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Long-term observations of autoimmune-prone mice treated for autoimmune disease by allogeneic bone marrow transplantation

[(NZB × NZW)F_1 mice/BXSB mice/MRL/Mr-lpr/lpr mice/renal disease/immunoreconstitution]

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ABSTRACT **Long-term effects of allogeneic bone marrow transplantation (ABMT) across major histocompatibility complex barriers were studied in (NZB × NZW)F_1 (B/W), BXSB, and MRL/Mr-lpr-lpr (MRL/lpr) mice with established autoimmune disease at the time of ABMT. In the BXSB or B/W mice, ABMT cured all aspects of autoimmune disease. Glomerular damage, revealed by histological study was dramatically improved. Serological abnormalities and immunologic functions also were normalized. Correction of autoimmune disease and advanced renal disease in BXSB and B/W mice regularly lasted >5–6 mo and even 1 yr after ABMT. In the MRL/lpr mice, however, autoimmune and renal disease at first improved but then recurred after ABMT, apparently because of intolerance of the mice for high doses of irradiation and a high degree of resistance of recipient stem cells to irradiation. In this model, *H-2* typing revealed that by the time of relapse, immunocompetent cells of the chimeric mice had been replaced by host (MRL/lpr; *H-2*^{k}) cells. B220$^+$ Ly-1$^+$ cells, present in increased numbers in untreated MRL/lpr mice, initially returned to normal levels after ABMT but then reappeared in the MRL/lpr mice that had received marrow from donors having few such lymphocytes. Thus, our results show that MRL/lpr mice possess abnormal radioresistant stem cells and provide impressive evidence that the origin of autoimmune diseases in this strain, as in the several other strains studied, resides in abnormalities present in stem cells.**

Evidence indicates that the etiopathogenesis of murine lupus can be attributed to defects that reside at the level of the stem cells (1–6). Morton and Siegel (2) first proposed this hypothesis based on experiments in which bone marrow cells (BMCs) from NZB (*H-2*^{d}) mice were transferred to autoimmune-resistant DBA/2 (*H-2*^{d}) mice, and the recipients developed autoimmune diseases similar to those found in NZB mice. Akizuki *et al.* (3) showed that T-cell-depleted BMCs from (NZB × NZW)F_1 (B/W) mice express autoimmune potential when transplanted into lethally irradiated autoimmune-resistant mice. Theofilopoulous and Dixon (1) supported this view with studies of BXSB and MRL/Mr-lpr/lpr (MRL/lpr) mice. Jyonouchi *et al.* (4) showed that hematopoietic abnormalities of NZB mice expressed early in development are corrected by marrow transplantation from major histocompatibility complex (MHC)-compatible donors.

T-cell deficiencies associated with premature thymic involution are attributable to abnormal stem cells in BXSB and MRL/lpr mice (5), and transfer of normal allogeneic BMCs to the MRL/lpr or BXSB mice reverses manifestations of autoimmune diseases in short-term (<5 mo) experiments (6). From experiments with >100 mice using renal biopsies, autopsy analyses, and immunologic studies before and after allogeneic bone marrow transplantation (ABMT), we show herein that ABMT ameliorates *established* lupus nephritis in B/W mice and BXSB mice. In addition, long-term observation (from >5 mo to 1 yr post-ABMT) revealed that autoimmune diseases of BXSB and B/W mice often remained corrected for at least 1 yr after ABMT. By contrast, in MRL/lpr mice treated by ABMT from BALB/c donors, relapse of autoimmune disease regularly occurred ≈5 mo after ABMT.

MATERIALS AND METHODS

Mice. MRL/lpr, NZB, BXSB, and NZW mice obtained from The Jackson Laboratory were maintained under specific pathogen-free conditions in the animal facility at Kansai Medical University, Osaka. BALB/c, BALB/c *nu/nu*, C3H/HeN, and C57BL/6J mice were obtained from CLEA Japan (Osaka, Japan). B/W mice were bred in our colony.

ABMT. MRL/lpr (*H-2*^{k}), BXSB (*H-2*^{b}), and B/W (*H-2*^{d}/*H-2*^{z}) mice were irradiated (doses ranged from 8.0 Gy to 9.5 Gy for the MRL/lpr mice and were >9.5 Gy for the other two strains using a ^{60}C source) and reconstituted with 2 × 10^7 BMCs of young BALB/c *nu/nu* donor mice (<2 mo) or 2 × 10^7 T-cell-depleted BMCs from BALB/c mice as described (6). In some experiments, 1–2 × 10^7 BALB/c fetal liver cells in lieu of BMCs were used as the source of hematopoietic stem cells (7).

Since it is difficult to obtain large numbers of autoimmune-prone mice at any one time, numerous sets of experiments using small groups of mice of varying ages were performed. Each group included at least 6 mice, and the total number of ABMT-treated mice exceeded 100. Although highly reproducible observations were obtained, in this report only representative data are included in *Results and Discussion*.

Histopathology. Mice were biopsied prior to ABMT or autopsied at intervals after ABMT, and sections of major organs were processed and stained with hematoxylin/eosin or periodic acid/Schiff reagent staining for histological examination. Glomerulonephritis was classified on a 1$^+$–4$^+$ scale based on severity and extent of histopathological

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Abbreviations: ABMT, allogeneic bone marrow transplantation; BMC, bone marrow cell; B/W, (NZB × NZW)F_1; C, complement; CIC, circulating immune complex; CTL, cytotoxic T lymphocyte; GVHR, graft-vs.-host reaction; IL-2, interleukin 2; MHC, major histocompatibility complex; MLC, mixed lymphocyte culture; MRL/lpr, MRL/Mr-lpr/lpr.

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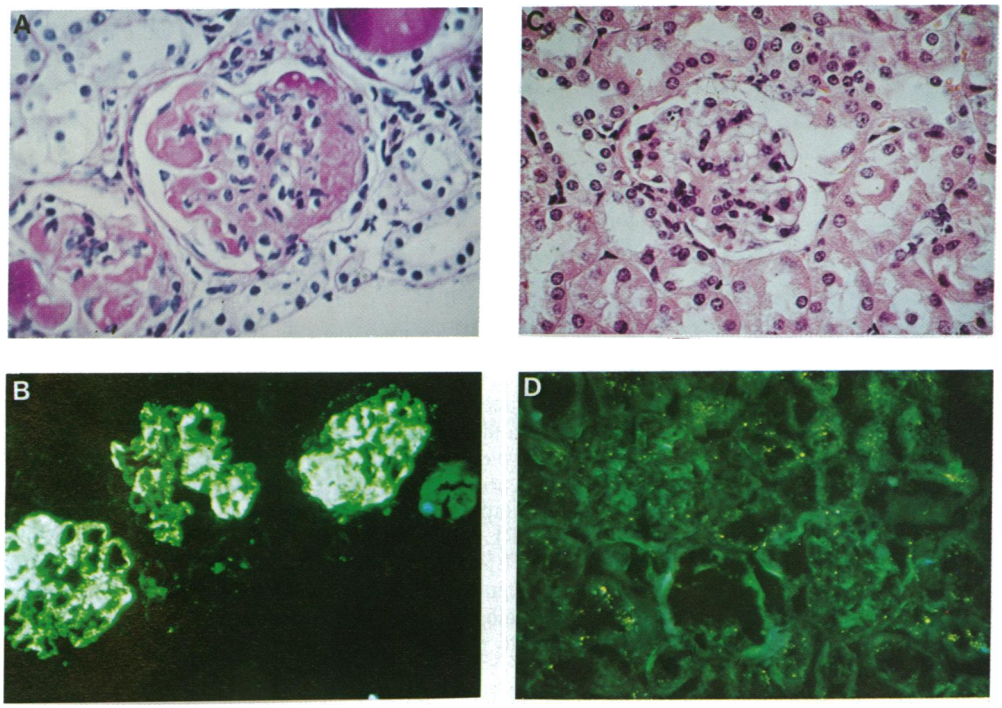

FIG. 1. Histopathological and immunofluorescence microscopy findings in the glomeruli of a B/W mouse before and after ABMT. Typical wire-loop lesions (*A*) and IgG deposits (*B*) were present in the glomeruli of this 8-mo-old B/W mouse before ABMT. When the same mouse was sacrificed at age 13 mo, 5 mo following ABMT from a BALB/c *nu/nu* donor, the glomeruli showed a normal appearance by hematoxylin/eosin staining (*C*) and markedly reduced deposition of IgG in the glomeruli (*D*).

changes. Grade 1^+ lesions showed minimal mesangial thickening; 2^+ lesions contained noticeable increases in mesangium and in capillary glomerular cellularity; 3^+ lesions were characterized by the preceding features plus superimposed inflammatory exudates and/or capsular adhesions; and 4^+ lesions showed obliteration of glomerular architecture involving ≥70% of the glomeruli. Lesions of grades 3^+ and 4^+ were considered to be those that contribute significantly as a cause of death associated with autoimmune diseases.

Immunofluorescence Study. Fresh renal biopsy or autopsy specimens were frozen in dry ice/acetone. Three-micron sections were incubated at room temperature with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse IgG or FITC-conjugated anti-mouse C3 (where C3 is the third component of complement) (Medical and Biological Laboratories, Nagoya, Japan), washed three times with phosphate-buffered saline, and then analyzed by means of standard methods of fluorescence microscopy using an Olympus fluorescence microscope (6).

The intensity of the fluorescence of IgG or C3 deposits in the mesangial areas or along capillary walls was graded as: −, no visible deposits; +, weakly staining deposits; ++, moderate deposits; and +++, heavy deposits. Evaluations were performed by two investigators who had not been provided with clinical or serological information about the mice.

gp-70 Anti-gp-70 Circulating Immune Complexes (CICs). CICs that contained the retrovirus-derived gp-70 were determined by application of polyethylene glycol to a gp-70 inhibition radioimmunoassay (8).

Table 1. Treatment of lupus nephritis in B/W mice by ABMT

ABMT	Age, mo	IgG deposition	Glomerular damage
Before	6–8	2.8 ± 0.12	2.6 ± 0.21
After	11–13	1.2 ± 0.13	0.6 ± 0.13

Twenty B/W mice received ABMT at age 6–8 mo and were sacrificed 5 mo later. Renal biopsies were performed and the intensity of the fluorescence of IgG deposits was graded as described in the text. The degree of glomerular damage was scored from 1^+ to 4^+ (also as described). The *P* value of difference in the severity of renal lesions before and after ABMT was <0.001. Values are presented as mean ± SD.

Cytotoxicity Tests, Assay for Cytotoxic T Lymphocytes (CTLs), and Mixed Lymphocyte Culture (MLC). Cytotoxicity tests using antibody plus C and assays for generation of CTLs were as described (6, 7). MLC reactivity was examined by measuring uptake of [^{3}H]thymidine into DNA after exposure to irradiated allogeneic cells as described (7).

Assay for Interleukin 2 (IL-2) Activity. Rat spleen cells were suspended at a concentration of 5×10^6 cells per ml in RPMI 1640 medium containing 5% fetal calf serum (GIBCO) in the

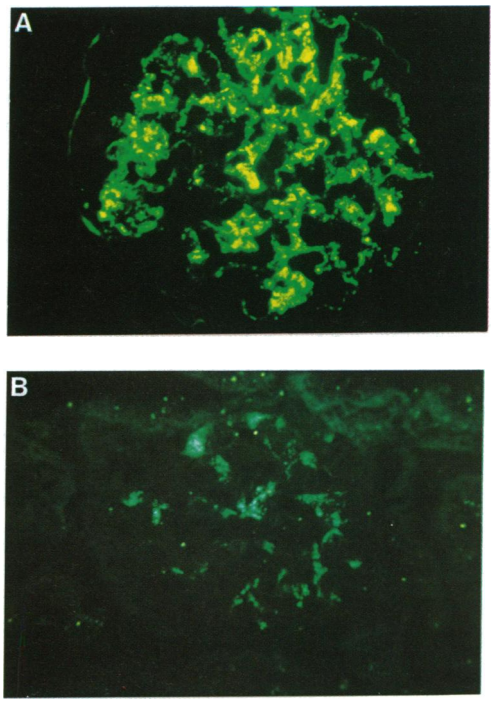

FIG. 2. Representative immunofluorescence microscopic analysis observed in a BXSB male mouse at age 8 mo (*A*) and at autopsy 5 mo following ABMT from a BALB/c *nu/nu* donor (*B*). Note the striking reduction of IgG deposits (present initially in a largely mesangial distribution) 5 mo following ABMT.

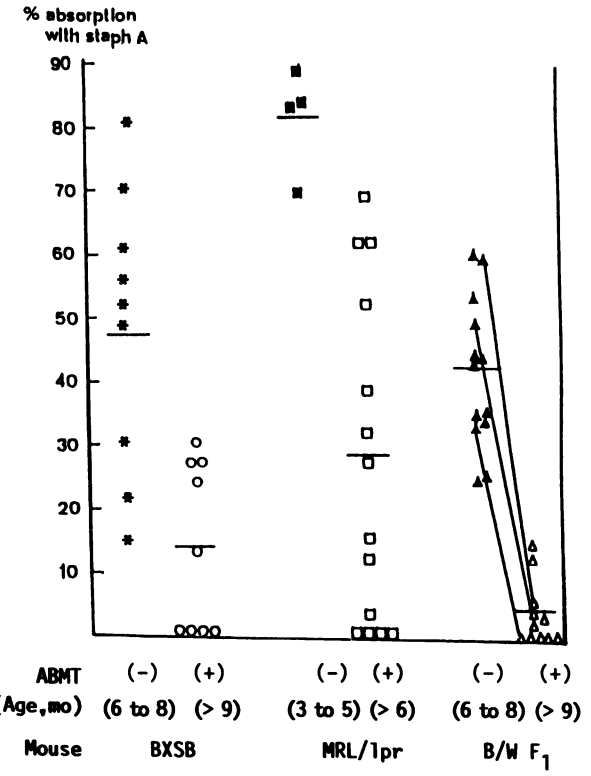

FIG. 3. Levels of gp-70 anti-gp-70 CICs before and after ABMT from BALB/c *nu/nu* donors. Levels were significantly reduced ($P < 0.001$) in BXSB and B/W mice and significantly reduced ($P < 0.01$) in MRL/lpr mice.

presence of 2.5 μg of concanavalin A per ml and cultured for 24 hr. Culture supernatant was harvested and stored at −70°C until used for standard IL-2 activity assays. Spleen cells from the various strains of mice, including autoimmune-prone mice reconstituted with BMCs, were cultured for 24 hr with 2.5 μg of concanavalin A (2.5×10^6 cells per ml) in 24-well culture plates (Costar Data Packaging, Cambridge, MA). Supernatants were stored at −70°C. The IL-2 content in culture supernatants was titrated using an IL-2-dependent cell line (CTLL-2) as described by Gillis *et al.* (9).

Staining Procedures and Enumeration of Cell Types. Methods for staining procedures and data analyses have been described in detail (10).

RESULTS AND DISCUSSION

Analysis of Chimerism. *H-2* typing after ABMT revealed that the radiation dose of 9.5 Gy was sufficient to permit apparent replacement of all hematopoietic and immunocompetent cells of B/W and BXSB mice by donor cells (BALB/c-derived cells). By contrast, MRL/lpr mice were found to be radiation-sensitive, a dosage of 8.0 Gy previously having been shown to be lethal in this strain (6). However, 5 mo after reconstitution of MRL/lpr mice with BALB/c BMCs, host MRL/lpr-derived cells (including T cells) gradually became dominant and autoimmune disease recurred (data not shown). To permit use of a larger radiation dosage in MRL/lpr mice, chlorinated water containing tetracycline (2 g/liter) was given ad libitum to the chimeric MRL/lpr mice transplanted with BALB/c *nu/nu* BMCs. The mice were kept in clean animal rooms in a laminar air flow system but were not maintained under specific pathogen-free conditions. In this way we were able to succeed regularly in treating MRL/lpr mice with as much as 9.5 Gy of total body irradiation, and this adjustment permitted long-term survival of [BALB/c *nu/nu* → MRL/lpr] mice. The increase to 9.5 Gy resulted in a progressive reduction of the incidence of rapidly occurring relapse (see Table 4). As positive controls, older (>3 mo) MRL/lpr mice were irradiated and then reconstituted with BMCs from MRL/lpr mice. However, these recipients regularly died of renal failure within 2 mo.

Histopathological Findings. Perivascular infiltration of lymphocytes and plasma cells was observed in the kidneys of female B/W (>6 mo of age), male BXSB (>6 mo), and female MRL/lpr mice (>3 mo). In each strain, the glomeruli exhibited typical advanced lesions, including wire-loop lesions and fibrinoid deposits that were demonstrable with hematoxylin/eosin or periodic acid/Schiff reagent staining. Immunofluorescence studies revealed the presence of granular deposits of both IgG and C3 in a capillary distribution in B/W mice, in a largely mesangial distribution in the BXSB male mice, and in both capillary and mesangial distributions in the MRL/lpr mice. Such IgG and C deposits also were found in mesangial areas of the glomeruli of mice of each strain.

To confirm that ABMT ameliorated the renal disease, renal biopsies were performed before and after ABMT, and autopsies were performed when the experiments were terminated on each mouse. As shown in Fig. 1 *A* and *B*, lesions representing typical wire-loop pathology and IgG deposits were present in the glomeruli of 6-mo-old B/W mice before ABMT. However, 5 mo after ABMT, deposits of IgG were markedly reduced and the glomeruli had a normal or nearly normal appearance (Fig. 1 *C* and *D*). Sample data from many experiments of this type are summarized in Table 1. ABMT-treated B/W and BXSB mice often survived >1 yr after ABMT; the longest survivors have now lived 1.5 yr following ABMT. In MRL/lpr mice, glomerular damage was temporarily improved when studied 3 mo after ABMT, but the glomerulonephritis exacerbated as early as 5 mo following

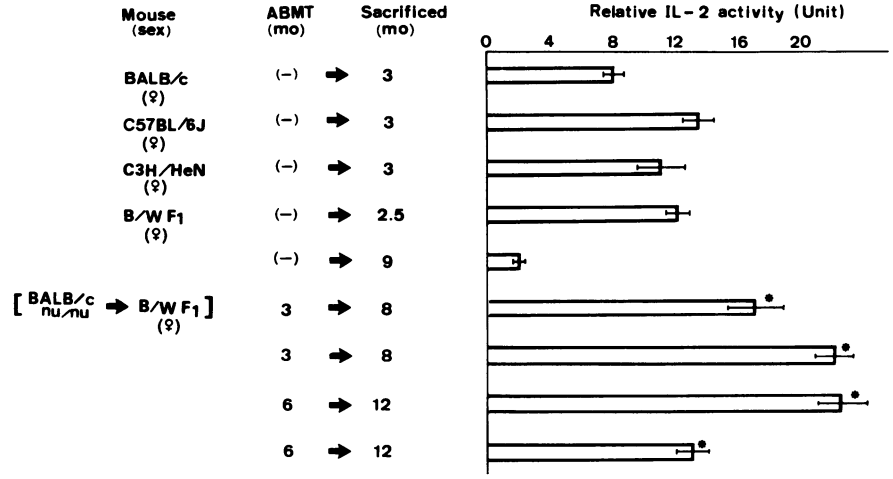

FIG. 4. A marked restoration of capacity for IL-2 production in spleen cells of B/W mice was observed 5–6 mo following ABMT ($P < 0.001$ vs. data for untreated 9-mo-old mice). B/W mice (at 3 or 6 mo of age) were irradiated and then reconstituted with BALB/c fetal liver cells in lieu of BMCs.

Table 2. Generation of CTLs in spleens of autoimmune-prone mice reconstituted with BALB/c marrow

Mice	ABMT, mo	Age at sacrifice, mo	% specific release from targets		
			P815(*H-2*^{d})	EL-4(*H-2*^{b})	X5563(*H-2*^{k})
BALB/c	—	3	0	40 ± 3	48 ± 2
B/W F_1(*H-2*^{d}/*H-2*^{z})	—	3	3 ± 1	25 ± 4	32 ± 6
	—	6	0	4 ± 1	6 ± 3
[BALB/c → B/W]*	6	19	5 ± 1	15 ± 2	18 ± 4
BXSB(*H-2*^{b})	—	3	63 ± 8	3 ± 1	56 ± 2
	—	6	8 ± 1	0	11 ± 3
[BALB/c → BXSB]	6	20	0	2 ± 1	31 ± 5
MRL/lpr(*H-2*^{k})	—	1.5	72 ± 6	26 ± 1	0
	—	6	8 ± 1	5 ± 1	2
[BALB/c → MRL/lpr]	4	7	0	46 ± 1	0
	5	11	55 ± 1	16 ± 3	0

Groups of at least six autoimmune-prone mice were transplanted with BALB/c marrow cells. Killing activity was measured at an effector:target ratio of 5:1. Values are presented as mean ± SEM. *B/W mice (age, 6 mo) were lethally irradiated (9.5 Gy), reconstituted with BALB/c BMCs, and sacrificed at 19 mo of age.

ABMT, and the mice proceeded to die of renal failure thereafter, showing extensive renal pathology (Fig. 2).

gp-70 Anti-gp-70 CICs. Izui *et al.* (11) demonstrated a correlation between levels of gp-70 CICs and the extent of glomerular damage in B/W mice. Thus, in the present experiments we measured levels of gp-70 anti-gp-70 CICs before and after ABMT and found these levels regularly to be reduced in the autoimmune-prone mice following ABMT (Fig. 3), the degree of reduction being greatest in B/W F_1 mice ($P < 0.001$) and considerably less, although still significant ($P < 0.01$), in MRL/lpr mice.

IL-2 Production. Older B/W mice (9 mo) produced less IL-2 than did young B/W mice (2.5 mo) (Fig. 4). However, >5 mo after ABMT, B/W mice reconstituted with BALB/c *nu/nu* BMC or BALB/c fetal liver cells showed significantly higher production of IL-2 and had levels of IL-2 production comparable to those observed in normal BALB/c, C57BL/6J, or C3H/HeN mice.

Generation of CTLs. As shown in Table 2, CTLs were not generated in older, untreated B/W female, BXSB male, or MRL/lpr female mice, although these cells could be generated in young mice of each strain. It is noteworthy that B/W female and BXSB mice reconstituted with BALB/c BMCs became unresponsive to both donor-type (BALB/c) and host-type (B/W female or BXSB male) MHC determinants but showed vigorous responses to third-party MHC determinants. These characteristics persisted for >1 yr following ABMT in mice of either strain. [BALB/c → MRL/lpr] chimeras also showed tolerance to both BALB/c-type and MRL/lpr-type MHC determinants 3 mo after ABMT. However, 5 mo after ABMT, spleen cells from such chimeras killed both EL-4(*H-2*^{b}) and P815(*H-2*^{d}) targets but not the X5563(*H-2*^{k}) target. These results were confirmed by MLC reactivity (data not shown). *H-2* typing demonstrated that in [BALB/c → MRL/lpr] chimeras, the T cells, B cells, and macrophages of these mice were all derived from stem cells of recipient strain 5 mo after BMT (data not shown). Immunohistopathological and serological studies revealed that the autoimmune disease and associated renal injury had recurred in such [BALB/c → MRL/lpr] mice (data not shown).

Abnormal Cells That Possess Markers for Both T Cells and B Cells. The number of Ly-1 B cells in B/W mice is known to increase with age (12). As shown in Table 3, an impressively high percentage of Ly-1 B cells was present in spleen of untreated B/W F_1 mice, but almost all such cells disappeared from the spleens of chimeric mice after ABMT. In MRL/lpr mice, the number of B220$^+$ Ly-1$^+$ also increases with age (13). Table 4 presents data showing that untreated 3-mo-old MRL/lpr mice have a high percentage of B220$^+$ Ly-1$^+$ cells in the spleen, and this population was decreased following ABMT (although not to the same degree as occurred in the ABMT-reconstituted B/W mice). However, at 5 mo following ABMT, B220$^+$ Ly-1$^+$ B cells reappeared in increased numbers in spleens of recipient MRL/lpr mice. The rate at which such cells reappeared correlated with the dose of irradiation given and with the number of months that had elapsed since ABMT. Ia^d-positive cells (derived from BALB/c stem cells) could not be detected in the majority of these ABMT-treated MRL/lpr mice >5 mo after ABMT (Table 4). Assays for host-derived cells and capacity to generate CTLs indicated that T cells were present that could react with cells of donor origin (BALB/c; *H-2*^{d}).

Table 4 also gives results of cytotoxicity analyses of the [BALB/c → MRL/lpr] chimeras. Spleen cells from chimeric mice assayed within 2 mo after ABMT showed donor (BALB/c) phenotype (*H-2*^{d}), whereas 5 mo after ABMT the host (MRL/lpr)-derived cells became dominant (except for one mouse that did not possess B220$^+$ Ly-1$^+$ cells). To ascertain that B cells and macrophages are also host-derived in these mice, the cells that had been treated with anti-Thy-1.2 plus C to eliminate T lymphocytes were used for *H-2* typing. Results showed that the B cells and macrophages were MRL/lpr host-derived (*H-2*^{k} cells >80%).

Several groups, including ours, have reported that transfer of T-cell-depleted BMCs or untreated fetal liver cells from MRL/lpr mice to other strains (even MRL/lpr +/+ mice) induces a graft-vs.-host reaction (GVHR)-like syndrome in recipients (7, 14, 15). Thus, the stem cells in MRL/lpr mice have a number of peculiarities, including an apparent resistance to radiation, as indicated by the fact that in the present

Table 3. The percentage of Ly-1 B cells in spleen of B/W mice decreases after ABMT

Mice	Age at ABMT, mo	Age at sacrifice, mo	% Ly-1 B
B/W	—	6	10
[BALB/c *nu/nu* → B/W]	3	12	<1
	3	15	<1
	3	15	<1
	6	10	4
	6	10	<1
	6	13	<1
	6	15	<1
	6	18	<1

B/W mice were lethally irradiated (9.5 Gy) and then reconstituted with BALB/c *nu/nu* marrow cells.

Table 4. Relationship between the percentage of B220$^+$ Ly-1$^+$ cells and chimerism in spleens of MRL/lpr mice reconstituted with BALB/c marrow cells

Mice	^{60}Co, Gy	ABMT, mo	Sacrifice, mo	% B220$^+$ Ly-1$^+$	% Ia^{d+} cells	% total IgM$^+$ cells	Cytotoxic index		
							H-2^k	*H-2^d*	Thy-1$^+$
BALB/c	—	—	3	2	48	46	10	80	24
MRL/lpr	—	—	3	32	<1	10	83	8	53
[BALB/c *nu/nu* → MRL/lpr]*	8.5	2	3	<1	38	42	8	85	10
	8.5	2	4	<1	35	45	15	78	18
	8.5	2	7	14	1	16	—	—	—
	8.5	3	7	7	<1	10	—	—	—
	8.5	3	8.5	18	<1	20	—	—	—
	8.5	3.5	7	12	<1	13	92	5	40
	8.5	4	6.5	10	<1	15	88	11	35
	9.0	5	11	4	<1	11	80	25	28
	9.0	5	11	<1	50	46	11	92	29
	9.5	5	11	9	<1	24	98	—	55

*MRL/lpr mice were irradiated and then reconstituted with BALB/c *nu/nu* marrow cells.

experiments a dose of 9.5 Gy was insufficient to eliminate such cells. Using a radiation dose of 1000 rads (1 rad = 0.01 Gy), Perkins *et al.* (16) reported marrow graft failure in MRL/lpr mice, suggesting that MRL/lpr mice are resistant to grafts of lymphoid but not erythroid stem cells from normal mice. Therefore, it is likely that MRL/lpr mice have abnormal lymphoid stem cells that are impressively radioresistant. Future investigations may establish a method to treat autoimmune diseases in this strain by a number of strategies—e.g., by repeated ABMT—since MRL/lpr mice (but not their stem cells) were radiosensitive and thus unsuited to experiments involving doses >9.5 Gy. Alternatively, combinations of myeloablative drugs, immunotoxic drugs, or monoclonal antibodies together with total body irradiation in maximal doses may be necessary to reverse the autoimmunities and immunologic abnormalities of MRL/lpr mice.

The long-lasting beneficial results we obtained with BXSB male and B/W female mice stand in striking contrast to the recurrent autoimmunity and evidence of recurring renal damage documented in the MRL/lpr mice. Indeed, even the advanced renal disease and/or advanced immunologic perturbations that are characteristic of mice of these strains were regularly corrected by ABMT using donor cells that crossed MHC barriers. Just how far renal disease can have advanced and yet still be reversible by ABMT in these two strains is a cogent question that must be addressed by these or similar methods in future studies.

Since autoimmune diseases are attributable to genetic abnormalities of stem cells, ABMT to correct autoimmunity using marrow from MHC-nonidentical donors might be preferable. In humans, however, ABMT across MHC barriers often fails, frequently due to GVHR arising from contamination with T cells of blood origin or to rejections based upon host-vs.-graft rejection or to hematopoietic competition. Herein we show that in autoimmune-prone mice, no such problems arise with ABMT if BMCs are completely depleted of T cells and if myeloablation and immunosuppression are adequate. It seems certain that successful ABMT in humans soon will be realized, making this a valuable strategy in therapy of lethal and/or highly morbid autoimmune diseases.

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1. Theofilopoulos, A. N. & Dixon, F. J. (1985) *Adv. Immunol.* **37**, 269–390.
2. Morton, J. I. & Siegel, B. V. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 2162–2165.
3. Akizuki, M., Reeves, J. P. & Steinberg, A. D. (1978) *Clin. Immunol. Immunopathol.* **10**, 247–259.
4. Jyonouchi, H., Kincade, P. W., Good, R. A. & Fernandes, G. J. (1981) *J. Immunol.* **127**, 1232–1235.
5. Nakamura, T., Ikehara, S., Good, R. A., Inoue, S., Sekita, K., Furakawa, F., Tanaka, H., Oo, M. M. & Hamashima, Y. (1985) *Thymus* **7**, 151–160.
6. Ikehara, S., Good, R. A., Nakamura, T., Sekita, K., Inoue, S., Oo, M. M., Muso, E., Ogawa, K. & Hamashima, Y. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 2483–2487.
7. Nakamura, T., Good, R. A., Yasumizu, R., Inoue, S., Oo, M. M., Hamashima, Y. & Ikehara, S. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4529–4532.
8. Maruyama, N., Furakawa, J., Nakai, Y., Sasaki, Y., Ohta, K., Ozaki, S., Hirose, S. & Shirai, T. (1983) *J. Immunol.* **130**, 740–746.
9. Gillis, S., Ferm, M. M., Ou, W. & Smith, K. A. (1978) *J. Immunol.* **120**, 2027–2032.
10. Hayakawa, K., Hardy, R. R. & Herzenberg, L. A. (1986) *Eur. J. Immunol.* **16**, 450–456.
11. Izui, S., McConahey, P. J., Theofilopoulos, A. N. & Dixon, F. J. (1979) *J. Exp. Med.* **149**, 1099–1116.
12. Hayakawa, K., Hardy, R. R., Parks, D. R. & Herzenberg, L. A. (1983) *J. Exp. Med.* **157**, 202–218.
13. Morse, H. C., Davidson, W. F., Yetter, R. A., Murphy, E. D., Roths, J. B. & Coffman, R. L. (1982) *J. Immunol.* **129**, 2612–2615.
14. Fujiwara, M. & Kariyone, A. (1984) *Immunology* **53**, 251–256.
15. Theofilopoulos, A. N., Balderas, R. S., Gozes, Y., Aguado, M. T., Hang, L. M., Morrow, P. R. & Dixon, F. J. (1985) *J. Exp. Med.* **162**, 1–18.
16. Perkins, D. L., Michaelson, J. & Marshak-Rothstein, A. (1987) *J. Immunol.* **138**, 466–469.