

# LETHAL GRAFT-VERSUS-HOST DISEASE AFTER BONE MARROW TRANSPLANTATION ACROSS MINOR HISTOCOMPATIBILITY BARRIERS IN MICE

Prevention by Removing Mature T Cells from Marrow\*

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Transplantation of HLA-matched bone marrow after total body irradiation in man is frequently followed by graft-versus-host disease (GVHD)<sup>1</sup> (1). The pathogenesis of this syndrome is controversial. Certain workers maintain that the disease, particularly its chronic form (secondary disease), reflects an attack against minor histocompatibility (H) antigens of the host by the progeny of stem cells in the marrow inoculum (1, 2). Although widely held, this viewpoint is difficult to reconcile with the following observations. First, irradiated F<sub>1</sub> mice given parental strain fetal liver cells (3, 4) or T-cell-depleted marrow cells (5) fail to develop GVHD even across major histocompatibility complex (MHC) barriers. Second, GVHD is not seen in embryo-fusion chimeras (6) or in natural hemopoietic chimeras (7-9). Production of blocking factors, etc. have to be invoked to explain these findings.

The opposing viewpoint is that the GVHD seen with HLA-matched marrow does not reflect alloaggression mediated by stem cells or their progeny but by mature T cells contaminating the marrow inoculum (5). The main objection to this notion is that, although T cells are known to control GVH reactions against MHC determinants, "... there is no evidence whatsoever that removal of the cells that produce GVH reactions influences the occurrence of the delayed type of GVH disease that is associated with weak incompatibilities" (10). The mouse is the obvious choice for examining this question since reagents with defined specificity for mature T cells are available only in this species. Surprisingly, although secondary disease to non-MHC determinants is well-documented in dogs (11), there are only isolated reports that transfer of MHC-identical marrow causes lethal GVHD in heavily irradiated mice (12). In certain situations spleen and lymph node cells cause heavy mortality in *H-2*-compatible mice given lethal irradiation (13), or sublethal irradiation plus cyclophosphamide (14); mortality was not seen in a recent study employing sublethal irradiation alone (15). In terms of splenomegaly and mortality assays in neonatal mice, it is well-accepted that minor H differences cause minimal reactions unless the donors are presensitized (16, 17). Similarly, cytotoxic reactions to minor H antigens are very weak except in secondary responses (18).

It is critical to distinguish between the above two theories for the cause of secondary disease. Thus, the first viewpoint is essentially pessimistic since it implies that with sufficient antigenic disparity between donor and host, GVHD is inevitable and amenable only to palliative treatment, e.g. immunosuppression. By contrast, the second viewpoint asserts that GVHD will not occur if the marrow inoculum is depleted of mature T cells.

In attempting to resolve this controversy the aim of the present paper was to study the effects of bone marrow transplantation across minor H barriers in mice. The data show that, at least with certain strain combinations, untreated marrow cells caused a high incidence of lethal

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<sup>1</sup> *Abbreviations used in this paper:* GVHD, graft-versus-host disease; H, histocompatibility; LN, lymph node; MHC, major histocompatibility complex; PFC, plaque-forming cells; SRC, sheep erythrocytes.

chronic GVHD when transferred to heavily irradiated allogeneic recipients. Significantly, GVHD did not occur when the marrow cells were depleted of mature T cells with anti-Thy 1.2 antiserum and complement.

### Materials and Methods

*Mice.* CBA/J, B10.BR/SgSn, C3H/HeJ, CBA/H-T6J, and AKR/J (all  $H-2^k$ ) and C57BL/6J (B6), C57BL/10J (B10) and 129/J (all  $H-2^b$ ) were obtained from The Jackson Laboratory, Bar Harbor, Maine. B10.K ( $H-2^k$ ) mice were a gift from W. L. Elkins, University of Pennsylvania. (B6  $\times$  CBA/J) $F_1$  mice were purchased from Cumberland View Farms, Clinton, Tenn. (B10  $\times$  B10.BR) $F_1$  and (C3H/He  $\times$  BALB/c ( $H-2^d$ )) $F_1$  mice were bred in our colony. Unless stated otherwise, male mice were used as donors and recipients. Mice 2–4 mo old were used as irradiated recipients of marrow cells after being maintained on a conventional diet and acid water ad libitum.

*Media.* RPMI-1640 (Microbiological Associates, Walkersville, Md.) supplemented with 10% fetal calf serum was used.

*Injections.* Cell suspensions were given intravenously in 0.5 ml via the tail vein.

*Viability.* Cell viability was assessed by dye exclusion.

*Cells.* Bone marrow cells were obtained from tibia and femura by gentle flushing with cold medium via a 25 gauge needle. Clumps were removed by passage over sterile cotton filters and the cells were washed three times by centrifugation before final resuspension. Spleen and lymph node (LN) cells were obtained by mincing the organs with curved scissors over an 80-mesh stainless steel sieve in cold medium. T-cell-enriched populations of LN cells were obtained by passage through nylon wool columns as described by Julius et al. (19). Over 90% of the effluent cells were T cells as tested by their susceptibility to lysis by anti-Thy 1.2 antiserum in the presence of guinea pig complement.

*Removal of Mature T Cells from Bone Marrow.* After centrifugation, marrow suspensions were resuspended in a 1:5 dilution of anti-Thy 1.2 serum (0.1 ml undiluted serum/ $5 \times 10^7$  cells) and incubated on ice for 30 min. The cells were then washed twice with at least 20 ml of medium and suspended in a 1:6 dilution of guinea pig complement (1.0 ml undiluted complement/ $3 \times 10^8$  cells). After a final incubation in a 37°C water bath for 30 min with occasional mixing, the cells were washed three times with medium and resuspended to the required concentration. Control experiments showed that this procedure lysed >98% of highly purified T cells, i.e. LN cells recirculated from blood to lymph through irradiated intermediate hosts (20).

LN cells were depleted of T cells by the same two-step procedure except that a higher concentration of anti-Thy 1.2 serum was used (0.1 ml of undiluted serum/ $2 \times 10^7$  cells).

*Antisera.* Anti-Thy 1.2 antiserum was made by injecting AKR/J mice with  $2 \times 10^7$  C3H/HeJ thymus cells intraperitoneally at weekly intervals for 12 wk. CBA anti-B6  $H-2$  alloantiserum was made as described elsewhere (20).

*Mortality Assay for GVHD.* Marrow recipients were given 750 rads of  $^{137}\text{Cs}$   $\gamma$ -irradiation at a dose rate of 106 rads/min. Groups of mice were split into separate cages and kept in a laminar flow room. Neomycin (50  $\mu\text{g}/1$ ) and Polymyxin B (50  $\mu\text{g}/\text{ml}$ ) were added to the drinking water for 3 wk after irradiation.

*Plaque-Forming Cell (PFC) Assays.* IgM (direct), IgG (indirect), and Ig<sup>b</sup>-allotype PFC to sheep erythrocytes (SRC) were assayed as described elsewhere (21). IgG PFC were developed with a polyvalent rabbit anti-mouse Ig antiserum; Ig<sup>b</sup> PFC were developed with a BALB/c anti-B6 Ig (anti-Ig<sup>b</sup>) anti-allotype serum (21).

### Results

*Experimental Design.* In all experiments mice received a lethal dose of irradiation (750 rads). 6–8 h later the mice were injected intravenously with  $10^7$  marrow cells alone or with the same dose of marrow cells mixed with other cell types. Unless stated otherwise, neither the donors nor the hosts were presensitized. The cell recipients were weighed thrice weekly and examined for signs of GVHD for 80 days or until death. Antibiotics (see Materials and Methods) were added to the drinking water for the

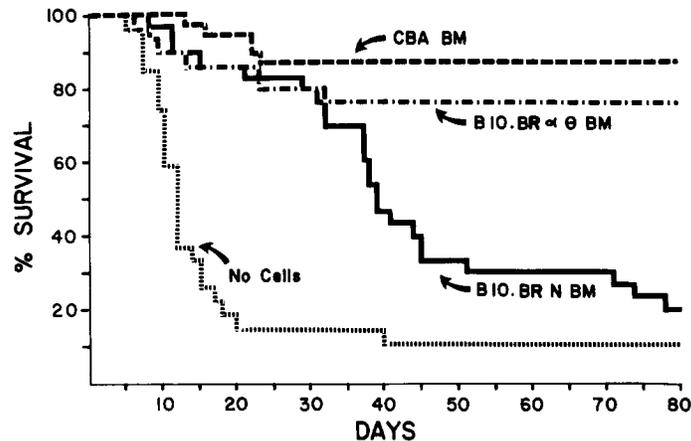


FIG. 1. Lethal GVHD in irradiated (750 rads) CBA/J mice given unfractionated B10.BR marrow cells. The data show cumulative mortality after injection of untreated syngeneic CBA/J marrow cells (CBA BM), B10.BR marrow cells treated with anti-Thy 1.2 serum and complement (B10.BR  $\alpha\theta$  BM), or with B10.BR marrow cells treated with normal AKR serum and complement (B10.BR N BM).  $10^7$  viable marrow cells injected intravenously. Data pooled from three separate experiments involving a total of 30 mice per group.

first 3 wk postirradiation. Greater than 80% of irradiated mice not protected with marrow cells died of hemopoietic failure within 3 wk.

*Transfer of B10.BR Marrow to Irradiated CBA/J Mice.* Fig. 1 illustrates mortality occurring in irradiated CBA/J ( $H-2^k$ ) mice reconstituted with syngeneic CBA/J marrow or with  $H-2$ -compatible B10.BR marrow (B10.BR and CBA/J express multiple non-MHC antigen differences (*vide infra*); the data are pooled from three experiments involving a total of 30 mice per group.) As expected, only low mortality occurred when syngeneic marrow was injected. Although a few mice died within the first 3–4 wk, there were no deaths after 30 days.

A very different pattern was observed with B10.BR marrow cells. If these cells were not pretreated with anti-Thy 1.2 antiserum and complement before injection, lethal chronic GVHD occurred (Fig. 1). Thus, with transfer of B10.BR marrow cells treated with control serum (normal AKR serum) and complement, most of the mice died after day 30 and only 20% survived beyond day 80. During this stage the mice showed typical signs of secondary disease before death, e.g. hunched posture, ruffled fur, weight loss, etc. Skin lesions were not seen.

Significantly, neither morbidity nor mortality occurred when B10.BR marrow cells were treated with anti-Thy 1.2 serum and complement before injection (Fig. 1). As with transfer of syngeneic marrow cells, isolated deaths were observed within the 1st mo but there were no deaths nor signs of ill-health after 30 days.

*Potential of GVH Disease by Adding T Cells to Marrow Inoculum.* The above data strongly implied that mature T cells in the marrow inoculum were the cause of secondary disease. The proportion of T cells in B10.BR marrow appeared to be very low since cytotoxic indices with anti-Thy 1.2 serum and complement were  $<5\%$  (data not shown). Accordingly, the addition of even small numbers ( $<5\%$ ) of purified T cells to inocula of anti-Thy 1.2-serum-treated B10.BR marrow cells would be expected to restore the incidence of secondary disease.

This prediction is verified by the data shown in Fig. 2 in which varying numbers

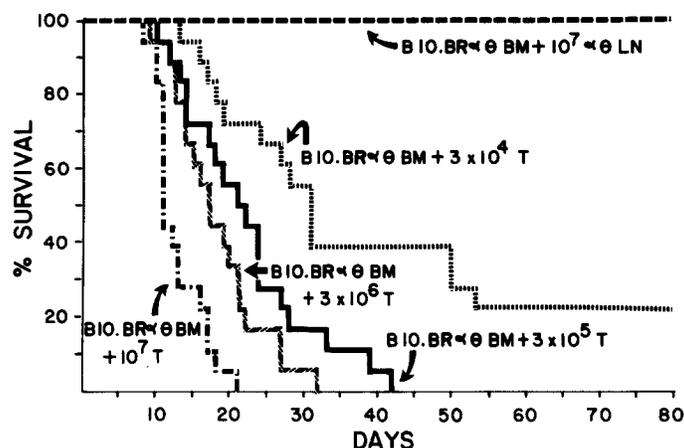


FIG. 2. Lethal GVHD in irradiated (750 rads) CBA/J mice given graded doses of purified B10.BR LN T cells. The data show cumulative mortality after transferring  $10^7$  anti-Thy 1.2-serum-treated B10.BR marrow cells (B10.BR  $\alpha\theta$  BM) supplemented with varying numbers of nylon-wool-purified B10.BR LN T cells or with anti-Thy 1.2 serum-treated B10.BR LN cells ( $\alpha\theta$  LN). Data pooled from three experiments involving a total of 18 mice per group.

of nylon-wool purified B10.BR LN T cells (>90% Thy 1.2-positive) were added to the dose of  $10^7$  T-cell-depleted B10.BR marrow cells. With the addition of high doses of T cells ( $10^7$ ), all of the recipients died within 21 days, presumably as the result of an acute GVH reaction. Smaller doses of T cells produced slower mortality, e.g. 100% mortality was delayed until day 32 with  $3 \times 10^6$  T cells and until day 42 with  $3 \times 10^5$  T cells. However, even doses as low as  $3 \times 10^4$  cells (equivalent to 0.3% T-cell contamination of the marrow) caused nearly 80% mortality by day 80. It is to be noted that the addition of high doses of anti-Thy 1.2-serum-treated B10.BR LN cells ( $10^7$  viable cells) caused no mortality.

**Inability of Tolerant T Cells to Cause Lethal GVH Disease.** Previous work has shown that T-cell tolerance to host-type MHC alloantigens occurs when parental strain marrow cells are transferred to irradiated H-2-different F<sub>1</sub> hybrid mice (22). To determine whether tolerance to non-H-2 determinants occurs in this situation, lethally irradiated (900 rads) (B6 ( $H-2^b$ )  $\times$  CBA/J)F<sub>1</sub> mice were reconstituted with  $10^7$  anti-Thy 1.2-serum-treated B10.BR marrow cells. Spleen cells taken from these mice 3 mo later were >95% of donor B10.BR origin, i.e. were resistant to lysis with CBA/J anti-B6 H-2 alloantiserum.

As shown in Fig. 3 a high dose of a mixture of  $10^7$  marrow cells and  $10^7$  pooled spleen and LN cells from these chimeras failed to cause mortality in irradiated CBA/J mice. As expected, the chimera cells were reactive to third-party alloantigens, i.e. they caused rapid mortality in irradiated (C3H/He  $\times$  BALB/c)F<sub>1</sub> ( $H-2^k \times H-2^d$ ) mice.

**GVHD with other H-2-Compatible Combinations.** Results obtained with a variety of other strain combinations fell into three categories (*vide infra*) (Table I).

**GVHD with Normal Marrow Cells.** As with transfer of B10.BR marrow to CBA/J mice, reciprocal transfer of CBA/J marrow to irradiated B10.BR mice led to chronic GVHD. Thus, in the experiment shown in Fig. 4, five of the six marrow recipients died between day 20 and day 45; the remaining mouse survived for >80 days. No deaths occurred with syngeneic marrow. Again, T-cell depletion of the allogeneic

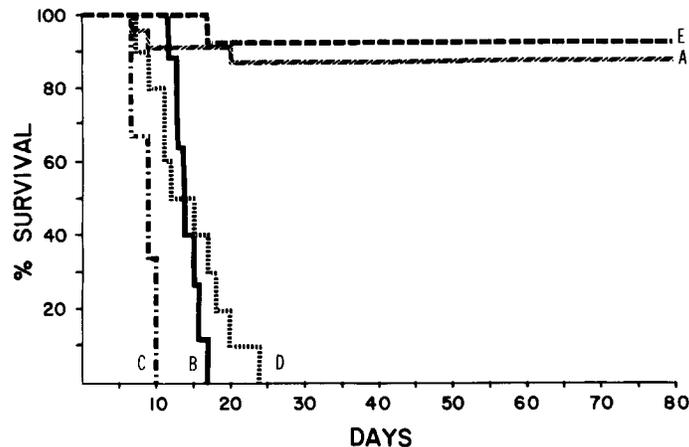


FIG. 3. Failure of specifically tolerant B10.BR spleen and LN cells to induce lethal GVHD in irradiated (750 rads) CBA/J mice. B10.BR cells tolerant of CBA/J determinants were obtained from heavily irradiated (900 rads) (B6  $\times$  CBA/J) $F_1$  mice reconstituted three months previously with anti-Thy 1.2-serum-treated B10.BR marrow cells (see text). The data show cumulative mortality in irradiated CBA/J mice given a mixture of  $10^7$  marrow cells plus  $10^7$  pooled spleen and LN cells derived from B10.BR  $\rightarrow$  (B6  $\times$  CBA/J) $F_1$  chimeras (group A) or from normal B10.BR mice (group B). Control experiments involved transferring the same mixture of B10.BR chimera cells into third-party irradiated (C3H/He  $\times$  BALB/c) $F_1$  mice with or without syngeneic (C3H/He  $\times$  BALB/c) $F_1$  marrow cells (groups C and D, respectively). Group E mice were irradiated (C3H/He  $\times$  BALB/c) $F_1$  mice given syngeneic marrow cells alone. Data pooled from four experiments. Groups A, B, C, D, and E contained totals of 23, 13, 10, 3, and 8 mice, respectively.

marrow with anti-Thy 1.2 serum prevented both mortality and morbidity and the addition of normal CBA/J spleen and LN potentiated mortality.

*GVHD with Spleen and LN Cells but not with Marrow Alone.* In most strain combinations tested, untreated marrow cells per se did not cause lethal GVHD. However, with six out of nine of these combinations, marked mortality occurred when the marrow was supplemented with spleen and LN cells of donor origin. A dose of  $10^7$  of these cells caused 70–100% mortality and  $10^8$  cells produced 100% mortality. With one combination (B10.K  $\rightarrow$  CBA/J), studies with nylon-wool-purified LN cells showed that as few as  $3 \times 10^5$  purified T cells led to >90% mortality (equivalent to 3% T-cell contamination of the marrow). It is to be noted that with all of these combinations there were at least three minor H antigen differences between the donor and host.

*No GVHD.* In the remaining three combinations, the addition of even high doses ( $10^8$ ) of spleen and LN cells caused only a minimal incidence of GVHD (<35% mortality). In two of these combinations, only a single minor H antigen difference was involved, i.e. H-9 for B6  $\rightarrow$  B10 and H-Y for B10 female  $\rightarrow$  B10 male (in the latter situation even presensitized cells were nonlethal [Table I]). In the third combination, i.e. CBA/H-T6  $\rightarrow$  CBA/J, there were no known minor H differences present.

*Immunocompetence and Extent of Chimerism in Mice given T-Cell-Depleted Allogeneic Marrow.* Most recipients of anti-Thy 1.2-serum-treated allogeneic marrow cells were killed after 3–4 mo for reasons of economy. However, certain groups were kept for long-term observation and are alive and well after 12 mo. Allogeneic chimeras killed after 3–4 mo had normal sized spleens and LN. Cell suspensions from pooled

TABLE I  
Summary of Mortality Observed after Transferring Untreated Bone Marrow to Lethally Irradiated (750 rads) H-2-Compatible Allogeneic Recipients

Donor	Recipient (H-2 haplo- type)	Some of the known genetic differences be- tween donor and recipient*	Deaths by 80 days after transfer of:				GVHD with:	
			Host mar- row alone	Donor mar- row alone	Donor marrow plus spleen and LN in dose of:		Donor mar- row alone	Donor mar- row plus spleen and LN
					10 <sup>7</sup>	10 <sup>8</sup>		
B10.BR	CBA/J (H-2 <sup>k</sup> )	H-1, -3, -7, -8, -9, -12 Tla, Mls, Ly 1, 2	6/40	24/30	18/18	12/12	+	+
CBA/J	B10.BR (H-2 <sup>k</sup> )	H-1, -3, -7, -8, -9, -12 Tla, Mls, Ly 1, 2	0/6	5/6	6/6	6/6	+	+
B10.K	CBA/J (H-2 <sup>k</sup> )	H-1, -3, -7, -8, -9, -12 Mls, Ly 1, 2	2/12	1/12	(11/12)‡	(12/12)§	-	+
B10.BR	CBA/H-T6 (H-2 <sup>k</sup> )	H-1, -3, -7, -8, -9, -12 Tla, Ly 2	0/5	0/5	5/5	5/5	-	+
AKR	CBA/J (H-2 <sup>k</sup> )	H-1, -7, -12 Mls, Ly 1, Thy 1	0/6	1/6	6/6	ND	-	+
C3H/He	CBA/J (H-2 <sup>k</sup> )	H-3, -7, -9, -13 Mls	1/12	0/12	8/12	ND	-	+
CBA/J	C3H/He (H-2 <sup>k</sup> )	H-3, -7, -9, -13 Mls	0/6	1/6	6/6	6/6	-	+
129	B10 (H-2 <sup>b</sup> )	H-1, -3, -4, -9, -11, -12, -13 Ly 4, 7	1/12	1/12	4/6	6/6	-	+
CBA H-T6	CBA/J (H-2 <sup>k</sup> )	Mls, Ly 1	0/6	0/6	2/6	2/6	-	-
B6	B10 (H-2 <sup>b</sup> )	H-9	1/6	0/6	3/6	2/6	-	-
B10♀	B10♂ (H-2 <sup>b</sup> )	H-Y	0/6	0/6	(1/6)	(0/6)	-	-

\* Drawn from references 23-25 and M. Cherry, personal communication. This list is far from complete; for example, Ig allotypes and Qa determinants (see footnote 2 in text) are not mentioned.

‡  $3 \times 10^5$  nylon-wool-purified LN T cells.

§  $3 \times 10^6$  nylon-wool-purified LN T cells.

|| Cells taken from donors presensitized with  $3 \times 10^7$  male spleen and LN cells intravenously 5 wk before. A prior experiment with unsensitized cells gave no GVHD.

peripheral and mesenteric LN were comparable in cellularity ( $\sim 10^8$  cells/mouse) and proportion of T cells ( $\sim 50\%$  Thy 1.2-positive) to LN suspensions from syngeneic chimeras.

The experiment illustrated in Table II shows primary anti-SRC responses by B10.BR  $\rightarrow$  CBA/J and CBA/J  $\rightarrow$  B10.BR chimeras measured at 4 mo after reconstitution. Both groups of chimeras gave high IgM and IgG responses which were

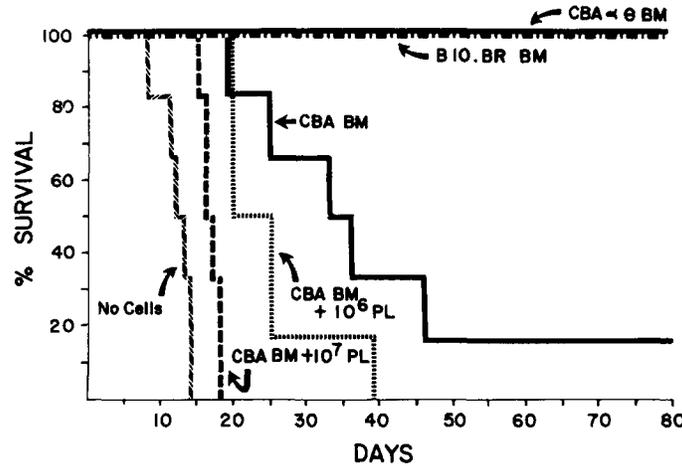


FIG. 4. Lethal GVHD in irradiated (750 rads) B10.BR mice given unfractionated CBA/J marrow cells. The data show cumulative mortality after injection of untreated syngeneic marrow cells (B10.BR BM), anti-Thy 1.2-serum-treated CBA/J marrow cells (CBA  $\alpha\theta$  BM), untreated CBA/J marrow cells (CBA BM) or CBA/J marrow cells supplemented with  $10^6$  or  $10^7$  peripheral lymphocytes (PL) from pooled spleen and LN cells of normal CBA/J mice. Data from a single experiment involving six mice per group.

TABLE II  
Identity of Anti-SRC PFC in B10.BR  $\rightarrow$  CBA/J and CBA/J  $\rightarrow$  B10.BR Chimeras Reconstituted with Anti-Thy 1.2-Serum-Treated Marrow Cells 3 mo Previously

Mice given 0.1 ml of 25% SRC intraperitoneally	Mice per group	Anti-SRC PFC/spleen at 7 days*			
		IgM	IgG	Ig <sup>b</sup>	Ig <sup>b</sup> /IgG
Normal CBA/J	2	9,350 (2.03)‡	67,490 (1.12)	0	0.00
Normal B10.BR	3	53,910 (1.30)	28,680 (1.22)	15,950 (1.32)	0.57
B10.BR $\rightarrow$ CBA/J	3	43,510 (1.11)	72,880 (1.22)	46,720 (1.27)	0.66
CBA/J $\rightarrow$ CBA/J	3	10,260 (1.09)	86,360 (1.29)	0	0.00
CBA/J $\rightarrow$ B10.BR	3	43,520 (1.27)	55,790 (1.26)	1,710 (2.62)	0.07
B10.BR $\rightarrow$ B10.BR	2	37,040 (1.32)	28,980 (1.21)	13,420 (1.12)	0.47

\* IgG PFC were enhanced with a rabbit anti-mouse Ig antiserum and Ig<sup>b</sup> PFC were enhanced with an anti-Ig<sup>b</sup> allotype antiserum as described in Materials and Methods.

‡ Geometric mean; number in parenthesis refers to value by which mean is multiplied or divided to give upper and lower limits, respectively, of SE.

roughly comparable to the responses of syngeneic chimeras or nonirradiated mice. Since B10.BR and CBA/J have different Ig allotypes (Ig<sup>b</sup> and Ig<sup>a</sup>, respectively), development of anti-SRC PFC with an anti-Ig<sup>b</sup> anti-allotype serum enabled the identity of the PFC to be determined. As shown in Table II, this antiserum developed PFC from B10.BR mice but not from CBA/J mice. In the case of the B10.BR  $\rightarrow$  CBA/J combination, it is evident that the numbers of PFC enhanced by the anti-Ig<sup>b</sup> serum were as high (or higher) as in syngeneic B10.BR  $\rightarrow$  B10.BR chimeras or in normal B10.BR mice. Thus, the vast majority of the PFC were of donor origin. This also appeared to be the case with CBA  $\rightarrow$  B10.BR chimeras since these mice contained only small numbers of Ig<sup>b</sup> PFC.

## Discussion

The present data show that in two situations (B10.BR  $\rightarrow$  CBA/J and vice versa), transplantation of untreated MHC-identical marrow cells into heavily irradiated allogeneic mice caused a high incidence of lethal chronic GVHD, i.e. mortality occurring between days 20–80 post-transfer. GVHD was not observed when the marrow cells were treated with anti-Thy 1.2 serum and complement before injection. A priori, the protective effect of anti-Thy 1.2 serum might have reflected activity against (a) T-cell precursors, (b) mature T cells, or (c) both. The first possibility is considered most unlikely because, in contrast to antisera raised in rabbits against mouse brain, there is no evidence that anti-Thy 1.2 serum prepared in mice has specificity for pre-T cells (26). The fact that GVHD was restored by the addition of even small numbers of purified LN T cells, but not by adding Thy 1.2-negative cells, strongly implies that mature T cells in marrow were the sole target of the antiserum.

Although marrow cells alone caused mortality in only two situations, it should be emphasized that 100% mortality occurred in six other combinations when spleen and LN cells were added to the marrow inoculum. Before discussing possible reasons for the differing effects of untreated marrow cells, it is first necessary to consider the nature of the antigenic determinants which led to GVHD with spleen and LN cells.

Although the precise identity of the non-MHC antigens evoking GVHD was not established, it seems almost certain that minor H determinants were responsible. Thus, it is evident from Table I that GVHD occurred only with differences at three or more minor H loci. By themselves, Mls determinants did not appear to be involved since GVHD was observed with two Mls-identical combinations (B10.BR  $\rightarrow$  CBA/H-T6 and 129/J  $\rightarrow$  B10). Moreover, Mls-incompatibility with identity at minor H loci (CBA/H-T6  $\rightarrow$  CBA/J) gave only minimal evidence of GVHD. Similarly, TLa and Ly antigens were of no apparent importance since GVHD occurred with a TLa-, Ly-identical combination (CBA/J  $\rightarrow$  C3H/HeJ).<sup>2</sup> However, the data do not exclude the possibility that responses to these antigens or Mls determinants can potentiate reactivity to minor H antigens. Establishing the precise identity of the determinants evoking lethal GVHD will require further work. Double congenic resistant strains differing only at defined minor H loci (29) would be required for this purpose.

Since T cells comprise only a minority population ( $\sim 2\%$ ) of mouse marrow cells (30), it is perhaps not surprising that lethal GVHD caused by untreated marrow cells alone was the exception rather than the rule. The degree of antigenic disparity between donor and recipient probably contributed to the variable effects of marrow cells per se. Strain variation in the proportion of T cells in marrow is another possibility; although this notion has not been tested it is of interest that certain rat strains show marked differences in numbers of T cells in marrow (31). A third possibility is that *Ir* genes contributed to the incidence of GVHD; here it is of interest that *Ir* genes control skin graft rejection (32), T-cell-mediated lympholysis (33, 34) and antibody production (35) to certain minor H determinants. Finally, strain variation in susceptibility to irradiation might have been involved. This was probably

<sup>2</sup> Qa determinants (which map between the *H-2D* and *Tla* loci) deserve consideration since these determinants evoke T-cell-mediated lympholysis (27, 28 and L. Flaherty, personal communication). Since CBA/J mice express the nonstimulating (b) *Qa* alleles, the GVHD seen in this strain presumably did not involve responses to *Qa* determinants.

not an important factor in the present experiments since in several situations the recipients were of the same strain (CBA/J). However, recently it has become apparent that the constitution of the host is indeed important since it has proved much more difficult to induce GVHD in heterozygous mice than in homozygous mice. Thus, whereas even small doses of B10.BR T cells killed CBA/J mice (Table I), even massive doses ( $>10^6$ ) of (B10.BR  $\times$  B10)F<sub>1</sub> T cells failed to kill MHC-compatible irradiated (B6  $\times$  CBA)F<sub>1</sub> mice in some experiments (data not shown).

It is clearly tempting to equate the GVHD seen in the present experiments with the syndrome of secondary disease occurring after marrow transplantation in man. Does it follow therefore that GVHD in man would be abolished by removing T cells from the marrow inoculum? Although this is an obvious prediction it has to be remembered that studies with healthy inbred mice are far removed from the clinical situation where the transplant recipient is generally in poor condition. Moreover, the fact that the recipient is frequently presensitized as the result of prior blood transfusions implies that a reciprocal HVG reaction is often involved. Finally, GVHD in man differs in certain respects from that seen in mice; for example, skin lesions are rare in mice.

Despite these reservations, the present findings would seem to provide strong support for the rationale for using T-cell-depleted populations as a source of hemopoietic stem cells in man, particularly because human marrow cells contain a much higher proportion of T cells (up to 30% [36]) than mouse marrow. Until specific anti-T-cell reagents are available in man, fetal liver cells might represent the best alternative (3, 37). However, although fetal liver cells often give good results, GVHD still occurs on occasions. While in some cases this might reflect contamination with small numbers of post-thymic T cells, an obvious complication with fetal liver transplants is that the *HLA* haplotype of the donor is usually unknown. In this respect, an intriguing alternative explanation is provided by the recent findings of Zinkernagel et al. (38, 39). These workers have shown that, even with T-cell-depleted marrow cells, allogeneic radiation chimeras in mice usually die after 2-3 mo unless the donor and host share I-region H-2 determinants (identity at the *H-2* complex is not necessary since  $a \times b \rightarrow a \times c$  chimeras show good survival). In essence this study shows that functional T cells develop only in situations where the hemopoietic stem cells share H-2 determinants with the thymus responsible for T-cell differentiation. Without such sharing, the chimeras remain immunoincompetent and most eventually develop the type of runt disease (closely resembling secondary disease) seen in other T-cell-deficient states, e.g. in neonatally thymectomized mice.<sup>3</sup> Two key predictions arise from these findings. First, fetal liver cell transplantation in man should fail on a long-term basis unless the donor and host share at least one *HLA-D* allele (the presumed counterpart of the mouse *I* region). Second, transplantation with *HLA-D*-disparate fetal liver should succeed if the thymus of the donor is also transplanted.

### Summary

In two situations, transfer of normal unsensitized bone marrow cells into heavily irradiated H-2-identical allogeneic mice caused a high incidence of lethal chronic graft-versus-host disease (GVHD), i.e. mortality occurring between days 20 and 80

<sup>3</sup> Whether the runt disease seen in bone marrow chimeras raised across complete *H-2* barriers is indeed the same as that found after neonatal thymectomy has yet to be proved. Nevertheless, it is striking that in both situations runt disease fails to develop in a germ-free environment (2, 40).

postirradiation. Minor histocompatibility determinants appeared to be the main target for eliciting GVHD. Removing mature T cells from the marrow with anti-Thy 1.2 serum and complement before injection prevented GVHD. On the basis of adding purified T cells to T-cell-depleted marrow cells, it was concluded that contamination of the marrow with as few as 0.3% T cells was sufficient to cause a high incidence of lethal GVHD in certain situations. No GVHD was found with the injection of non-T cells (Thy 1.2-negative cells) or with tolerant T cells. Irradiated recipients of T-cell-depleted marrow cells remained in good health for prolonged periods. These mice showed extensive chimerism with respect to the donor marrow, normal numbers of T and B cells and were immunocompetent.

The data provide no support for the view that chronic GVHD developing after bone marrow transplantation in man is the result of an attack by the progeny of the donor stem cells. The results imply that mature T cells contaminating marrow inocula are probably the main cause of GVHD seen in the clinical situation.

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### References

1. Thomas, E. D., R. Storb, R. A. Clift, A. Fefer, F. L. Johnson, P. E. Neiman, K. G. Lerner, H. Gluckaberg, and C. D. Buckner. 1975. Bone marrow transplantation. *N. Engl. J. Med.* **292**:832 and 895.
2. van Bekkum, D. W. 1974. The double barrier in bone marrow transplantation. *Semin. Hematol.* **11**:325.
3. Uphoff, D. E. 1958. Preclusion of secondary phase of irradiation syndrome by inoculation of fetal hematopoietic tissue following lethal total body x-irradiation. *J. Natl. Cancer Inst.* **20**:625.
4. Yunis, E. J., G. Ferrandes, J. Smith, and R. A. Good. 1976. Long survival and immunologic reconstitution following transplantation with syngeneic or allogeneic fetal liver and neonatal spleen cells. *Transplant. Proc.* **8**:521.
5. Sprent, J., H. von Boehmer, and M. Nabholz. 1975. Association of immunity and tolerance of host *H-2* determinants in irradiated  $F_1$  hybrid mice reconstituted with bone marrow cells from one parental strain. *J. Exp. Med.* **142**:321.
6. Mintz, B. 1970. Allophenic mice as test animals to detect tissue-specific histocompatibility alloantigens or  $F_1$  hybrid antigens. *Transplantation (Baltimore)*. **9**:523.
7. Owen, R. D. 1945. Immunogenetic consequences of vascular anastomoses between bovine twins. *Science (Wash. D. C.)*. **102**:400.
8. Race, R. R., and R. Sanger. 1975. Blood groups in man. 6th edition. Blackwell Scientific.
9. Benirsche, K., J. M. Anderson, and L. E. Brownhill. 1962. Marrow chimerism in the marmosets. *Science (Wash. D. C.)*. **138**:54.
10. van Bekkum, D. W. 1978. Natural resistance systems against foreign cells, tumors, and microbes. G. Cudkowicz, M. Landy, and G. M. Shearer, editors. Academic Press, Inc., New York. 69.
11. Storb, R., R. H. Rudolph, H. J. Kolb, T. C. Graham, E. Mickelson, V. Erickson, K. G. Lerner, H. Kolb, and E. D. Thomas. 1973. Marrow grafts between DLA matched canine littermates. *Transplantation (Baltimore)*. **15**:92.
12. Trentin, J. J. 1958. Tolerance and homologous disease in irradiated mice protected with homologous bone marrow. *Ann. N. Y. Acad. Sci.* **73**:799.
13. Simonsen, M., and E. Jensen. 1959. The graft versus host assay in transplantation

- chimaeras. *In Biological Problems of Grafting*, Université De Liège (Belgium). 214-238.
14. Rodey, G. E., M. M. Bortin, F. H. Bach, and A. A. Rimm. 1974. Mixed leukocyte culture reactivity and chronic graft-versus-host reactions (secondary disease) between allogeneic *H-2<sup>k</sup>* mouse strains. *Transplantation (Baltimore)*. 17:84.
  15. Elkins, W. L. 1976. Correlation of graft-versus-host mortality and positive CML assay in the mouse. *Transplant. Proc.* 8:343.
  16. Elkins, W. L. 1971. Cellular immunology and the pathogenesis of graft-versus-host reactions. *Prog. Allergy*. 15:78.
  17. Cantrell, J. L., and W. H. Hildemann. 1972. Characteristics of disparate histocompatibility barriers in congenic strains of mice. I. Graft-versus-host reactions. *Transplantation (Baltimore)*. 14:761.
  18. Bevan, M. J. 1975. The major histocompatibility complex determine susceptibility to cytotoxic T cells directed against minor histocompatibility antigens. *J. Exp. Med.* 142:1349.
  19. Julius, M. H., E. Simpson, and C. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* 3:645.
  20. Sprent, J., and H. von Boehmer. 1976. Helper function of T cells depleted of alloantigen-reactive lymphocytes by filtration through irradiated *F<sub>1</sub>* hybrid recipients. I. Failure to collaborate with allogeneic B cells in a secondary response to sheep erythrocytes measured *in vivo*. *J. Exp. Med.* 144:617.
  21. Sprent, J. 1978. Restricted helper function of *F<sub>1</sub>* hybrid T cells positively selected to heterologous erythrocytes in irradiated parental strain mice. II. Evidence for restriction affecting helper cell induction and T-B collaboration, both mapping to the K-end of the *H-2* complex. *J. Exp. Med.* 147:1159.
  22. von Boehmer, H., and J. Sprent. 1976. T cell function in bone marrow chimeras: absence of host reactive T cells and cooperation of helper T cells across allogeneic barriers. *Transplant. Rev.* 29:3.
  23. Graff, R. J., and P. W. Bailey. 1973. The non-*H-2* histocompatibility loci and their antigens. *Transplant. Rev.* 15:26.
  24. Festenstein, H. 1973. Immunogenetic and biological aspects of *in vitro* lymphocyte allotransformation (MLR) in the mouse. *Transplant. Rev.* 15:62.
  25. Frelinger, J. A., D. B. Murphy, and J. F. McCormick. 1974. *Tla* types of *H-2* congenic and recombinant mice. *Transplantation (Baltimore)*. 18:292.
  26. Roelants, G. E., F. Loor, H. von Boehmer, J. Sprent, L. Hagg, K. S. Mayor, and A. Rydén. 1975. Five types of lymphocytes ( $Ig^{-}\theta^{-}$ ,  $Ig^{-}\theta^{+}$  weak,  $Ig^{-}\theta^{+}$  strong,  $Ig^{+}\theta^{-}$ , and  $Ig^{+}\theta^{+}$ ) characterized by double immunofluorescence and electrophoretic mobility. Organ distribution in normal and nude mice. *Eur. J. Immunol.* 5:127.
  27. Forman, J., and L. Flaherty. 1978. Identification of a new CML target antigen controlled by a gene associated with the *Qa-2* locus. *Immunogenetics*. 6:227.
  28. Klein, J., and C. L. Wang. 1978. A new locus (*H-2T*) at the *D* end of the *H-2* complex. *Immunogenetics*. 6:235.
  29. Wettstein, P. J., and G. Haughton. 1977. The production, testing and ability of double congenic mouse strains. II. *B10-H-2<sup>a</sup> H-7<sup>b</sup>/Wts* and *B10-H-2<sup>d</sup> H-7<sup>b</sup>/Wts*. *Immunogenetics*. 5:85.
  30. Farrar, J. J., B. E. Loughman, and A. A. Nordin. 1974. Lymphopoietic potential of bone marrow cells from aged mice: comparison of the cellular constituents of bone marrow from young and aged mice. *J. Immunol.* 112:1244.
  31. Howard, J. C., and D. W. Scott. 1972. The role of recirculating lymphocytes in the immunological competence of rat bone marrow cells. *Cell. Immunol.* 3:421.
  32. Gasser, D. L., and W. K. Silvers. 1971. Genetic control of the immune response in mice. III. An association between *H-2* type and reaction to *H-Y*. *J. Immunol.* 106:875.
  33. Simpson, E., and R. D. Gordon. 1977. Responsiveness to *H-Y* antigen, *Ir* gene complementation and target cell specificity. *Immunol. Rev.* 35:59.

34. von Boehmer, H., C. G. Fathman, and W. Haas. 1977. *H-2* gene complementation in cytotoxic T cell responses of female against male cells. *Eur. J. Immunol.* **7**:443.
35. Zink, G. L., and S. Heyner. 1978. Further studies on non-*H-2* histocompatibility alloantibodies in the mouse. *Immunogenetics.* **6**:269.
36. Gale, R. P., G. Opelz, M. Kinchi, and D. W. Golde. 1975. Thymus dependent lymphocytes in human bone marrow. *J. Clin. Invest.* **56**:1491.
37. O'Reilly, R. J., R. Pahwa, B. Dupont, and R. A. Good. 1978. Severe combined immunodeficiency: transplantation approaches for patients lacking an HLA genotypically identical sibling. *Transplant. Proc.* **10**:187.
38. Zinkernagel, R. M., G. N. Callahan, A. Althage, S. Cooper, P. A. Klein, and J. Klein. 1978. On the thymus in the differentiation of "*H-2* self recognition" by T cells: evidence for dual recognition? *J. Exp. Med.* **147**:882.
39. Zinkernagel, R. M., G. N. Callahan, A. Althage, S. Cooper, J. W. Streilein, and J. Klein. 1978. The lymphoreticular system in triggering virus plus self specific cytotoxic T cells: evidence for T help. *J. Exp. Med.* **197**:897.
40. Miller, J. F. A. P., and D. Osoba. 1967. Current concepts of the immunological function of the thymus. *Physiol. Rev.* **47**:437.