Influence of dimethyl myleran on tolerance induction and immune function in major histocompatibility complex-haploidentical murine bone-marrow transplantation

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ABSTRACT To study murine major histocompatibility complex (MHC)-haploidentical bone-marrow transplantation (BMT), B6C3F1 mice (H-2^{b/k}) underwent BMT using syngeneic [B6C3F1 (H-2^{b/k})], haploidentical [CB6F1 (H-2^{d/b})], or fully allogeneic [DBA/2 (H-2^d)] donor mice. As pretreatment, dimethyl myleran (DMM), an alkylating agent that produces effective myeloablation but little immunosuppression, was used with total body irradiation (TBI). Four conditioning regimens were studied: TBI 800 rads (1 rad = 0.01 Gy), TBI 950 rads, TBI 800 rads plus DMM (0.2 mg per mouse), and TBI 950 rads plus DMM. Survival rates, chimerism, proliferative responses in mixed-lymphocyte culture, specific cell-mediated lympholysis, and *in vivo* plaque-forming cell responses to several antigens were compared. TBI 800 rads plus DMM was maximally effective. Haploidentical BMT was as successful in inducing long-term survival and immune and hematologic reconstitution as was syngeneic BMT. This regimen plus haploidentical BMT of T-cell-purged marrow yielded survivors tolerant of donor and recipient major histocompatibility complex. Such myeloablation and immunosuppression prevented graft rejection, immunodeficiency due to histoincompatibility, and damage to a radiosensitive cell population. A microenvironmental influence crucial to some antibody responses was thus revealed. Delayed recovery of antibody production after BMT in humans may be due partly to suboptimal myeloablation or excess irradiation.

Bone-marrow transplantation (BMT) can cure many life-threatening human diseases (1). In model systems BMT creates long-term immunological tolerance that can permit organ transplantation without continuous immunosuppression (2). T-cell-purged haploidentical BMT is used regularly to cure children with severe combined immunodeficiency disease (3, 4) but often fails when used to treat leukemia, metabolic diseases, or aplastic anemias.

Lapidot *et al.* (5) showed that myeloablation with dimethyl myleran (DMM) coupled with total body irradiation (TBI) permitted establishment of long-lasting tolerance using fully allogeneic, major histocompatibility (MHC)-mismatched BMT in a conventional environment (5). We compared survival, chimerism, and immune reconstitution after TBI (800–950 rads; 1 rad = 0.01 Gy) alone or with DMM. T-cell-purged haploidentical BMT after midlethal TBI plus DMM permits long-term survival without immunodeficiency and with rare graft rejection. Antibody (Ab) responses to various classes of antigens differed with the pretreatment used. The 800-rad TBI plus DMM regimen in mice, followed by BMT with T-cell-purged haploidentical marrow, yielded impressive reconstruction of a range of immune responses, including Ab responses to four different classes of antigens.

MATERIALS AND METHODS

Animals. (C57BL/6J × C3H/HeJ)F₁ (B6C3F1, H-2^{b/k}), (BALB/cJ × C57BL/6J)F₁ (CB6F1, H-2^{d/b}), DBA/2J (H-2^d), and SJL/J (H-2^s) mice (The Jackson Laboratory) were kept in a sterile environment before BMT (6).

TBI. Female 12-week-old B6C3F1 mice were exposed to 800 or 950 rads (¹³⁷Cs γ -irradiation source at 80 rads/min).

Administration of DMM. DMM (2,5-hexanediol dimethanesulfonate, NSC-23890, National Cancer Institute, a gift from C. D. Buckner, Seattle) was dissolved in dimethyl sulfoxide (Sigma) diluted with prewarmed phosphate-buffered saline at 1 mg/ml. Then 0.2 mg of DMM ($\approx$ 10 mg/kg) was injected *i.v.* 1 day after TBI (5, 6); 0.2 ml of 10% dimethyl sulfoxide in phosphate-buffered saline was injected *i.v.* into controls.

BMT. Three million T-cell-depleted bone-marrow cells ($\approx 1.5 \times 10^8$ cells per kg of body weight) from H-2 syngeneic (B6C3F1, H-2^{b/k}), haploidentical (CB6F1, H-2^{d/b}), or mismatched (DBA/2, H-2^d) donor were transplanted *i.v.* 1 day after pretreatment. Marrow was obtained from long bones (7). T-cell-depleted marrow (TCDM) was prepared by complement-dependent cytotoxicity with rat anti-Thy 1.2 monoclonal Ab (Becton Dickinson) plus low-toxic rabbit complement (Cedarlane, Ontario, Canada).

Survival Rate. Survival was determined for 100 days after BMT; curves were compared by a logarithm-rank method (8).

Chimerism Analysis. Spleen cells prepared on day 100 after BMT were used for immunostaining and flow cytometry (Epics Elite, Coulter) with mouse anti-H-2K^d monoclonal Ab conjugated with fluorescein isothiocyanate (IgG2a, Pharmingen, San Diego, CA) or mouse IgG2 control conjugated with fluorescein isothiocyanate (Becton Dickinson).

Mixed Lymphocyte Culture (MLC). Spleen cells (2×10^5) were distributed with 2×10^5 irradiated stimulating spleen cells (2500 rads) into wells of 96-well round-bottomed plates containing 200 μ l of RPMI 1640 medium/10% fetal calf serum/penicillin/streptomycin/50 μ M 2-mercaptoethanol (Sigma). Cells were cultured in 5% CO₂ at 37°C for 4 days. Four hours before harvest, 1 μ Ci of [³H]thymidine (1 Ci = 37 GBq) (DuPont) was added to each well. Plates were harvested on glass-fiber papers, placed in scintillation fluid, and counted. Results were expressed as stimulation index calculated as mean cpm of experimental culture/mean cpm of culture with autologous cells. Assays were done in triplicate using the medium and conditions described.

Cell-Mediated Lympholysis (CML). Effector spleen cells were prepared as for MLC. Target cells were prepared by

Abbreviations: Ab, antibody; BMT, bone-marrow transplantation; CML, cell-mediated lympholysis; DMM, dimethyl myleran; MHC, major histocompatibility complex; MLC, mixed lymphocyte culture; PFC, plaque-forming cell; SRBC, sheep red blood cells; TBI, total body irradiation; TCDM, T-cell-depleted marrow; TNP, trinitrophenyl; LPS, lipopolysaccharide; KLH, keyhole limpet hemocyanin. *To whom reprint requests should be addressed at: All Children's Hospital, 801 Sixth Street South, St. Petersburg, FL 33701.

Table 1. Survival rate and chimerism on day 100 after BMT

BMT, pretreatment	Survival at 100 days (% survival)	Engraftment, %	H-2 k ^{d+} spleen cells in engrafted mice, %
B6C3F1 → B6C3F1			
TBI 800 rads	44/45 (97.8)		5.4 ± 2.6
TBI 950 rads	43/45 (95.5)		4.9 ± 0.8
TBI 800 rads			
+ 0.2 mg of DMM	44/45 (97.8)		4.7 ± 2.7
TBI 950 rads			
+ 0.2 mg of DMM	23/28 (82.1)*		3.9 ± 0.9
CB6F1 → B6C3F1			
TBI 800 rads	39/40 (97.5)	72.0	80.9 ± 2.2
TBI 950 rads	51/65 (78.4)†	100	94.7 ± 0.3
TBI 800 rads			
+ 0.2 mg of DMM	52/55 (94.5)	100	91.9 ± 0.6
TBI 950 rads			
+ 0.2 mg of DMM	25/35 (71.4)†	100	95.9 ± 0.4
DBA/2 → B6C3F1			
TBI 800 rads	35/35 (100)	23.5	76.7 ± 4.6
TBI 950 rads	31/45 (68.9)‡	100	91.2 ± 0.5
TBI 800 rads			
+ 0.2 mg of DMM	52/55 (94.5)	82.4	85.9 ± 0.9
TBI 950 rads			
+ 0.2 mg of DMM	26/45 (57.8)‡	100	91.2 ± 0.9

Survival rate is the number of mice alive on day 100 per total mice transplanted; engraftment rate is the number of mice engrafted per mice surviving 100 days. Percent H-2k^{d+} spleen cells (mean ± SEM) in normal control mice was as follows: B6C3F1, 5.9 ± 0.6; CB6F1, 97.5 ± 0.4; and DBA/2, 98.5 ± 0.5%.

*Significant difference ($P = 0.02$) between group D and other groups.

†Significant difference ($P = 0.02$) between 800 and 950 rads.

‡Similar difference seen in mismatched BMT recipients.

incubating spleen cells (1×10^6 /ml) in culture medium/Con A at 2.5 μ g/ml (Pharmacia) for 2 days and labeled with ⁵¹Cr (Na₂CrO₄, DuPont). Effector cells (4×10^5) were distributed with 1×10^4 target cells into wells of 96-well round-bottomed culture plates and cultured for 4 hr in CO₂. The amount of ⁵¹Cr released was determined in 100- μ l aliquots of the supernatant from each well in a γ counter. Percentage specific lysis (% CML) was calculated as follows: $100 \times [(\text{experimental}$

release – spontaneous release)/(maximal release after incubation with 0.7% Triton-X – spontaneous release)].

Plaque-Forming Cell (PFC) Response to *in Vivo* Immunization with Sheep Red Blood Cells (SRBC). Mice were immunized by i.p. injection of 0.5 ml of a 2% suspension of freshly collected SRBC in phosphate-buffered saline. To study primary Ab response, 5 days after immunization spleen cell suspensions were used for PFC assay (9). For secondary responses, mice were reimmunized 9 days after the first injection; PFC assay was done on day 13. To enumerate IgG PFC, rabbit anti-mouse IgG, prepared by immunization with the 7S fraction of immunoglobulin (10), was used.

PFC Responses to *in Vivo* Immunization with Trinitrophenyl (TNP)-Conjugated Antigens. Ficoll (Sigma) was aminoethyl-carboxymethylated (AECM₈₃-Ficoll) and conjugated with 2,4,6-trinitrobenzenesulfonate (Sigma) using Inman's method (11) (TNP₈-Ficoll). Keyhole limpet hemocyanin (KLH, Calbiochem) was conjugated with trinitrobenzenesulfonate (TNP₁₀-KLH). TNP-lipopolysaccharide (TNP-LPS) was from Sigma. Two hundred micrograms of TNP-KLH, 15 μ g of TNP-LPS, or 10 μ g of TNP-Ficoll in 0.2 ml of phosphate-buffered saline was injected i.v. into mice. Spleen suspensions were prepared 5 days later. PFC assays were done by using SRBC conjugated with 2,4,6-trinitrobenzenesulfonate (12). Responses were TNP-specific based on absence of PFC specific for underivatized SRBC (13).

PFC Response to *in Vitro* Immunization with SRBC and TNP-Conjugated Antigens. Spleen cell suspensions were prepared from DBA/2 mice and divided into three groups; nontreated, T-cell depleted (see above), and macrophage-depleted. Macrophages were depleted by passage over a Sephadex G-10 column (Pharmacia) (14). Cells (5×10^6) of each group were cultured with 1 ml of medium containing 30 μ l of 1% SRBC suspension, 50 μ g of TNP-KLH, 2 μ g of TNP-LPS, or 10^{-2} μ g of TNP-Ficoll in the wells of a 24-well plate. After 4 days, anti-SRBC or anti-TNP-SRBC PFC assay was as described. Cells at 1×10^5 per 200 μ l of medium were cultured with each antigen at the concentration described for 2 days, and incorporation of [³H]thymidine was studied to determine B-cell mitogenicity *in vitro*.

Table 2. MLC and CML responses in recipients on day 100 after BMT

Mice (n)	B6C3F1		CB6F1		DBA/2		SJL	
	MLC SI	CML, %	MLC SI	CML, %	MLC SI	CML, %	MLC SI	CML, %
Normal controls								
B6C3F1 (15)	1.0 ± 0.0	0.0 ± 1.3	3.7 ± 0.4	50.2 ± 3.8	5.1 ± 1.1	67.4 ± 19.5	3.8 ± 0.3	63.3 ± 1.3
CB6F1 (12)	3.4 ± 0.4	46.6 ± 4.0	1.0 ± 0.0	4.9 ± 1.4	ND	ND	3.9 ± 0.8	50.8 ± 3.8
DBA/2 (5)	3.4 ± 0.6	49.0 ± 11.4	ND	ND	1.0 ± 0.0	-6.0 ± 1.1	4.1 ± 0.6	51.4 ± 5.0
B6C3F1 → B6C3F1								
A (3)	1.2 ± 0.1	2.1 ± 1.5	3.4 ± 0.7	41.7 ± 7.3	ND	ND	3.5 ± 0.3	61.6 ± 9.3
B (4)	1.0 ± 0.3	3.2 ± 1.2	2.2 ± 0.2	52.8 ± 6.2	ND	ND	2.2 ± 0.4	57.0 ± 9.7
C (3)	1.2 ± 0.1	2.3 ± 2.1	3.6 ± 0.3	43.3 ± 2.1	ND	ND	4.5 ± 1.3	61.4 ± 5.0
D (2)	1.2 ± 0.0	0.9 ± 0.6	2.2 ± 0.2	35.7 ± 1.8	ND	ND	2.3 ± 0.2	64.2 ± 1.6
CB6F1 → B6C3F1								
A (13)	1.0 ± 0.1	-1.2 ± 1.4	1.1 ± 0.1	-2.8 ± 1.6	ND	ND	2.8 ± 0.3	59.7 ± 5.5
B (15)	1.2 ± 0.1	2.4 ± 0.9	1.1 ± 0.1	2.5 ± 0.9	ND	ND	3.2 ± 0.4	52.0 ± 4.7
C (15)	1.2 ± 0.1	-0.1 ± 0.8	1.1 ± 0.1	-0.3 ± 0.1	ND	ND	2.9 ± 0.2	46.2 ± 3.2
D (7)	1.0 ± 0.1	-0.8 ± 0.2	1.0 ± 0.1	-4.1 ± 0.5	ND	ND	2.5 ± 0.4	60.9 ± 2.5
DBA/2 → B6C3F1								
A (2)	1.1 ± 0.2	-4.3 ± 1.0	ND	ND	0.9 ± 0.1	-6.2 ± 1.5	2.9 ± 0.5	34.7 ± 4.5*
B (9)	1.2 ± 0.3	-3.7 ± 0.4	ND	ND	1.0 ± 0.0	-4.6 ± 0.4	3.6 ± 0.4	38.5 ± 1.6†
C (7)	1.1 ± 0.3	-3.1 ± 0.4	ND	ND	1.1 ± 0.1	-4.5 ± 0.4	4.2 ± 0.8	38.1 ± 4.7‡
D (9)	1.2 ± 0.1	-3.6 ± 0.2	ND	ND	1.0 ± 0.4	-5.4 ± 1.0	3.9 ± 0.7	32.9 ± 3.2§

Pretreatments were as follows: A, TBI 800 rads; B, TBI 950 rads; C, 800 rads + 0.2 mg of DMM; D, 950 rads + 0.2 mg of DMM. SI, stimulation index (mean ± SEM). Difference between normal DBA/2 mice and DBA/2 → B6C3F1: *0.1 < P < 0.25; †0.005 < P < 0.01; ‡0.05 < P < 0.1; § P < 0.005.

FIG. 1. Direct and indirect PFC responses to SRBC. Mice (numbers beneath bars) were immunized i.p. with SRBC; PFC assay was done 5 days later. A, control; B, syngeneic BMT; C, haploidentical BMT; D, fully allogeneic BMT. Treatment regimens (Rx): 1, TBI 800 rads; 2, TBI 950 rads; 3, 800 rads plus DMM; 4, 950 rads plus DMM. Group D mice showed impaired vigorous direct or indirect PFC responses compared with group A. Response is MHC restricted. Mice subjected to Rx 2 or 4 had decreased responses to SRBC; this response is radiosensitive. DMM addition did not change responses. B6C3F1 mice differed significantly from group B mice in direct responses to Rx 2 at $P < 0.005$ and to Rx 4 at $P < 0.01$ (a); in indirect responses to Rx 2 and Rx 4, the significance levels were $P < 0.001$ and $P < 0.05$, respectively (b). n.d., not done.

Statistical Analyses. A two-tailed Student's *t* test was used to determine the significance of differences.

RESULTS

Survival Rates and Engraftment After BMT Using TCDM. Engraftments and survival rates for B6C3F1 mice (H-2^{b/k}) transplanted with 3×10^6 TCDM cells of syngeneic (B6C3F1), H-2 haploidentical (CB6F1, H-2^{d/b}), or H-2 mismatched (DBA/2, H-2^d) mice are shown in Table 1. To determine lethality of each protocol, groups of 15–25 mice were given each treatment without BMT; all died within 23 days, except for 3 of 20 mice treated with the TBI 800-rad protocol that survived >100 days post-BMT. All mice transplanted with syngeneic TCDM survived, except for the group that received TBI 950 rads plus DMM at 0.2 mg. Five of 28 mice in this group died between days 11 and 26. In both haploidentical and fully mismatched groups, fewer ($P < 0.02$) mice given 950 rads TBI than mice given 800 rads survived. Percentages of engraftment on day 100 after BMT in mice treated with TBI 800 rads alone were 72.0% with haploidentical BMT and 23.5% with MHC-mismatched BMT. Mice given 950 rads in both BMT groups showed 100% engraftment. However, DMM greatly increased engraftment without decreasing survival in mice given TBI 800 rads. With

FIG. 2. Direct (a) and indirect (b) PFC responses to TNP-KLH. Mice (numbers beneath bars) were injected i.v. with 200 μ g of TNP-KLH; PFC assay was done 5 days later. Group D mice showed impaired direct or indirect PFC responses compared with DBA/2 mice; this response is MHC restricted. Direct responses were all significant at $P < 0.01$; indirect responses were all significant at $P < 0.005$. Differences were not so large between Rx 1 and 2 when syngeneic (B) or haploidentical (C) donors were used; this response is not significantly radiosensitive. For B6C3F1 controls vs. group B direct responses were all significant at $P > 0.05$, except Rx 4 ($0.025 < P < 0.05$); indirect responses were significant at $P > 0.05$. For CB6F1 vs. group C mice direct responses were significant at $P > 0.1$; indirect responses were significant at $P > 0.1$.

haploidentical BMT, mice conditioned with TBI 800 rads plus DMM at 0.2 mg had a survival rate (94.5%) equal to that of syngeneic BMT recipients, and the 100% engraftment rate equaled that achieved in mice conditioned with 950 rads of TBI.

MLC and CML Assays. To assess tolerance to MHC antigens, proliferative and cytotoxic responses to donor (H-2^{b/k}, H-2^{d/b}, or H-2^d), host (H-2^{b/k}), or third party (H-2^s) antigens were evaluated by MLC and CML assays. Spleen cells of long-lived recipients engrafted with donor marrow cells did not proliferate in response to *in vitro* stimulation by irradiated spleen cells of donor or host type (Table 2). Proliferative responses to third party were vigorous. MLC-sensitized spleen cells of engrafted recipients were not cytotoxic for donor or host Con A-stimulated blasts but readily lysed third-party blastoid targets. Neither differences of preconditioning nor differences between host and donor strain influenced the MLC. However, recipients of fully allogeneic BMT exhibited decreased cytotoxic responses toward third-party antigens compared with recipients of syngeneic BMT preconditioned as reported (15). Haploidentical BMT recipients did not show reduced cytotoxic responses. Haploidentical BMT alone among the methods used induced immunotolerance to donor and recipient but did not decrease cytotoxic response to alloantigens.

FIG. 3. Direct PFC responses to TNP-LPS and TNP-Ficoll. Mice (numbers beneath bars) were immunized i.v. with 15 μ g of TNP-LPS (a) or 10 μ g of TNP-Ficoll (b); PFC assay was done 5 days later. See legend for Fig. 1. (a) PFC numbers in recipients are comparable to those in donors; response is neither MHC-restricted nor radiosensitive. (b) PFC responses to TNP-Ficoll were radiosensitive; no MHC restriction was seen. For B6C3F1 vs. group B mice with Rx 2 or Rx 4 results were significant at $P < 0.025$. For CB6F1 vs. group 3 mice with Rx 2 or Rx 4, results were significant at $P < 0.05$. For DBA/2 vs. group D mice with Rx 2 or Rx 4 results were significant at $P < 0.05$. This is a radiosensitive Ab response. n.d., not done.

PFC Responses to *in Vivo* Immunization with SRBC. To evaluate T-cell-dependent Ab production, direct and indirect PFC responses to SRBC after primary or secondary immunization were analyzed. Recipients of fully mismatched BMT showed lower responses after primary immunization than normal mice or recipients of either syngeneic or haploidentical BMT (Fig. 1). Even mice transplanted from fully mismatched donors responded normally to SRBC after secondary immunization (data not shown) (6, 16).

Among recipients of syngeneic or haploidentical BMT, mice pretreated with TBI 950 rads had lower PFC responses after primary immunization than mice given 800 rads. Addition of DMM to 800 rads TBI did not impair responses (Fig. 1). Mice given 950 rads showed normal responses after secondary immunization with SRBC, as observed in mice given allogeneic BMT (data not shown).

Primary PFC responses to SRBC showed MHC restriction and radiosensitivity. Secondary responses were not affected by MHC mismatch or by radiation injury. Syngeneic or haploidentical BMT after low-dose-TBI plus DMM generated normal primary and secondary PFC responses to SRBC.

PFC Responses to Immunization *in Vivo* with TNP-Conjugated Antigens. Ab production against hapten-carrier antigens was studied by evaluating PFC responses to TNP-SRBC after immunization *in vivo* with three TNP-conjugated antigens: TNP-KLH, TNP-LPS, or TNP-Ficoll. TNP-KLH is a thymus-dependent antigen. Like SRBC, it requires cooperation between

FIG. 4. Effects of T-cell or macrophage depletion on PFC responses to four antigens with normal DBA/2 mice. Spleen cells (5×10^6) were cultured with 0.05% SRBC, 50 μ g of TNP-KLH, 2 μ g of TNP-LPS, or 10^{-2} μ g of TNP-Ficoll in 24-well plates with 1 ml of medium ($\square$). Responses to SRBC were decreased either by T-cell depletion ($\blacksquare$, $P < 0.025$) or macrophage depletion ($\hbar$, $P < 0.005$). Responses to TNP-KLH were decreased by T-cell depletion ($P < 0.005$) or macrophage depletion ($P < 0.05$). Responses to TNP-LPS were not affected by either treatment ($P > 0.5$). Responses to TNP-Ficoll were not decreased by T-cell depletion ($P > 0.25$) but were reduced by macrophage depletion ($P < 0.025$). SRBC and TNP-KLH responses are thus T-cell dependent and macrophage dependent, but TNP-Ficoll responses are only macrophage dependent when immunization is done *in vitro*.

B and T cells and an influence of macrophages (17). Recipients of fully allogeneic BMT showed lower antibody responses after immunization with TNP-KLH than did normal, unmanipulated donor (DBA/2) mice (Fig. 2). This response was less radiosensitive than that seen with SRBC. TNP-LPS is a type 1 thymus-independent antigen that can activate some B cells polyclonally (18). The number of PFC in recipients immunized with TNP-LPS was similar to that in each donor strain and not influenced by different pretreatments (Fig. 3). TNP-Ficoll is a type 2 thymus-independent antigen that can stimulate B cells directly when presented on specialized macrophages—e.g., those among marginal zone cells of splenic follicles (13). Unlike the anti-TNP-LPS response, responses against TNP-Ficoll were decreased after larger doses of TBI (Fig. 3). DMM plus 800 rads TBI did not depress these responses. These results were similar to those for SRBC, yet different because no decrease of primary PFC responses to TNP-Ficoll occurred in H-2-mismatched BMT, whereas responses to SRBC were depressed.

Primary responses to TNP-KLH are fully MHC restricted. Response to TNP-LPS was vigorous in all BMT recipients studied. Responses to TNP-Ficoll are not MHC restricted but were strongly radiosensitive. Either syngeneic or haploidentical BMT after 800 rads TBI plus DMM allowed normal production of Abs against all antigens studied. Deficiencies of Ab responses to these antigens except for TNP-LPS were seen when 950 rads TBI was used alone or with DMM.

***In Vitro* Characterization of Antigens Used in PFC Assays.** To analyze cell-cell interactions among T cells, B cells, and macrophages in forming PFC responses to SRBC and TNP-antigens, we studied the influences of T-cell or macrophage depletion. Response to TNP-KLH was the most T-cell dependent of those studied (Fig. 4). Response to SRBC was T-cell dependent, but response to TNP-LPS was not T-cell dependent. T-cell depletion did not significantly affect response to TNP-Ficoll. Responses to SRBC or TNP-Ficoll were strongly macrophage-dependent; response to TNP-LPS was not. Response to TNP-KLH was macrophage dependent but less so than SRBC or TNP-Ficoll.

B-cell mitogenic activity *in vitro* was tested by measuring uptake of [3 H]thymidine by spleen cells after culture (2 days) with the antigens (Fig. 5). TNP-KLH and TNP-LPS strongly stimulated B-cell proliferation, but SRBC and TNP-Ficoll did not do so. TNP-KLH is a T-cell-dependent antigen in PFC

FIG. 5. *In vitro* mitogenic activities of four antigens. Spleen cells (1×10^5) from 4–6 DBA/2 (□) mice were cultured with 0.05% SRBC, 50 μ g of TNP-KLH, 2 μ g of TNP-LPS, or 10^{-2} μ g of TNP-Ficoll in 200 μ l of medium in 96-well flat-bottomed plates. After 44 hr, 1 μ Ci of [3 H]thymidine was added to wells and cultured for 4 hr. Cells were harvested, and [3 H]thymidine uptake was measured. TNP-LPS-stimulated polyclonal B-cell proliferation without T cells (■) or macrophages (□) is shown. TNP-KLH stimulated B-cell proliferation with the help of macrophage ($P < 0.01$). SRBC and TNP-Ficoll could not stimulate B-cell proliferation.

responses but can induce nonspecific B-cell proliferation without T-cell help. TNP-Ficoll was not mitogenic for isolated B cells, although it acts as a T-cell-independent antigen in PFC responses. To test possible inhibitory effects of SRBC, TNP-Ficoll, or Ficoll on proliferative response to phytohemagglutinin P, Con A, or LPS, these antigens were added individually to culture medium along with the mitogens. None of the antigens suppressed proliferative responses to the phytohemagglutinins (data not shown).

PFC responses *in vitro* against SRBC or TNP-Ficoll were much lower than those generated *in vivo* (Fig. 6), but no difference between *in vivo* or *in vitro* immunization with TNP-KLH or TNP-LPS was seen. Neither SRBC nor TNP-Ficoll suppressed Ab production against other antigens *in vitro* (data not shown).

DISCUSSION

Clinical development of BMT is hampered by difficulties in locating a fully MHC-matched donor. Haploidentical BMT regularly cures children with severe combined immunodeficiency disease (3, 4), but attempts to use haploidentical BMT to treat leukemias, inherited hematological abnormalities, aplastic anemias, or certain metabolic diseases frequently fail. A genetically constructed mouse model designed to

FIG. 6. *In vivo* vs. *in vitro* PFC number per spleen in DBA/2 mice. PFC responses after *in vitro* (■) or *in vivo* (□) immunization with TNP-KLH or TNP-LPS were comparable. Responses to *in vivo* immunization with SRBC or TNP-Ficoll were much greater than *in vitro* responses to these antigens. Thus, PFC responses to SRBC or TNP-Ficoll are readily elicited only *in vivo*.

mimic haploidentical BMT showed that haploidentical BMT can be successful. After Lapidot *et al.* (5), we employed T-cell depletion of donor marrow plus TBI and DMM. This pretreatment permitted haploidentical BMT with full immune reconstitution, tolerance of donor and recipient MHC, full reactivity to third-party MHC determinants, sustained full chimerism, and long-term survival in conventional environments. Such mice had immunologic responses equal to those of mice given BMT from a syngeneic donor. Perhaps as prompt and complete immunologic reconstruction cannot be achieved in larger animals or humans by haploidentical BMT. Alternatively, perhaps the ideal regimen of myeloablation and immunosuppression can be identified.

It is surprising how fragile immunologic reconstruction after BMT can be. If the dose of irradiation is too high, crucial cell-cell interactions are compromised. The compromising conditions may vary for different cell-mediated immunities and Ab responses to various antigens. However, using various amounts of TBI and DMM, we identified for mice a preparatory regimen for T-cell-purged haploidentical marrow that permits prompt restoration of both cellular and humoral immunities that often are fragile toward TBI.

Fully allogeneic BMT produces recipients that are severely deficient in primary responses to SRBC and other antigens, although secondary responses may be vigorous. When too much irradiation is used, radiosensitivity of antibody responses is observed with haploidentical or syngeneic BMT. We observed consistent differences between cellular and humoral immune responses in susceptibility to MHC restrictions and to irradiation-induced injury that correlated to the cell-cell interactions needed.

It seems important to adapt similar myeloablative and/or immunosuppressive influences to other species and ultimately to humans. If haploidentical BMT can thus succeed, the achievement would represent a long stride toward making BMT more widely applicable and effective.

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