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Immunology

Effective treatment of autoimmune disease and progressive renal disease by mixed bone-marrow transplantation that establishes a stable mixed chimerism in BXSB recipient mice

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ABSTRACT **Male BXSB mice spontaneously develop autoimmune disease with features similar to systemic lupus erythematosus. To determine whether this autoimmune disease can be treated as well as prevented by bone-marrow transplantation (BMT) and, at the same time, whether the immunity functions of lethally irradiated recipients can be reconstituted fully, male BXSB mice were engrafted with mixed T cell-depleted marrow (TCDM) both from fully allogeneic autoimmune-resistant BALB/c mice and from syngeneic autoimmune-prone BXSB mice, after the onset of autoimmune disease in the recipient mice. BMT with mixed TCDM from both resistant and susceptible strains of mice (mixed BMT) established stable mixed chimerism, prolonged the median life span, and arrested development of glomerulonephritis in BXSB mice. BMT with mixed TCDM also reduced the formation of anti-DNA antibodies that are observed typically in male mice of this strain. Furthermore, mixed BMT reconstituted the primary antibody production in BXSB recipients impressively. These findings indicate that transplantation of allogeneic autoimmune-resistant TCDM plus syngeneic autoimmune-prone TCDM into lethally irradiated BXSB mice can be used to treat autoimmune and renal disease in this strain of mice. In addition, this dual bone-marrow transplantation reconstitutes the immunity functions and avoids the immunodeficiencies that occur regularly in fully allogeneic chimeras after total body irradiation. This report describes an effective treatment of progressive renal disease and autoimmunity by establishing a stable mixed chimerism of TCDM transplantation from allogeneic autoimmune-resistant BALB/c mice plus syngeneic autoimmune-prone BXSB mice into BXSB mice.**

BXSB mice spontaneously develop a human-lupus-like autoimmune disease and die from immune-complex-mediated glomerulonephritis. This disease is somewhat different in distribution and manifestation than the renal disease characteristic of mice from other autoimmune-prone strains (1). A mutant gene, *Yaa*, located in the Y chromosome of BXSB mice, has been found to enhance autoimmune abnormality profoundly in this strain of mice (2, 3).

The etiologic and pathogenic bases of many autoimmune diseases in relatively short-lived inbred strains of mice ultimately reside in the primitive self-renewing hematopoietic stem cell population. The effect of bone-marrow transplantation (BMT) and other forms of cellular engineering as a treatment and/or prevention of these autoimmune diseases in mice has been investigated extensively (4–12). Cellular engineering by BMT, which replaces the primitive self-renewing hematopoietic stem cells of the recipient with those of the donor, can be used to treat or prevent many autoimmune diseases in mice. Fully allogeneic BMT, after the marrow is purged of potentially damaging and destructive T cells, can prolong the life spans, inhibit the production of serum autoantibodies, and either treat or prevent the development of the autoimmune-associated histopathological lesions in autoimmune-prone strains of mice (4–9). However, when donors and recipients are mismatched fully at the MHC, the fully allogeneic chimeras prepared experience immunodeficiencies after total body irradiation (TBI) followed by BMT. Although these fully allogeneic chimeras are specifically tolerant of both donor and recipient and fully reactive to third-party cells and tissue grafts, they lack normal primary humoral immune responses (13) and have deficient cellular immune responses to certain intracellular pathogens (14).

Ildstad *et al.* (15) discovered that chimeras transplanted with mixed T cell-depleted marrow (TCDM) from both allogeneic and syngeneic donors can reconstitute hematopoietic and immunologic function fully after supralethal TBI; these chimeras do not express the immunological deficits observed after TBI plus BMT with fully allogeneic bone marrow. El-Badri and Good (16, 17) extended Ildstad's research by showing survival in high frequency with stable mixed chimerism and normally vigorous, functioning immune systems in C57BL/6 mice transplanted with TCDM from both BALB/c allogeneic donors and C57BL/6 syngeneic donors that differed from each other across the entire MHC barrier. These stable mixed chimeras did not have the immunodeficiencies that are observed regularly in fully allogeneic chimeras.

Previously, we have described that mixed BMT can prevent the development of autoimmune disease in BXSB mice (18). Our present investigation represents an attempt to treat autoimmune diseases and, at the same time, to reconstruct full immunity functions of irradiated mice by transplanting mixed TCDM from both allogeneic autoimmune-resistant donor mice and syngeneic autoimmune-prone donor mice into lethally irradiated BXSB recipients. This method of treatment at once corrects the propensity to autoimmune disease and also avoids the annoying immunodeficiencies that may otherwise be produced by allogeneic BMT. This treatment has been successful, and it has produced a dramatic correction of autoimmune disease in the BXSB mice.

After the apparent manifestation of autoimmune disease, lethally irradiated 16-week-old recipient BXSB mice were transplanted with mixed TCDM cells from both allogeneic autoimmune-resistant BALB/c donor mice and syngeneic autoimmune-prone BXSB mice. The transplanted mice had longer life spans, lower levels of serum anti-double-stranded (ds)DNA antibodies, and higher numbers of primary antibody-producing plaque-forming cells (PFC) than mice transplanted with syngeneic BXSB TCDM cells. These mice also produced

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Abbreviations: BMT, bone-marrow transplantation; ds, double-stranded; PFC, plaque-forming cell; SRBC, sheep red blood cells; TBI, total body irradiation; TCDM, T cell-depleted marrow.

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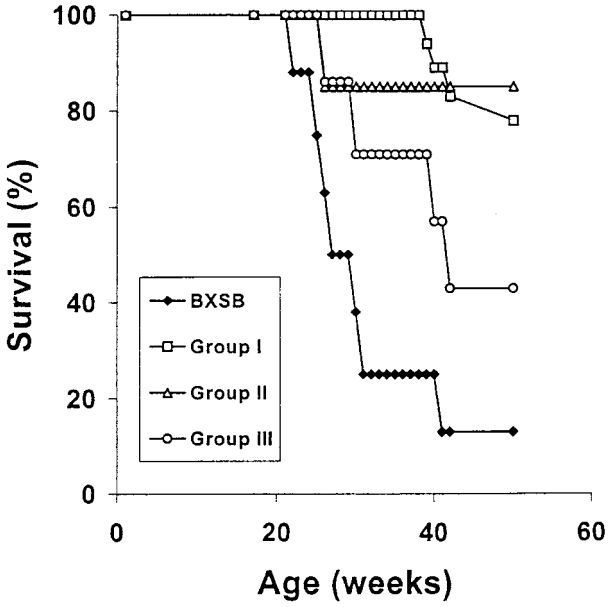

FIG. 1. Survival curves of male BXSB mice exposed to 9.5 Gy of TBI (^{137}Cs irradiation; 0.70 Gy/min) and given TCDM cells intravenously at 16 weeks of age, after the occurrence of autoimmune disease: BALB/c + BXSB → BXSB group (squares; group I; $n = 18$); BALB/c → BXSB group (triangles; group II, $n = 8$); and BXSB → BXSB group (circles; group III; $n = 7$). Untreated BXSB mice served as controls (diamonds; $n = 8$).

primary antibody responses that were significantly greater than those produced by mice that had been transplanted with bone marrow from fully allogeneic donors that differed from recipients across the entire MHC antigen barrier. This transplantation of mixed TCDM also arrested and reversed the development of autoimmune-associated glomerulonephritis in the BXSB mice.

MATERIALS AND METHODS

Mice. Male BXSB, BALB/c, and C57BL/6 mice were purchased from The Jackson Laboratory and maintained in a pathogen-free environment. Recipient mice were 16 weeks old and had manifested apparent autoimmune disease, which was confirmed by renal biopsy 10 days before BMT. Donor mice were 8–10 weeks old.

BMT. Bone-marrow cells were harvested from the femurs and tibias of donors and were depleted of T cells by complement-dependent cytotoxicity with purified anti-Thy 1.2 monoclonal antibody (PharMingen) plus rabbit complement (Cedarlane Laboratories). Recipients, 16-week-old male BXSB mice, were given 9.5 Gy of TBI (^{137}Cs irradiation; 0.70 Gy/min) and reconstituted intravenously by BMT of mixed TCDM cells in a standard fashion. Different donor TCDM cells were used for three transplantation groups: 15×10^6 allogeneic autoimmune-resistant BALB/c TCDM cells plus 5×10^6 syngeneic autoimmune-prone BXSB TCDM cells for the BALB/c + BXSB → BXSB experimental group (18 recipients); 20×10^6 allogeneic BALB/c TCDM cells for the BALB/c → BXSB treatment control group (8 recipients); and 15×10^6 syngeneic BXSB TCDM cells for the BXSB → BXSB autoimmune-disease control group (7 recipients). BALB/c → C57BL/6 (three recipients) served as fully allogeneic BMT controls. C57BL/6 → C57BL/6 (three recipients) were used as normal syngeneic BMT controls. Untreated BXSB mice (eight mice) were also used as controls. All studies with animals were conducted in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care, in compliance with the principles of the *Guide for the Care and Use of Laboratory Animals* (19).

Assay of Chimerism. Single-cell suspensions of spleen cells from chimeric mice at the age of 50 weeks (34 weeks after BMT) were incubated with fluorescein-conjugated anti-K^b and fluorescein-conjugated anti-K^d monoclonal antibodies (PharMingen). Subpopulations of allogeneic donor cells (K^d positive) and syngeneic cells (K^b positive) were quantified with flow-cytometric analysis.

Histopathology. Samples of kidneys were obtained at biopsy (when BXSB recipients were 16 weeks and 30 weeks old) as well as at autopsy. These kidneys were fixed in 10% neutral formalin for histopathologic examination, and histological sections were stained with either the periodic acid-Schiff reagent or with hematoxylin eosin. Glomerulonephritis was scored on a 0–4 scale, based on the intensity and extent of histopathological changes. A grade of 0 was given to kidney without glomerular lesions; a grade of 1 was given when there was minimal thickening of the mesangium; a grade of 2 was given when there was a noticeable increase in both mesangial and glomerular capillary cellularity; a grade of 3 was given when the preceding conditions were observed, along with superimposed inflammatory exudates and capsular adhesions; and a grade of 4 was given when the obliteration of the glomerular architecture included >70% of glomeruli. These classifications were used to grade 20 glomeruli within one area, and the mean glomerular histopathological score was calculated for each mouse. In turn, these scores were used to calculate mean scores for each experimental cohort.

Anti-Sheep Red Blood Cell (SRBC) PFC Assay. Primary antibody production against a cellular antigen was tested in BXSB BMT recipient mice as well as untreated control mice when the mice were 50 weeks old. For this purpose, each treated or control mouse was injected intraperitoneally with 0.5 ml of 2% SRBCs. After 5 days, the injected mice were killed, and a single-cell suspension of spleen cells was adjusted to a concentration of 5×10^6 cell per ml. A mixture of 500 μl of 0.5% agarose solution (Sigma), 100 μl of spleen-cell suspension, and 50 μl of 1% SRBC suspension was poured onto a precoated microslide. Dried slides were inverted carefully in a tray and incubated with guinea-pig complement (Cappel). After being incubated at 37°C for 3 h, hemolytic plaques were counted and expressed as number of PFCs per 5×10^5 cells.

Assay of Anti-dsDNA AutoAntibodies. Serum antibodies specific for dsDNA were determined by using an enzyme-linked immunosorbent assay as described (20). The serum was diluted to 1:100 for the assay. The concentration of anti-dsDNA was determined by reading the OD at 410 nm.

Statistical Analysis. Statistical significance was determined by either a log-rank Mantel–Haenszel test or Student's t test. P values <0.05 were considered significant.

RESULTS

Longevity. Within 34 weeks of BMT (age of 50 weeks), 72% of the BXSB recipients of TCDM from autoimmune-prone

Table 1. Chimerism of BXSB recipients transplanted with mixed TCDM

Mice	Cell phenotype, %	
	H-2d*	H-2b†
BALB/c + BXSB → BXSB‡	49.8 ± 16.6	40.4 ± 14.2
BALB/c → BXSB	97.3 ± 0.2	2.0 ± 0.6

Chimeric spleen cells were analyzed 34 weeks after BMT. Data are given as means ± SD.
*Phenotype of BALB/c.
†Phenotype of BXSB.
‡In the BALB/c + BXSB → BXSB mixed TCDM group, 2 of 18 transplants did not engraft allogeneic cells of BALB/c origin. The chimeric data from these two mice were excluded.

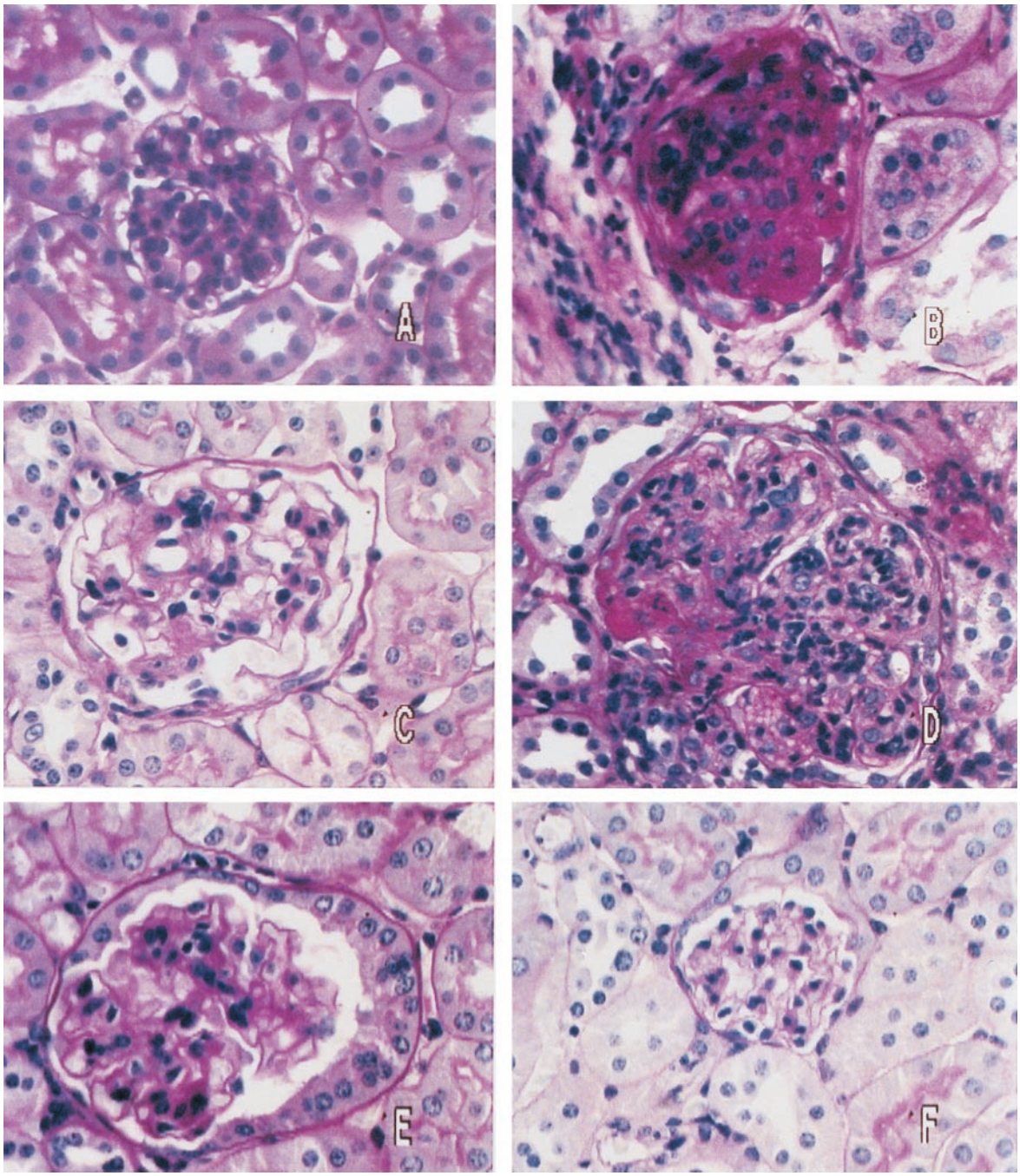

FIG. 2. Histopathology of kidneys from BXSB recipients transplanted with mixed TCDM. Representative histological appearance of glomerular lesions of kidneys from 50-week-old untreated mice or chimeras, taken from biopsies of 16-week-old BXSB mice (*A*), untreated BXSB mice (*B*), BALB/c + BXSB → BXSB mice (*C*), BXSB → BXSB mice (*D*), BALB/c → BXSB mice (*E*), and C57BL/6 mice (*F*). The tissues were stained with periodic acid-Schiff reagent (×400).

BXSB donors had developed and died of fulminant lethal glomerulonephritis (Fig. 1). In contrast, only 22% of the BXSB recipients of mixed BMT (transplanted with mixed TCDM from autoimmune-resistant BALB/c donors plus TCDM from the autoimmune-prone BXSB donors) had developed fatal renal disease at this time. This result is comparable to the percentage (15%) of the treatment control group of BXSB recipients that had been transplanted with TCDM from autoimmune-resistant allogeneic BALB/c donors. Median survival age of recipients of syngeneic BXSB TCDM was 41 weeks, whereas that of mice engrafted with mixed TCDM was >48 weeks of age, at which point the study was terminated. Median survival age of untreated BXSB chimeric mice was 32 weeks.

Chimeric Analysis. As shown in Table 1, spleens from BXSB mice transplanted with allogeneic BALB/c TCDM cells were repopulated almost completely with cells of donor origin (H-2d). The percentages of cells of donor origin from allogeneic BALB/c → BXSB chimeras were comparable to those of cells of donor origin (H-2d) from BALB/c → C57BL/6 allogeneic chimeras (data not shown). The percentages of H-2d positive cells (allogeneic origin) from BXSB mice transplanted with mixed TCDM were 49.8% from BALB/c + BXSB → BXSB chimeric mice.

Histopathology. Glomerulonephritis within each kidney of chimeric mice or untreated BXSB mice at ages of 16 weeks (biopsy) and 50 weeks (autopsy) was graded according to the intensity and extent of renal lesion. Mean glomerular lesion

scores for each mouse were determined, and mean glomerular lesion scores for each experimental cohort were calculated and compared. For those biopsy samples from 16-week-old BXSB mice, mean glomerular lesion scores were 1.9 ± 0.56. At the time of terminating this experiment (when recipient BXSB mice were 50 weeks old), glomerular lesions of mice engrafted with mixed TCDM (BALB/c + BXSB → BXSB) as well as with allogeneic TCDM (BALB/c → BXSB) were significantly less severe compared with those of recipients of BXSB TCDM or BXSB untreated controls (Fig. 2). Mean glomerular lesion scores of BALB/c + BXSB → BXSB chimeric mice and those of BALB/c → BXSB chimeric mice were comparable (means of 2.17 and 2.25 with SDs of ±0.69 and ±0.83, respectively). Mean glomerular lesion scores of BXSB control mice and those of the recipients of BXSB TCDM were comparable (means of 4.0 and 3.5 with SDs of ±0.0 and ±0.50, respectively).

Primary Antibody Response. BXSB mice transplanted with TCDM were tested for the ability to mount a primary antibody response against the cellular antigen SRBC in a PFC assay. As shown in Fig. 3, BXSB recipients transplanted with mixed TCDM from the BALB/c + BXSB → BXSB group and from the C57BL/6 → C57BL/6 syngeneic control group as well as normal C57BL/6 mice produced a primary antibody response against SRBCs. However, inhibition of primary antibody formation was present in BXSB untreated control mice, BXSB → BXSB transplants, and BALB/c → BXSB transplants, as well as BALB/c → C57BL/6 fully allogeneic chimeras.

Anti-dsDNA AutoAntibodies. Serum levels of anti-dsDNA autoantibodies were determined for mice from each group of recipients as well as from the BXSB control group when mice were 50 weeks of age. As shown in Fig. 4, recipient BXSB mice transplanted with mixed TCDM from BALB/c plus BXSB had lower levels of anti-dsDNA than did BXSB → BXSB and BXSB mice.

DISCUSSION

We have shown recently that it is possible to prevent autoimmune disease in BXSB mice by creating a stable mixed bone-marrow chimerism by transplanting T cell-purged allogeneic autoimmune-resistant marrow from BALB/c with T cell-purged syngeneic autoimmune-prone marrow (18). In the present study, we investigated whether such a mixed BMT that establishes a stable mixed chimerism can be used effectively to treat autoimmune disease in BXSB mice.

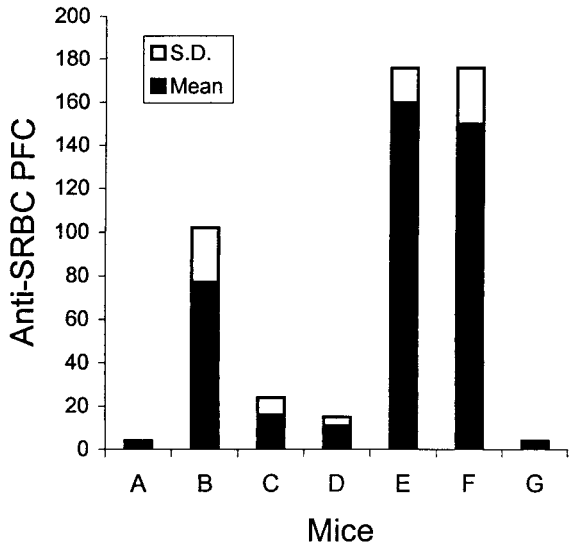

FIG. 3. Primary anti-SRBC antibody response in BXSB recipients transplanted with TCDM cells. Bars: BXSB (*A*), BALB/c + BXSB → BXSB (*B*), BALB/c → BXSB (*C*), BXSB → BXSB (*D*), C57BL/6 (*E*), C57BL/6 → C57BL/6 (*F*), and BALB/c → C57BL/6 (*G*). PFC data are given per 5×10^5 spleen cells.

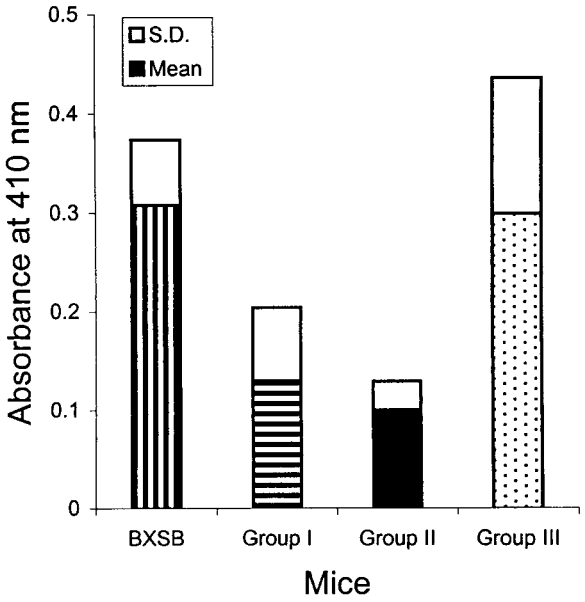

FIG. 4. Serum levels of anti-dsDNA autoantibodies of TCDM-recipient male BXSB mice at 50 weeks of age. Untreated BXSB mice served as controls. BXSB, untreated BXSB mice; Group I, BALB/c + BXSB → BXSB chimeras; Group II, BALB/c → BXSB chimeras; Group III, BXSB → BXSB transplants.

The autoimmune diseases of humans may be ultimately disabling and/or may cause premature death. The murine models of autoimmune diseases, such as BXSB mice, are invaluable for assessing the efficacy of various therapeutic modalities, because these models have histologic and serologic similarities to human autoimmune diseases. Although it has been shown that BMT can establish a stable fully allogeneic chimerism and can be used to prevent and/or cure autoimmune diseases in mice when fully allogeneic BMT chimeras are produced in these experiments, the mice often have deficiencies of antibody production to primary antigenic stimulation after TBI and BMT.

Stable mixed chimerism has advantages over fully allogeneic BMT-induced stable chimerism. Mixed chimeras have fully reconstituted and normal immune functions, which are often missing in fully allogeneic stable chimeras. Mixed chimerism may also prevent fatal aplasia, because the syngeneic bone-marrow component seldom fails to engraft. We found that autoimmune disease of BXSB mice could be treated by transplantation of mixed TCDM from both autoimmune-resistant BALB/c and autoimmune-susceptible BXSB mice. The advancement of progressive destructive renal histopathological lesions and the increase of serum anti-dsDNA level, as observed in untreated BXSB mice and BXSB → BXSB transplant controls, were arrested in BXSB recipients that had been transplanted with a mixed TCDM from both BALB/c and BXSB donors. Median survival of BALB/c + BXSB → BXSB chimeric mice was comparable to that of BALB/c → BXSB and was extended significantly compared with that of BXSB recipients transplanted with BXSB TCDM or that of untreated BXSB mice. These findings showed that transplantation of mixed TCDM from allogeneic autoimmune-resistant donor mice plus syngeneic autoimmune-prone donor mice can be used as an effective treatment of autoimmune diseases and progressive renal disease in BXSB mice.

BXSB-recipient mice in which stable mixed chimerism transplanted with mixed TCDM (from allogeneic BALB/c and from a syngeneic recipient strain) produced a primary antibody

response against the cellular antigen SRBC *in vivo*, as did mice with syngeneic transplants of C57BL/6 → C57BL/6 and untreated C57BL/6 mice. Fully allogeneic chimeras, such as BALB/c → BXSB and BALB/c → C57BL/6, did not produce an impressive antibody response against SRBC, nor did BXSB → BXSB chimeric mice and untreated BXSB mice. Because all BXSB → BXSB mice or untreated BXSB mice at the termination of these experiments had far more severe renal histopathological lesions and higher anti-dsDNA titer than did the stable mixed chimeras, they were not able to mount a normal response to T cell-dependant antigens like SRBC. Transplantation of mixed TCDM from both allogeneic autoimmune-resistant donor mice plus syngeneic autoimmune-prone donor mice caused a full reconstitution of the immunity functions and also was an effective treatment for preexisting autoimmune-based renal disease and rapidly progressive renal disease in the BXSB mice.

In our BALB/c + BXSB → BXSB mixed BMT experiment group, only 2 of 18 transplanted animals did not engraft the bone marrow cells derived from the BALB/c allogeneic donor origin. We could not explain why allogeneic BALB/c donor cells failed to engraft in these two recipient BXSB mice. However, these two transplants showed in another way that syngeneic hematopoietic stem cells from the autoimmune-prone donor alone could not be used to treat autoimmune and renal disease in BXSB mice, because these two mice, like those syngeneic BXSB → BXSB transplants, had larger spleens, higher anti-dsDNA titers, and more renal histopathological lesions of greater severity compared with those in stable mixed chimeras.

Hematopoietic stem cells derived from the bone marrow, peripheral blood, or umbilical cord blood have been transplanted as a means to treat a number of genetic and acquired diseases (9). Recently, transplantation of highly purified hematopoietic stem cells has been investigated extensively (21–23). Future studies using highly purified hematopoietic stem cells or stable mixed chimerism to prevent or treat autoimmune disease should permit an improved understanding of the cellular origin of autoimmune disease.

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