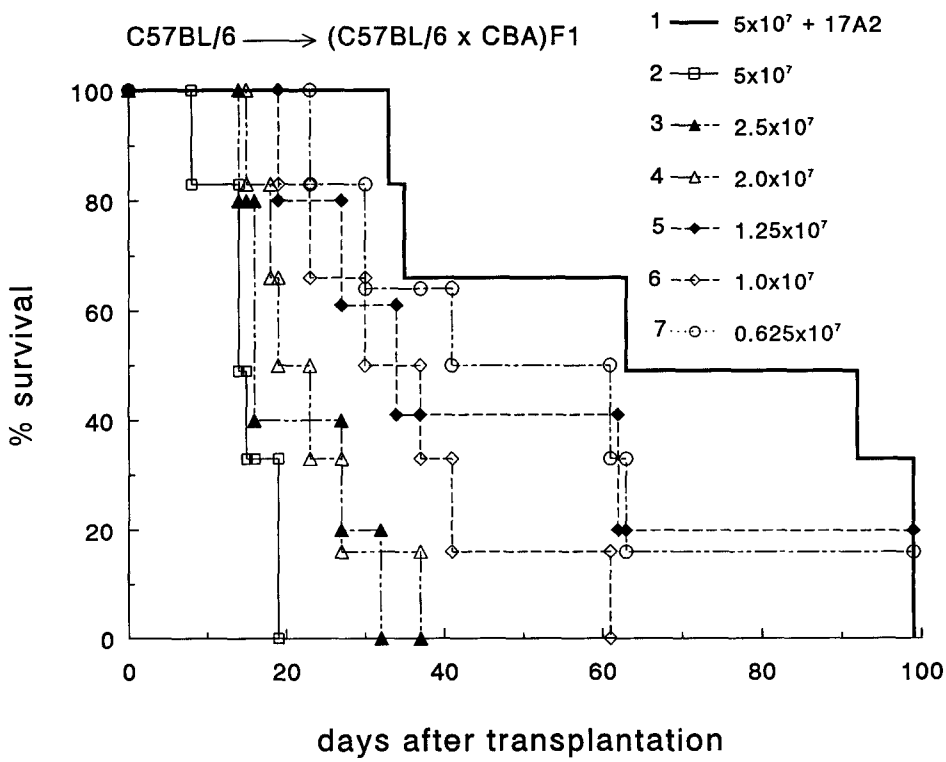

Fig 4. Survival of lethally irradiated (C57BL/6 × CBA)F1 mice (6 per group) after transplantation of 2×10^7 bone marrow cells and different numbers of spleen cells as indicated from 17A2-treated donors or control mice pretreated with MoAb of the same isotype but irrelevant specificity. Statistical analysis was identical as in Fig 3: 1 *v* 2, $P = .017$; 1 *v* 3, $P = .009$, 1 *v* 4, $P = .018$; 1 *v* 5, $P = .623$; 1 *v* 6, $P = .049$; 1 *v* 7, $P = .643$.

as they cause sufficient T-cell elimination.[17-21] The 50% T-cell–depletory effect of 17A2 is clearly insufficient to explain its suppression of GVHD in fully mismatched mice. A rat IgG2c antimouse Thy-1 MoAb, causing 92% T-cell suppression, hardly delayed GVHD in fully mismatched mice, in contrast to the IgG2b isotype with 98% depletion and prevention of GVHD.[21] Therefore, we conclude that TCR modulation in addition to T-cell depletion must be operative in suppressing GVHD with anti-CD3 MoAb. Our observation that prolonged recipient treatment with 17A2 can lead to specific transplantation tolerance is more difficult to understand. Whether transferred donor T cells underwent programmed cell death (apoptosis)[31] or were gradually substituted by tolerant T cells deriving from transplanted stem cells and differentiated in the marrow recipient's thymus was not further investigated. Even subclinical GVHD cannot be dismissed completely, considering the reduced frequency of $CD4^+$ and $CD8^+$ cells that we had observed in some chimeras beyond day 100 posttransplantation.

OKT3 is presently used for treatment of GVHD symptoms after bone marrow transplantation. It may not be completely academic to discuss conditioning marrow recipients with anti-CD3 MoAb for GVHD prophylaxis. Bone marrow engraftment, a clinical problem when T-cell–depleting MoAbs are used, has been shown to be promoted by anti-CD3 treatment.[32] Mitogenic side effects of anti-CD3 MoAb can be mitigated or avoided by isotype-matching,[33] steroids,[34] pentoxphilline,[35] by antibodies to tumor necrosis factor,[36,37] or by anti-CD3 $F(ab')_2$ fragments.[15,38]

ACKNOWLEDGMENT

The authors express their gratitude to Dr H. Robson MacDonald (Ludwig Institute for Cancer Research, Lausanne Branch, Epaliges, Switzerland) for making available the antimouse CD3 MoAb–producing hybridoma. The authors thank U. Bamberg, H. Jennen, and J. Jasny for excellent technical assistance.

REFERENCES

1. Kung PC, Goldstein G, Reinherz EL, Schlossman SF: Monoclonal antibodies defining distinctive human T cell surface antigens. Science 206:347, 1979

2. van Agthoven A, Terhorst C, Reinherz EL, Schlossman S: Characterization of T cell surface glycoproteins T 1 and T 3 present on all human peripheral T lymphocytes and functionally mature thymocytes. Eur J Immunol 11:18, 1981

3. Janossy G, Tidman N, Papageorgiou ES, Kung PC, Goldstein G: Distribution of T lymphocyte subsets in the human bone marrow and thymus: An analysis with monoclonal antibodies. J Immunol 126:1608, 1981

4. Hirsch RL, Layton PC, Barnes LA, Kremer AB, Goldstein G: Orthoclone OKT3 treatment of acute renal allograft rejection in patients receiving maintenance cyclosporine therapy. Transplant Proc 19:32, 1987

5. Todd PA, Brogden RN: Muromonab CD3. A review of its pharmacology and therapeutic potential. Drugs 37:871, 1989

6. Prentice HG, Blacklock HA, Janossy G, Bradstock KF, Skeggs D, Goldstein G, Hoffbrand AV: Use of anti–T-cell monoclonal antibody OKT3 to prevent acute graft-versus-host disease in allogeneic bone-marrow transplantation for acute leukaemia. Lancet 1:700, 1982

7. Filipovich AH, McGlave PB, Ramsay NK, Goldstein G, Warkentin PI, Kesey JH: Pretreatment of donor bone marrow with monoclonal antibody OKT3 for prevention of acute graft-versus-host disease in allogeneic histocompatible bone-marrow transplantation. Lancet 1:1266, 1982

8. Blacklock HA, Prentice HG, Gilmore MJ, Price-Jones E, Schey S, Tidman N, Ma D, Goldstein G, Janossy G, Hoffbrand AV: Attempts at T cell depletion using OKT3 and rabbit complement to prevent acute graft versus host disease in allogenic bone marrow transplantation. Exp Hematol 11:37, 1983

9. Gratama JW, Jansen J, Lipovich RA, Tanke HJ, Goldstein G, Zwaan FE: Treatment of acute graft-versus-host disease with monoclonal antibody OKT3. Clinical results and effect on circulating T lymphocytes. Transplantation 38:469, 1984

10. Reinherz EL, Meuer S, Fitzgerald KA, Hussey RE, Levine H, Schlossman SF: Antigen recognition by human T lymphocytes is linked to surface expression of the T3 molecular complex. Cell 30:735, 1982

11. Chatenoud L, Baudrihaye MF, Kreis H, Goldstein G, Schindler J, Bach JF: Human in vivo antigenic modulation induced by the anti-T cell OKT3 monoclonal antibody. Eur J Immunol 12:979, 1982

12. Leo O, Foo M, Sachs DH, Samelson LE, Bluestone JA: Identification of a monoclonal antibody specific for a murine T3 polypeptide. Proc Natl Acad Sci USA 84:1374, 1987

13. Hirsch R, Eckhaus M, Auchincloss HJ, Sachs DH, Bluestone JA: Effects of in vivo administration of anti-T3 monoclonal antibody on T cell function in mice. I. Immunosuppression of transplantation responses. J Immunol 140:3766, 1988

14. Hirsch R, Gress RE, Pluznik DH, Eckhaus M, Bluestone JA: Effects of in vivo administration of anti-CD3 monoclonal antibody on T cell function in mice. II. In vivo activation of T cells. J Immunol 142:737, 1989

15. Hirsch R, Bluestone JA, DeNenno L, Gress RE: Anti-CD3 $F(ab')_2$ fragments are immunosuppressive in vivo without evoking either the strong humoral response or morbidity associated with whole mAb. Transplantation 49:1117, 1990

16. Ferran C, Sheehan K, Dy M, Schreiber R, Merite S, Landais P, Noel LH, Grau G, Bluestone JA, Bach JF, Chatenoud L: Cytokine-related syndrome following injection of anti-CD3 monoclonal antibody: Further evidence for transient in vivo T cell activation. Eur J Immunol 20:509, 1990

17. Thierfelder S, Cobbold SP, Kummer U, Waldmann H, Schuh R: Antilymphocytic antibodies and marrow transplantation. VII. Two of nine monoclonal anti–Thy-1 antibodies used for pretreatment of donor marrow suppressed graft-versus-host reactions without added complement. Exp Hematol 13:948, 1985

18. Antica M, Hoffmann Fezer G, Thierfelder S: Antilymphocytic antibodies and marrow transplantation. IX. T-cell depletion in marrow donors with Clq high and low affinity antibodies for suppression of GVHD in fully mismatched mice. Exp Hematol 17:942, 1989

19. Thierfelder S, Kummer U, Schuh R, Mysliwietz J: Antilymphocytic antibodies and marrow transplantation. VIII. Recipient conditioning with Clq-affine monoclonal anti-pan T antibodies prevents GVHD in homozygous fully mismatched mice. Blood 68:818, 1986

20. Kummer U, Thierfelder S, Hoffmann Fezer G, Schuh R: In vivo immunosuppression by pan-T cell antibodies relates to their isotype and to their Clq uptake. J Immunol 138:4069, 1987

21. Thierfelder S, Mysliwietz J, Hoffmann Fezer G, Kummer U: Antilymphocytic antibodies and marrow transplantation. XIV.

Antibody-induced suppression of graft-versus-host disease in C3-decomplemented mice differentiates two T-cell-depletion pathways. Blood 77:2285, 1991

22. Miescher GC, Schreyer M, MacDonald HR: Production and characterization of a rat monoclonal antibody against the murine CD3 molecular complex. Immunol Lett 23:113, 1989

23. Cobbold SP, Thierfelder S, Waldmann H: Immunosuppression with monoclonal antibodies. A model to determine the rules for effective serotherapy. Mol Biol Med 1:285, 1983

24. Cobbold SP, Martin G, Qin S, Waldmann H: Monoclonal antibodies to promote marrow engraftment and tissue graft tolerance. Nature 323:164, 1986

25. Mysliwietz J, Thierfelder S, Hoffmann Fezer G, Kummer U: Antilymphocytic antibodies and bone marrow transplantation. XI. Evidence that reduced Thy-1 expression in Thy-1.1 mice prevents suppression of graft-versus-host disease with anti–Thy-1 monoclonal antibodies. Transplantation 49:749, 1990

26. Reik LM, Maines SL, Ryan DE, Levin W, Bandiera S, Thomas PE: A simple, non-chromatographic purification procedure for monoclonal antibodies. Isolation of monoclonal antibodies against cytochrome P450 isozymes. J Immunol Methods 100: 123, 1987

27. Nargessi RD, Landon J: Methods in Enzymology, vol 74. New York, NY, Academic, 1981

28. Kremmer E, Thierfelder S, Kummer U, Lederer R, Mysliwietz J: Neutralization of immunosuppression by antibodies against variable as well as constant regions of monoclonal anti–Thy-1 xenoantibodies and their ability to be suppressed by initial T cell depletion. Transplantation 47:641, 1989

29. Peto R, Pike MC, Armitage P, Breslow NE, Cox DR, Howard SV, Mantel N, McPherson K, Peto J, Smith PG: Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. Br J Cancer 35:1, 1977

30. Rodt H, Thierfelder S, Eulitz M: Antilymphocytic antibodies and marrow transplantation. IV. Comparison of the effects of antibody fragments directed against immunoglobulin or lymphocyte antigens on acute secondary disease. Exp Hematol 2:195, 1974

31. Janssen O, Wesselborg S, Kabelitz D: Immunosuppression by OKT3-induction of programmed cell death (apoptosis) as a possible mechanism of action. Transplantation 53:233, 1992

32. Blazar BR, Hirsch R, Gress RE, Carroll SF, Vallera DA: In vivo administration of anti-CD3 monoclonal antibodies or immunotoxins in murine recipients of allogeneic T cell-depleted marrow for the promotion of engraftment. J Immunol 147:1492, 1991

33. Parleviet KJ, Jonker M, ten Berge RJ, van Lier RA, Wilmink JM, Strengers PF, Aarden LA, Schellekens PT: Anti-CD3 murine monoclonal isotype switch variants tested for toxicity and immunologic monitoring in four chimpanzees. Transplantation 50:889, 1990

34. Alegre ML, Vandenbeele P, Depierreux M, Florquin S, Deschodt-Lanckman M, Flamand V, Moser M, Leo O, Urbain J, Fiers W, Goldman M: Cytokine release syndrome induced by the 145-2C11 anti-CD3 monoclonal antibody in mice: Prevention by high doses of methylprednisolone. J Immunol 146:1185, 1992

35. Alegre ML, Gastaldello K, Abramowicz D, Kinnaert P, Vereerstraeten P, De Pauw L, Vandenabeele P, Moser M, Leo O, Goldman M: Evidence that pentoxifylline reduces anti-CD3 monoclonal antibody-induced cytokine release syndrome. Transplantation 52:674, 1991

36. Holler E, Thierfelder S, Behrends U, Daum L, Hintermeier-Knabe R, Kolb HJ, Kempeni J, Wilmanns W: Anti–TNF-alpha and pentoxifylline for prophylaxis of aGvHD in murine allogeneic bone marrow transplantation. Onkologie 15:31, 1992

37. Ferran C, Sheehan K, Schreiber R, Bach JF, Chatenoud L: Anti-TNF abrogates the cytokine-related anti-CD3 induced syndrome. Transplant Proc 23:849, 1991

38. Woodle ES, Thistlethwaite JR, Ghobrial IA, Jolliffe LK, Stuart FP, Bluestone JA: OKT3 $F(ab')_2$ fragments—Retention of the immunosuppressive properties of whole antibody with marked reduction in T cell activation and lymphokine release. Transplantation 52:354, 1991

blood 1992 80: 2661-2667

Antilymphocytic antibodies and marrow transplantation. XII. Suppression of graft-versus-host disease by T-cell-modulating and depleting antimouse CD3 antibody is most effective when preinjected in the marrow recipient

J Mysliwietz and S Thierfelder

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.