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Enhancement of bone marrow allografts from nude mice into mismatched recipients by T cells void of graft-versus-host activity

(T-cell-depleted bone marrow/bone marrow transplantation)

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ABSTRACT **Transplantation of 8 × 10^6 C57BL/6-Nu$^+$/Nu$^+$ (nude) bone marrow cells into C3H/HeJ recipients after conditioning with 8 Gy of total body irradiation has resulted in a markedly higher rate of graft rejection or graft failure compared to that found in recipients of normal C57BL/6 or C57BL/6-Bg$^+$/Bg$^+$ (beige) T-cell-depleted bone marrow. Mixing experiments using different numbers of nude bone marrow cells with or without mature thymocytes (unagglutinated by peanut agglutinin) revealed that engraftment of allogeneic T-cell-depleted bone marrow is T-cell dependent. To ensure engraftment, a large inoculum of nude bone marrow must be supplemented with a trace number of donor T cells, whereas a small bone marrow dose from nude donors requires a much larger number of T cells for engraftment. Marked enhancement of donor type chimerism was also found when F_1 thymocytes were added to nude bone marrow cells, indicating that the enhancement of bone marrow engraftment by T cells is not only mediated by alloreactivity against residual host cells but may rather be generated by growth factors, the release of which may require specific interactions between T cells and stem cells or between T cells and bone marrow stroma cells.**

Graft rejection and leukemia relapse present significant obstacles for transplantation of T-cell-depleted allogeneic bone marrow in leukemia patients (1). In mice, unlike in man, large numbers of healthy and immunologically vigorous allogeneic chimeras across a complete H-2 barrier have been produced (2, 3).

One possibility that could explain the differing results in mice and man involves the large bone marrow inoculums traditionally used in mice, usually ranging from 10 × 10^6 to 30 × 10^6 cells per mouse [the equivalent of 5–15 × 10^8 cells per kg (body weight)]. To obtain an equivalent inoculum in man, the bone marrow dose should be even larger, considering that mouse bone marrow is obtained by flushing marrow from the bones, whereas in man bone marrow aspirates are withdrawn from the iliac crest and are highly contaminated with peripheral blood lymphocytes.

Titration of a T-cell-depleted bone marrow (TDBM) dose in the "classical" mouse model [in which animals are conditioned with a single dose of 8–9 Gy of total body irradiation (TBI)] revealed a range of cell doses in which bone marrow allograft rejection is similar to that found in man (4). Allogeneic transplants consisting of TDBM cells from C57BL/6 donors were injected into C3H/HeJ recipients who had previously undergone conditioning with 8 Gy of TBI. Chimerism status was defined by testing the H-2 type of spleen cells in each transplanted mouse 1–2 months after transplant. The results revealed that a transplant of 2–4 × 10^6 cells led to graft rejection in 50–80% of the mice, whereas a transplant of 8 × 10^6 cells or more resulted in 100% donor type chimerism. This dose-dependent relationship may result from a proportional increase in the total number of transplanted stem cells. Alternatively, it could be mediated by the 0.1–0.5% of T cells left after T-cell depletion.

The question of whether T cells play a critical regulatory role in hematopoiesis has been controversial for more than two decades. A considerable number of studies have demonstrated the possible influence of the thymus (5–8) or thymus-derived cells (9–13) on hematopoiesis *in vivo* as well as *in vitro* (14–17).

On the other hand, there are two major arguments against such a role: (*i*) relatively normal hematopoiesis takes place, despite the absence of T-cell function, in human patients and in mice with severe combined immunodeficiency, as well as in athymic nude mice, and (*ii*) the demonstration that T-cell-depleted mismatched bone marrow can be fully engrafted in lethally irradiated mice. This discrepancy was largely created by quantitative differences in the extent of T-cell depletion and in the total number of bone marrow cells used for transplantation.

In the present study, the relative role of stem cells versus that of T cells in the process that leads to the establishment of durable donor type chimerism was evaluated using C57BL/6-Nu/Nu (nude) mice as bone marrow donors. The use of bone marrow from nude mice, coupled with T-cell depletion, offers a stem-cell source with minimal T-cell contamination, thereby enabling us to study the role of T cells by reconstituting this highly depleted bone marrow with graduated numbers of purified T cells.

MATERIALS AND METHODS

Animals used were 8- to 12-week-old female C3H/HeJ, (C57BL/6 × C3H/HeJ)F_1, C57BL/6, and C57BL/6-Bg$^+$/Bg$^+$ mice obtained from The Jackson Laboratory; and C57BL/6-Nu$^+$/Nu$^+$ mice obtained from Bomholtgard Breeding and Research Center (Bomholtgard, Denmark). All mice were kept in small cages (five animals per cage) and fed sterile food and acid water.

Mice were exposed to a single dose of 8 Gy of TBI from a Gammabeam 150-A ^{60}Co source (produced by the Atomic Energy of Canada; Ottawa) at a focal skin distance of 75 cm and at a dose rate of 65 cGy/min. Bone marrow cells were fractionated by differential agglutination with soybean agglutinin, according to Reisner *et al.* (3), with minor modifications (4). Twenty-four hours after irradiation, the mice were transplanted with various bone marrow preparations [i.v. in 0.2 ml phosphate-buffered saline (P_i/NaCl)].

Mouse thymocytes were fractionated by differential agglutination with peanut agglutinin (PNA) (18), and the unagglu-

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Abbreviations: GvHD, graft-versus-host disease; PNA, peanut agglutinin; TDBM, T-cell-depleted bone marrow; TBI, total body irradiation.

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tinated cells (PNA^-) were subsequently used as a source of pure competent T cells.

Blood testing was performed 12–14 days after bone marrow transplantation (19). Chimerism analysis was performed 4–9 weeks after bone marrow transplantation (19).

RESULTS

Effect of Mature Thymocytes on Durable Engraftment of Nude Bone Marrow Cells. To investigate the possible effect of the minute T-cell number contaminating preparations of TDBM, we compared such preparations from normal mice to those of TDBM cells from nude mice. C3H/HeJ mice were transplanted with 8×10^6 TDBM cells, either from C57BL/6 donors or from C57BL/6 nude donors, and, 6–8 weeks after bone marrow transplantation, donor type chimerism was tested.

In four experiments (summarized in Table 1), it was found that durable engraftment of nude donor type cells was not achieved in 50 of the 56 mice tested (89%) despite the use of large bone marrow inoculums which, in recipients of normal C57BL/6 TDBM, resulted in more than 90% donor type chimerism. Donor type engraftment after transplantation of TDBM cells from C57BL/6-Bg/Bg donors, which are deficient in natural killer cells but possess normal numbers of T cells, was achieved in 13 out of 17 recipients (Table 1) and did not differ significantly from that obtained from transplants of normal C57BL/6 bone marrow cells. A similar pattern was also revealed by the test for hematopoietic reconstitution on day 12 after bone marrow transplantation (Table 1). These results strongly indicate that T cells and not natural killer cells do, indeed, play a unique regulatory role in the engraftment of bone marrow allografts.

To address this critical question more directly, graduated numbers of PNA^- mature thymocytes from C57BL/6 mice were added to 8×10^6 TDBM cells from C57BL/6-Nu/Nu mice. To avoid graft-versus-host disease (GvHD), only small numbers of PNA^- thymocytes were used in these experiments, but they proved sufficient for promotion of engraftment in that as little as 8×10^4 PNA^- thymocytes could greatly enhance donor type chimerism 1–2 months after bone marrow transplantation.

In three experiments (Table 2), 49 out of 55 mice receiving 8×10^6 TDBM cells from C57BL/6-Nu/Nu mice survived 2 months after bone marrow transplantation, but donor type chimerism was found in only one mouse (2%). On the other hand, among mice receiving 8×10^6 nude bone marrow cells plus $8–20 \times 10^4$ PNA^- thymocytes, survival was 50–75%, with donor type chimerism ranging between 36% and 53%. The significantly lower survival rate found in one of two experiments among mice receiving 16×10^4 T cells resulted in a significantly reduced overall survival rate (44 of 62 mice), compared to survival in the control group that did not receive PNA^- thymocytes (49 of 55 mice). This enhanced mortality could be associated with GvHD, although signs of GvHD were not observed among graft recipients that survived the immediate period after transplant.

Thus, although clinical or subclinical GvHD cannot be ruled out completely in recipients of nude bone marrow contaminated with small numbers of T cells, a substantial number of mice have survived in good health for a long enough period to allow evaluation of long-term chimerism. It is, therefore, possible to conclude that a very small number of T cells is indeed required for the establishment of a durable allogeneic hematopoietic system, provided that a large enough number of stem cells is transplanted.

Enhancement of Bone Marrow Engraftment Mediated by T Cells Void of Graft-Versus-Host Activity. The effect of T cells on durable donor type chimerism could be mediated by two

Table 2. Effect of C57BL/6 PNA^- thymocytes on engraftment of C57BL/6-Nu/Nu TDBM cells in C3H/HeJ mice after conditioning with 8 Gy of TBI

Exp.	Cells: TDBM (8×10^6 cells)	Cells: C57BL/6 PNA^- thymocytes, no.	No. surviving mice/no. total mice	Engrafted with donor cells, %
1	C57BL/6	–	9/10*	77.8†
	C57BL/6-Nu/Nu	–	20/20	25.0
	C57BL/6-Nu/Nu	8×10^4	6/7*	50.0†
	C57BL/6-Nu/Nu	16×10^4	19/20*	52.6†
2	C57BL/6	–	7/7*	85.7‡
	C57BL/6-Nu/Nu	–	17/20	0
	C57BL/6-Nu/Nu	16×10^4	11/20†	36.4‡
3	C57BL/6-Nu/Nu	–	12/15	0
	C57BL/6-Nu/Nu	20×10^4	8/15*	37.5†

Percent of mice engrafted with donor cells represents the fraction of mice that was found to be either mixed or full donor type chimeras. Staining (%, mean ± SD) of spleen cells from donor type chimeras with anti-H-2^b and anti-H-2^k antibodies was 65 ± 7 and 9 ± 3, respectively ($P < 0.01$). Statistical significance of donor type chimerism was established according to the Student's t test. Staining with anti-H-2^b of each experimental group was compared to that of the control group (C57BL/6-Nu/Nu bone marrow recipients). Statistical significance of survival was established according to the χ^2 test. *Not significant. †$P \leq 0.05$. ‡$P \leq 0.01$.

Table 1. Hematopoietic reconstitution and donor type chimerism after transplantation of TDBM from different donor strains

Exp.	Bone marrow source	Hematopoietic reconstitution 12 days after transplantation: WBC ($\times 10^{-9}$), no. per liter	Hb, g/dl	Platelets ($\times 10^{-12}$), no. per liter	No. surviving mice/no. total mice	Chimerism status 60 days after transplantation: Host	Mixed	Donor	Engrafted with donor cells, %
1	C57BL/6	3.5 ± 1.2	12.4 ± 2.6	371 ± 107	15/15	1	–	14	93.3
	C57BL/6-Nu/Nu	1.6 ± 0.5*	11.0 ± 1.5*	228 ± 86†	4/5	3	1	–	25.0‡
2	C57BL/6	2.6 ± 1.5	11.7 ± 1.8	435 ± 202	13/15	2	–	11	84.6
	C57BL/6-Nu/Nu	0.8 ± 0.1‡	8.2 ± 1.0‡	65 ± 30‡	17/20	17	–	–	0‡
	C57BL/6-Bg^+/Bg^+	2.4 ± 0.8*	12.1 ± 1.2*	414 ± 181*	17/20	4	–	13	76.5*
3	C57BL/6-Nu/Nu	ND	2.9 ± 2.2	114 ± 78	12/15	12	–	–	0
4	C57BL/6	ND	ND	ND	9/10	2	–	7	77.8
	C57BL/6-Nu/Nu	ND	ND	ND	20/20	15	4	1	25.0†

TDBM cells (8×10^6 cells in 0.2 ml of P_i/NaCl) were transplanted into C3H/HeJ recipients that had received 8 Gy of TBI. The percent of mice engrafted with donor cells represents the fraction of mice that were found to be either mixed or full donor type chimeras. Staining (%, mean ± SD) of spleen cells from donor type chimeras with anti-H-2^b and anti-H-2^k antibodies was 68 ± 9.9 and 10 ± 3, respectively ($P < 0.01$). Statistical significance was established according to the Student's t test. Staining with anti-H-2^b and hematological values of each experimental group were compared to those of the control group (C57BL/6 bone marrow recipients). ND, not determined. *Not significant. †$P \leq 0.05$. ‡$P \leq 0.01$.

mechanisms. One possibility concerns the alloreactivity of donor type T cells, which may eliminate resistance to engraftment mediated by host T cells or stem cells (19), although GvHD was not observed in graft recipients that survive the initial period after transplant. Alternatively, added donor type T cells may be required in trace amounts as a source of lymphokines, which might be missing during the initial period after transplant.

To reduce the possible effect of the former mechanism, which involves alloreactivity of donor cells against host cells, we chose to use (C3H/HeJ × C57BL/6)F_1 PNA^- thymocytes. As can be seen in Fig. 1, these T cells were found to have a marked effect on donor type chimerism as well as on rate of engraftment, when administered together with TDBM cells from C57BL/6-Nu/Nu mice. The relationships among stem-cell dose, number of F_1 thymocytes added to the bone marrow, and their combined effect on donor type chimerism were as follows.

When a constant large number (8×10^6) of nude TDBM cells was transplanted with various numbers of F_1 thymocytes (ranging from 0.2×10^6 to 4×10^6 cells), the incidence of mice that failed to achieve donor type chimerism after transplantation was reduced from 100% to 38.5%, resulting in 23% donor type chimera and 38.5% mixed chimera. No GvHD was observed, even when the largest F_1 PNA^- thymocyte inoculum was used. When the number of nude TDBM cells was increased from 2×10^6 to 8×10^6 cells, and a constant number (1×10^6) of F_1 PNA^- thymocytes was used, the incidence of mice without donor type chimerism was reduced from 100% to 57%, resulting in 29% donor type chimera and 14% mixed chimera. In recipients of 2×10^6 nude TDBM cells plus 1×10^6 F_1 PNA^- thymocytes, platelet number and hemoglobin levels on day 12 after bone marrow transplantation were 86 ± 47 platelets and 7.6 ± 1.2 g/dl, respectively, whereas in recipients of 8×10^6 nude TDBM cells plus 1×10^6 F_1 PNA^- thymocytes, they were elevated to 191 ± 129 platelets and 10 ± 2.6 g/dl, respectively.

In leukemia patients, it is possible to obtain only a limited stem cell dose due to the method of bone marrow aspiration, which is restricted to collection from the iliac crest. At present, therefore, it is more reasonable to attempt to enhance bone marrow engraftment by means other than by an increase in stem-cell dose.

Is it possible to enhance engraftment of low doses of stem cells by increasing the number of T cells in the bone marrow transplant? This question may be answered by using F_1 thymocytes void of graft-versus-host activity. In two independent experiments involving a transplant of 2×10^6 TDBM cells from C57BL/6 donors, engraftment was markedly enhanced by adding large numbers of F_1 PNA^- thymocytes to the bone marrow inoculum. In one experiment (Table 3), 38% (5 of 13 mice) donor type chimerism resulted from an inoculum to which T cells had not been added. When 8×10^6 F_1 PNA^- thymocytes were added to the inoculum, donor type chimerism was enhanced to 89% (16 of 18 mice). In the second experiment (detailed data not shown), the addition of 4×10^6 F_1 PNA^- thymocytes to the bone marrow inoculum enhanced donor type chimerism from 25% to 59%. Thus, when a relatively low number of stem cells is used, the number of T cells required to guarantee durable engraftment seems to be larger than the bone marrow inoculum.

Interestingly, transplanted mice were primarily of host type or donor type. Mixed chimera were rare (1 of 13 mice in the control group and 1 of 18 mice among the recipients of F_1 thymocytes), ruling out the possibility that enhancement by F_1 thymocytes was induced by contaminating F_1 stem cells in the thymocyte preparation.

DISCUSSION

In current clinical trials involving HLA-identical bone marrow transplants in leukemia patients, TDBM cells are supplemented with very small numbers of donor type T cells, so as to avoid GvHD and to gain, in part, the beneficial effect of T cells that has been hypothesized. Preliminary results suggest that these attempts have not met with much success, more often resulting in GvHD rather than in improved engraftment (N. A. Kernan, C. Bordigon, G. Geller, I. Cun-

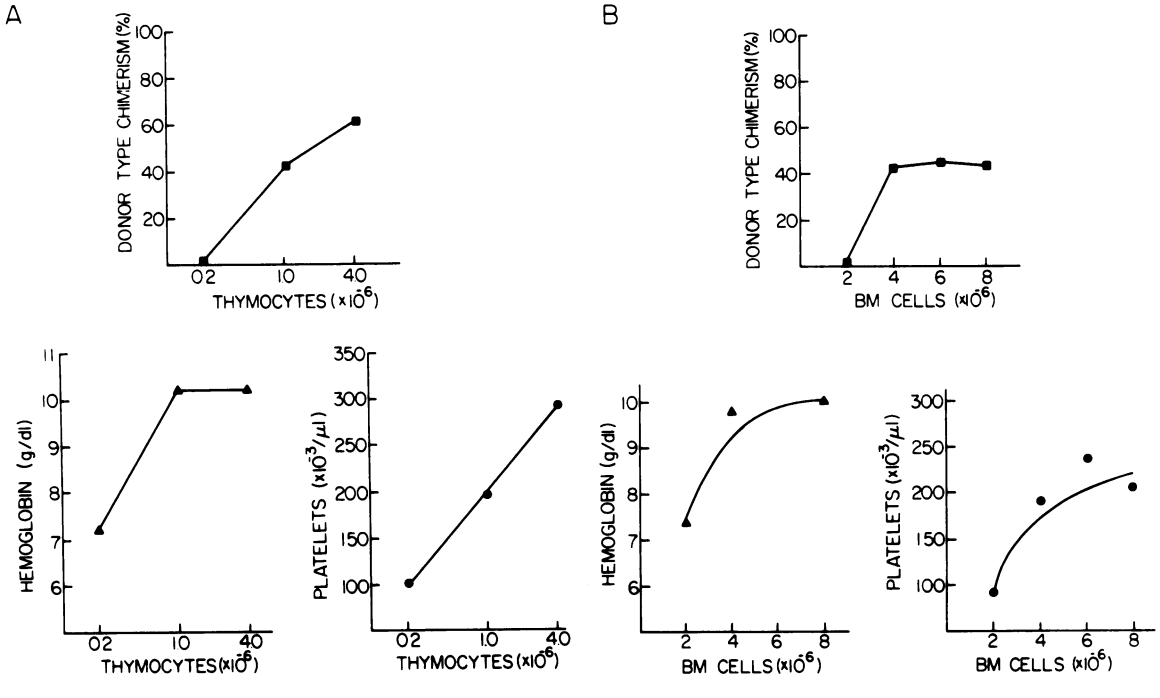

FIG. 1. Effect of increasing numbers of F_1 PNA^- thymocytes (*A*) or T-cell-depleted C57BL/6-Nu/Nu bone marrow (BM) cells (*B*) on hematopoietic parameters after transplantation of 8×10^6 T-cell-depleted C57BL/6-Nu/Nu bone marrow cells (*A*) or 1×10^6 PNA^- F_1 thymocytes (*B*). Hemoglobin (▲) and platelet (●) counts were recorded on day 12 after transplantation. Donor type chimerism (■) was recorded 6–8 weeks after transplantation.

Table 3. Effect of 8×10^6 (C57BL/6 × C3H/HeJ)F_1 PNA^- thymocytes on engraftment of low-dose C57BL/6 TDBM (2×10^6 cells) in C3H/HeJ mice after conditioning with 8 Gy of TBI

Animal	Typing by cytofluorimetry, %			Type of chimera
	Control	Anti-H-2^k	Anti-H-2^b	
	Bone marrow cells alone			
1	10	21	68	D
2	11	81	17	H
3	12	29	75	D
4	15	87	24	H
5	16	24	86	D
6	17	81	21	H
7	12	22	56	D
8	8	60	14	H
9	10	70	18	H
10	12	22	60	D
11	13	80	18	H
12	10	70	16	H
13‡	11	35	40	M
	Bone marrow cells and PNA^- thymocytes			
14	17	26	70	D
15	9	17	78	D
16	12	30	62	D
17	15	23	69	D
18	18	24	76	D
19	15	19	60	D
20	8	17	69	D
21	8	15	59	D
22	9	17	61	D
23	11	43	55	M
24	13	19	65	D
25	13	20	68	D
26	12	19	67	D
27	10	19	65	D
28	13	21	72	D
29	12	70	16	H
30	14	23	81	D
31	17	25	80	D

Control samples were treated with P_i/NaCl and thereafter with the second antibody (fluorescein isothiocyanate-conjugated goat anti-mouse IgG2A). Donor type chimeras (D) were scored when staining with anti-H-2^k was $\leq 30\%$ and staining with anti-H-2^b was $\geq 30\%$. Host type chimeras (H) were scored when staining with anti-H-2^k was $\geq 30\%$ and staining with anti-H-2^b was $\leq 30\%$. Mixed chimeras (M) were scored when staining with both anti-H-2^b and anti-H-2^k was $>30\%$. Staining (%, mean ± SD) with anti-H-2^b of spleen cells alone or with PNA^- thymocytes was 39 ± 26 and 65 ± 14, respectively ($P < 0.01$ in Student's t test). Staining (%, mean ± SD) of spleen cells from donor type chimeras with anti-H-2^b and anti-H-2^k antibodies was 67 ± 10 and 22 ± 4, respectively ($P < 0.01$).

ningham, H. Casro-Malaspina, B. Shank, N. Flomenberg, J. Burns, S. Y. Yang, P. Black, N. H. Collins & R. J. O'Reilly, personal communication).

It would seem that a significant improvement in clinical transplantation requires either a significant increase in stem cell number or a much larger dose of T cells than is currently employed, an option that is limited due to GvHD. Our data with F_1 thymocytes suggest that enhancement of engraftment can be achieved by T cells void of graft-versus-host activity. It is, therefore, possible that a T-cell subpopulation incapable of producing GvHD may enhance engraftment of bone marrow allografts. However, these activities may be inseparable.

It could be argued that the enhancement of engraftment of nude bone marrow cells might be mediated by pluripotent stem cells present in the PNA^- F_1 thymocyte preparation. However, this possibility is unlikely, as such F_1 stem cells would give rise to progenies that would be stained by both anti-H-2^b and anti-H-2^k antibodies and, therefore, could not account for the marked reduction in host type cells found in our assay.

Still, it is possible that some cell types of the thymic environment other than T lymphocytes could be responsible in part for the effect. Preliminary cell-sorting experiments with specific T-cell markers (data not shown) further support the suggestion that the enhancement of donor type chimerism is mediated by F_1 PNA^- thymocytes.

Augmentation of bone marrow engraftment by thymocytes has been demonstrated in various murine models of bone marrow transplantation. Goodman and Shinpock (9, 10) have shown that donor type thymocytes can somewhat abrogate hybrid resistance and enhance hematopoiesis in fully irradiated F_1 recipients of parental bone marrow. Although interpretation of these studies was difficult, due to the occurrence of GvHD-induced splenomegaly, it was established that the addition of donor type thymocytes in large numbers resulted in an increase in iron incorporation as well as in circulating erythrocytes.

This augmentative effect was also seen in allogeneic and xenogeneic chimeras (20). Again, morbidity was also increased due to an enhanced incidence of GvHD. However, Goodman and Shinpock (21) found that in the few mice surviving GvHD, donor type erythrocytes persisted for extended periods of time. Whether this enhancement is mediated by donor anti-host responses or by non-GvHD-producing T cells could not be determined by these studies.

In line with these data, Zipori and Trainin (7) demonstrated that bone marrow cells from both neonatally thymectomized or athymic nude mice manifested a reduced radioprotective capacity when inoculated into lethally irradiated syngeneic recipients. Zipori and Trainin (7) suggested that this was due to a lower rate of proliferation among hematopoietic cells originating in the bone marrow of thymectomized mice. Implantation with intact thymic tissue reversed the reduction of colony forming units associated with thymectomy, whereas injection of thymocytes was not followed by any noticeable change. The discrepancy between the study of Zipori and Trainin (7) and other *in vivo* studies (9–13) regarding the effect of thymocytes has not been explained but may be associated with the type of bone marrow recipient (e.g., syngeneic in the former versus semiallogeneic or allogeneic in the latter) used in each study.

Other evidence for the role of T cells in hematopoiesis was demonstrated by Sharkis *et al.* (12, 13), who found that depletion of bone marrow T cells by means of anti-θ antiserum impaired the ability of the W/W bone marrow to cure the macrocytic anemia of the W/W^v mouse. Moreover, a very small number of thymocytes were found to be capable of restoring the curative capacity of these anti-θ-treated bone marrow cells. Although these results were obtained with H-2 compatible transplants in a murine model involving nonirradiated mice, they are in accordance with our finding regarding the critical role of T cells in promoting sustained engraftment of fully allogeneic donor type stem cells in radiation chimera.

Sachs and coworkers (21) found that tolerance to both host and donor type H-2 antigens could be induced by transplanting into lethally irradiated recipients, mixtures of T-cell-depleted host type bone marrow plus unseparated allogeneic bone marrow. Such tolerance could also be achieved by using T-cell depletion for both host and donor type bone marrow preparations, although the chimerism resulting from such transplants was largely of host type. Similar results were obtained when T-cell-depleted bone marrow cells from F_1 mice were mixed with T-cell-depleted parental bone marrow cells and transplanted into lethally irradiated parental-type recipients. Donor type chimerism was significantly higher in the recipients of unseparated F_1 bone marrow cells plus host type T-cell-depleted parental cells, indicating that this effect

may be mediated by non GvHD-producing T cells. However, add-back experiments with purified F_1 T cells were not performed. Further support for the effect of F_1 T cells on the hematopoietic engraftment of allogeneic pluripotent stem cells was provided by Touraine *et al.* (22), who found that partially purified spleen T cells could enhance engraftment of allogeneic fetal liver cells.

Our experiments that made use of nude mice as a source of allogeneic TDBM cells, and PNA^- thymocytes as a source of purified mature T cells, enabled us to demonstrate the regulatory role of T cells in allogeneic recipients after conditioning similar to that used in the treatment of leukemia patients. This role could not be detected in our studies in which a high dose (8×10^6 cells) of normal T-cell-depleted C57BL/6 bone marrow cells was used as a source of stem cells, and it seems likely, therefore, that it was also overlooked in several murine models that made use of doses of TDBM cells equal to or greater than 1×10^7 in recipients irradiated with more than 8 Gy of TBI. When the stem-cell dose is relatively large, a trace amount of T cells is required, whereas, with a relatively low number of stem cells, a very large number of T cells may be needed to ensure donor type chimerism (Table 3). Thus, the controversy regarding the role of T cells in donor type engraftment may be attributed to quantitative differences.

In line with our findings, it may be possible to interpret two intriguing clinical reports. Martin *et al.* (23) described extremely poor results when using TDBM cells for transplantation in leukemia patients. They took extra care to drastically purge T cells from their bone marrow preparations, and it is possible that this led to the higher rate (over 50%) of graft rejection experienced in their series compared to what is commonly found in other centers using less-extensive T-cell depletion. At the other extreme, Bacigalupo *et al.* (24) reported the results of clinical trials involving partial T-cell depletion that made use of a mouse monoclonal antibody (anti-BT5/9, specific for 15% of peripheral blood T cells and 5–10% of bone marrow T cells; CD not yet defined) (25) to eliminate only a small subset of inducer/helper T cells. Surprisingly, the incidence of graft failure and leukemia relapse was significantly higher among these patients than among those treated at other centers with conventional T-cell depletion techniques that used pan-T-cell antibodies. These results would suggest either that treatment with this antibody was especially toxic to pluripotent bone marrow stem cells or that $BT5/9^+$ T cells may play an important regulatory role in bone marrow engraftment. Bacigalupo *et al.* (24) support the latter possibility, as removal of $BT5/9^+$ T cells does not affect the growth of myeloid or erythroid colonies *in vitro*.

Our long-term assay for durable hematopoietic engraftment, which uses add-back experiments mixing purified thymocytes with TDBM cells from nude donors, should enable isolation of such hypothetical T-cell subpopulations. It should permit the investigation of whether the role of T cells shown in our model is mediated or could be replaced by lymphokines. Since F_1 T cells are not present in larger animals or in man, the study of the latter possibility holds greater potential for refining bone marrow transplantation procedures in leukemia patients.

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