Marrow transplantation from tolerant donors to treat and prevent autoimmune diseases in BXSB mice

(tolerance/autoimmunity/resistance genes/bone marrow transplantation)

KUNISUKE HIMENO* AND ROBERT A. GOOD†

*Medical Institute of Bioregulation, Department of Immunology, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan; and †University of South Florida, Department of Pediatrics/St. Petersburg, All Children's Hospital, 801 6th Street South, St. Petersburg, FL 33701

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ABSTRACT Autoimmune-prone BXSB male mice were supralethally irradiated and transplanted with CBA/H bone marrow cells. A complete and long-term chimerism was established when donor mice had been induced to develop tolerance of BXSB male antigens by combined treatment with BXSB male spleen cells and cyclophosphamide. Such chimeras did not express autoimmune phenomena or develop lethal autoimmune manifestations. Nor did the recipient mice develop the wasting syndrome or evidence of persistent immunodeficiencies that have been seen in other strains of autoimmune-resistant mice that had been transplanted with bone marrow cells across major histocompatibility complex barriers following an initial purging of the bone marrow of Thy-1⁺ cells using anti-Thy-1⁺C.

Mice of certain strains develop autoimmune disease early in life (1). Transplantation of bone marrow cells from mice resistant to autoimmunity or from mice susceptible to autoimmunity provides an opportunity to elucidate the role of donor genes and/or thymic environment on expression of immunologic abnormalities and susceptibility to disease in mice of these strains (2-9). Bone marrow transplants matched at the major histocompatibility complex (MHC) from resistant donors to susceptible recipients correct autoimmune disease and many autoimmune abnormalities expressed early in life in the autoimmune-prone mice (2, 3, 5, 7-9). However, no one has analyzed the potential of MHC mismatched marrow transplants in these model systems.

Marrow transplantation across major MHC barriers heretofore has introduced complex issues of graft versus host (GVH) reaction, chronic wasting syndrome, and defective interaction of the marrow-derived cells (10-13). Indeed, fully allogeneic marrow chimeras in autoimmune-susceptible mice from autoimmune-resistant mice have been difficult to produce without producing lethal GVH reaction rather than chimeras between autoimmune-resistant strains. Further, with BXSB and MRL/l mice, few studies exist of the influence of resistance genes introduced by marrow transplants even from H-2-compatible donors. Irradiation itself exerts some therapeutic and/or prophylactic influences on development of autoimmune diseases (14-16). Thus, to evaluate the influences of resistance genes versus autoimmunities and cancers introduced by bone marrow transplantations that cross MHC barriers, long-term chimerism is required. If long-term chimeras can be produced, crucial genetic influences on susceptibility to autoimmune disease can be studied.

Recent evidence suggests that elimination not only of T cells but of certain prethymic precursor T cells (PTC) from the marrow inoculum is essential to making allogeneic chimerism without GVH reaction (17-19). Moreover, tolerance

of MHC-mismatched alloantigens may be induced in PTC by injecting allogeneic spleen cells into athymic nude mice (20). Such a treatment permits prolonged specific survival of engrafted skin and is MHC specific (20). A similar tolerance was induced by injection of alloantigen-bearing cells concomitant with cyclophosphamide (CY) treatment (21, 22) or anti-lymphocyte serum treatment (23, 24).

In the present studies, autoimmune-prone BXSB males were supralethally irradiated and reconstituted with marrow from H-2-mismatched donors after donor mice had been made tolerant of the BXSB recipients. The recipients were long-lived, autoimmune-resistant, and free of GVH disease. Such chimeras, between autoimmune-resistant donors and autoimmune-susceptible recipients, were persistently immunocompetent and could even exhibit a primary response to sheep erythrocytes (SRBC). The late wasting syndrome and associated profound immunodeficiency described by Rayfield and Brent (13) and Longley *et al.* (12) were not observed.

MATERIALS AND METHODS

Mice. Male BXSB (BX, H-2^b), CBA/H-T6.T6 (CBA/H, H-2^k), and DBA/2 (H-2^d) mice were purchased from The Jackson Laboratory, maintained in sterilized cages on laminar air-flow racks with acidified water, and fed sterilized food *ad libitum*.

Preparation of Bone Marrow Chimeras. BXSB male, 8 wk old, were the recipients. The mice were supralethally irradiated in a plastic box with 950 rads (1 rad = 0.01 gray) by using a ⁶⁰Co-γ-irradiator (Theratron 80, Atomic Energy of Canada; Ottawa, ON) at a dose rate of 77 rads/min. Twenty-four hours later the mice were given an intravenous injection of 25 × 10⁶ donor bone marrow cells treated as follows. Donor mice, 8 wk of age, were sacrificed by bleeding. Bone marrow cells were obtained from tibia, femur, and humerus by gently flushing with cold Hanks' balanced salt solution (HBSS). After centrifugation, marrow suspensions were adjusted to 3 × 10⁷ per ml, mixed with an equal volume of 1:10 diluted anti-Thy-1.2 alloantiserum, and incubated on ice for 45 min with occasional mixing. Then, one-half of the volume of 1:10 diluted rabbit complement (Low-Tox-M; Cedarlane Laboratories, Hornby, ON) was added, incubation was continued for an additional 30 min at 37°C, and, after washing three times with HBSS, 25 × 10⁶ viable bone marrow cells were injected into irradiated recipient mice.

Abbreviations: MHC, major histocompatibility complex; PTC, prethymic precursor T cells; SRBC, sheep erythrocyte(s); CY, cyclophosphamide; MLC, mixed lymphocyte culture; PHA, phytohemagglutinin; LPS, lipopolysaccharides from *Escherichia coli*; PWM, pokeweed mitogen; ANA, anti-nuclear antibody; GVH, graft versus host; PFC, plaque-forming cells; ds, double-stranded; C, complement.

†To whom reprint requests should be addressed.

Pretreatment of Donor Mice with Recipient Spleen Cells. Some of the donor mice were injected intravenously at 5 wk of age with 2.2×10^8 BXSB male spleen cells that had been first irradiated with 200 rads by using a ^{137}Cs - γ -irradiator (Gammacell 1000, Atomic Energy of Canada). Donors were then injected intraperitoneally with CY at 150 mg/kg 2 days later. This procedure was repeated three times at 7-day intervals. However, in the second and third treatments, CY was injected 1 day later and then on the same day after injection of stimulating spleen cells. Bone marrow cells of mice fully tolerant of BXSB were harvested 9 days after the last injection and used for treatment of the lethally irradiated BXSB mice.

Chimerism of Mice Given H-2-Incompatible Bone Marrow from Tolerant or Intolerant Donors. Bone marrow cells, spleen cells, and thymus cells were used to determine H-2 haplotype. Contaminating erythrocytes were removed by centrifugation on a Ficoll/hypaque cushion and by NH_4Cl lysis. The cells were washed twice with HBSS supplemented with 10% fetal calf serum (GIBCO). The anti-H-2^b and anti-H-2^k sera used were purchased from Cedarlane Laboratories, and all antisera were used at a 1:20 dilution. The assays were performed in triplicate in 60-well Terasaki trays (Robins Scientific; Palo Alto, CA). One microliter of cell suspensions and 1 μl of antisera were added to each well and the trays were incubated for 30 min at room temperature. Five microliters of Low-Tox-M rabbit complement diluted 1:10 was added to each well and the incubation was continued for an additional 60 min. Four microliters of 2% eosin Y was then added. Five minutes later, 5 μl of 10% formalin was added to each well. Dead cells were larger than viable cells and had irregular cell borders and a pinkish cytoplasm. Cytotoxic index (CI) was calculated as follows: $\text{CI} = (\% \text{ killed with antiserum plus complement (C)} - \% \text{ killed in C control}) / 100 - \% \text{ killed in C control} \times 100$.

Mixed Lymphocyte Culture (MLC). Responder (4×10^5) as well as responder (2×10^5) and 2000 rad-irradiated stimulator spleen cells (2×10^5) were cultured in 200 μl of medium for 72 hr at 37°C in a 96-well flat-bottomed microplate (Limbro, Flow Laboratories), pulsed with 0.4 μCi (1 Ci = 37 GBq) of [^3H]thymidine (New England Nuclear) for the final 8 hr, harvested on glass-fiber filter paper with a Titertek cell harvester 6 (Flow Laboratories) and assayed for radioactivity in a scintillation counter. RPMI 1640 medium supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 5 mM Hepes buffer (all from GIBCO), 1% fresh mouse serum, and 0.05 mM 2-mercaptoethanol (Sigma) was used as the culture medium.

Mitogen Response. Spleen cells (4×10^5) were cultured for 72 hr with 0.4 μg of phytohemagglutinin (PHA), 0.4 μg of Con A, 20 μg of lipopolysaccharides from *Escherichia coli* (LPS), or 1:250 diluted pokeweed mitogen (PWM) (all from Difco) in 200 μl of the same culture medium as that used in the MLC assay. Eight hours prior to harvest, 0.4 μCi of [^3H]thymidine was added to each well.

In Vitro Anti-SRBC Antibody Production. Spleen cells (4×10^5) were cultured with 1×10^6 SRBC in 0.2 ml of conditioned RPMI 1640 medium in 96-well flat-bottomed microplates for 5 days. Direct plaque-forming cells (PFC) were assessed by using a Jerne hemolytic plaque assay as modified by Mishell and Dutton (25).

Assays for Autoantibodies. (i) Anti-nuclear antibody (ANA). ANA was assayed by an indirect immunofluorescence method using murine kidney nuclei as antigen. A 1:40 diluted fluorescein-conjugated goat anti-mouse IgG serum (Sigma) was used. Samples of unfixed murine kidney were placed in a small tissue capsule with embedding medium and frozen in liquid nitrogen. The capsule was then mounted in a cryostat and sections were cut at 5 μm . (ii) Anti-double-stranded DNA (dsDNA) antibody. A solid-phase ELISA was employed as

described by Rubin *et al.* (26). A serocluster plate (Costar, Cambridge, MA) was precoated with methylated bovine serum albumin at 2 $\mu\text{g}/0.2 \text{ ml}$ (Sigma) for 90 min, rinsed with phosphate-buffered saline (PBS), and then coated with a solution of dsDNA at 0.5 $\mu\text{g}/0.2 \text{ ml}$ (Sigma) overnight. Uncoated sites on the plate were blocked with 0.1% bovine serum albumin (Sigma) in carbonate buffer (pH 9.2). The plate was then washed twice with 0.05% Tween 80 in PBS (PBS/Tween 80). Sera diluted with 0.05% Tween 80 and 0.1% bovine serum albumin in PBS were incubated on the plate for 16 hr at 4°C. After washing with PBS/Tween 80, the IgG fraction of goat anti-mouse IgG was diluted 1:10 and conjugated to the alkaline phosphatase substrate *p*-nitrophenylphosphate (Sigma). Chromatophore development was followed by using an MR 580 Microelisa Auto Reader (Dynatek) set at 405 nm.

RESULTS

Effect of "Pretolerization" of Donor Mice with BXSB Male Spleen Cells on the Survival in BXSB Recipient Mice Supralethally Irradiated and Transplanted with Allogeneic Bone Marrow Cells. Fig. 1 presents survival curves of BXSB male mice supralethally irradiated and transplanted with BXSB male or CBA ($H-2^k$) bone marrow cells. Nontreated BXSB male mice began to die from the 8th wk after the start of the experiment (when 16 wk old), and all mice were dead by the 36th wk (42 wk of age). [CBA $\rightarrow$ BXSB] mice died progressively from the 5th wk after transplantation, and most had died by the 16th wk (13/16), suffering from chronic wasting manifestations. All BXSB mice transplanted with syngeneic male bone marrow cells [BX $\rightarrow$ BX] survived for 20 wk (28 wk old) after the transplantation and then began to die. By the 38th week after transplantation, all such mice died and, in most, death was clearly attributable to autoimmune disease. All of these mice exhibited severe splenomegaly. By contrast, of the mice transplanted with allogeneic bone

FIG. 1. Effect of allogeneic chimeras on the survival of autoimmune-prone BXSB male mice. Supralethally irradiated BXSB male mice ($H-2^b$) at 8 wk of age were transplanted with 2.5×10^7 bone marrow cells from CBA/H ($H-2^k$) mice. Donor bone marrow cells were always treated with Thy-1 and rabbit complement before transplantation. [CBA(pre-BX) $\rightarrow$ BX]: donor CBA/H were made tolerant to BXSB male antigen by the combination treatment of BXSB male spleen cells and CY prior to transfer to 950-rad-irradiated BXSB male recipients. [CBA $\rightarrow$ BX]: nontreated CBA/H mice were used as donors of bone marrow cells. [BX $\rightarrow$ BX]: nontreated BXSB male mice were used as donors. [CBA $\rightarrow$ CBA]: bone marrow cells from nontreated CBA/H mice were transferred to 950-rad-irradiated CBA/H mice. [CBA/H(pre-BX) $\rightarrow$ CBA]: bone marrow cells from CBA/H mice tolerant to BXSB male antigen were transferred to 950-rad-irradiated CBA/H mice. $\downarrow$, Five to eight mice in each group were sacrificed for immunological assays 40 wk after transplantation (48 wk of age). Parentheses show the number of recipient mice used in each group.

Table 1. Cell numbers and *H-2* haplotype in lymphoid organs of BXSB male mice, supralethally irradiated and reconstituted with bone marrow cells from autoimmune-resistant CBA/H mice

Mice	Age, wk	n	Spleen			Thymus			Bone marrow	
			Cells,* no. $\times 10^{-6}$	<i>H-2^b</i> ,† %	<i>H-2^k</i> ,† %	Cells,* no. $\times 10^{-6}$	<i>H-2^b</i> ,† %	<i>H-2^k</i> ,† %	<i>H-2^b</i> ,† %	<i>H-2^k</i> ,† %
CBA	34	8	88.5 $\pm$ 19.5	0	>95	32.1 $\pm$ 6.9	0	>95	0	>95
BX	34	5	383.8 $\pm$ 68.2	>95	0	0.6 $\pm$ 0.2	>95	0	>95	0
[CBA(pre-BX) $\rightarrow$ BX]	34	8	90.2 $\pm$ 18.9	0	>95	29.5 $\pm$ 9.5	0	>95	0	>95
[BX $\rightarrow$ BX]	34	8	298.5 $\pm$ 42.5	>95	0	2.5 $\pm$ 0.3	>95	0	>95	0
CBA	48	8	82.5 $\pm$ 9.8	0	>95	20.5 $\pm$ 7.3	0	>95	0	>95
[CBA $\rightarrow$ CBA]	48	8	92.9 $\pm$ 25.8	0	>95	35.0 $\pm$ 9.8	0	>95	0	>95
[CBA(pre-BX) $\rightarrow$ CBA]	48	8	79.5 $\pm$ 8.9	0	>95	24.4 $\pm$ 4.3	0	>95	0	>95
[CBA(pre-BX) $\rightarrow$ BX]	48	8	84.9 $\pm$ 8.5	0	>95	29.6 $\pm$ 8.9	0	>95	0	>95
C57BL/6J	10	2	72.3 $\pm$ 3.8	>95	0	42.5 $\pm$ 5.7	>95	0	>95	0

n, Number of mice.

*Values are the mean $\pm$ SD.†% of *H-2^b*- or *H-2^k*-positive lymphoid cells.

marrow cells from CBA donors that had been made tolerant with BXSB spleen cells and CY [CBA(pre-BX) $\rightarrow$ BX], 4 died within 8 wk after transplantation. Forty weeks following transplantation, when the mice were 48 wk old, 13 of 20 mice had survived and all were free of symptoms or signs of autoimmune disease or GVH reaction (Table 1).

To assess the influence of bone marrow cells from mice retreated with allogeneic spleen cells and CY, bone marrow cells from such mice were transplanted from CBA mice into CBA mice: [CBA(pre-BX) $\rightarrow$ CBA]. CBA mice transplanted with such marrow cells survived almost as well as did [CBA $\rightarrow$ CBA] mice. On the 40th week following transplantation, eight mice from each group were sacrificed to examine the *H-2* haplotype of lymphoid cells and to assay for the presence and vigor of immunological functions and for autoantibodies.

Lymphoid Cell Number and *H-2* Haplotype of the Chimeric Mice. The number of cells in the lymphoid organs and *H-2* haplotype of the chimeric mice were determined by using the lymphoid cells from spleen, thymus, or bone marrow as described in *Materials and Methods* (Table 1). Prominent splenomegaly and increased spleen cell numbers were observed in untreated BXSB male mice. Such mice showed severe thymic atrophy. A similar pattern was also present in [BX $\rightarrow$ BX] mice. In [CBA(pre-BX) $\rightarrow$ BX] mice, the size as well as the cell number of thymus and spleen were almost the same as in untreated CBA mice. Further, the *H-2* type of the cells in the spleen, thymus, and bone marrow was completely attributable to that of donor (CBA)-type *H-2^k*. Thus, the lymphoid cells of these mice had been completely replaced with those of donor type.

MLC in Chimeric Mice. The same experimental groups as described in Fig. 1 were prepared for immunological studies.

At 26 wk following bone marrow transplantation, four mice were sacrificed for *in vitro* assay.

For MLC, spleen cells irradiated *in vitro* with 2000 rads from male BXSB (*H-2^b*), CBA/H (*H-2^k*), or DBA/2 (*H-2^d*) mice were used as stimulator cells (Fig. 2). As would be expected, the spleen cells of CBA/H mice responded to *H-2^k* and *H-2^d* antigens of the BXSB and DBA/2 mice but not to CBA/H cells, and BXSB responded, although rather weakly, to CBA/H and DBA/2 spleen cells. [CBA(pre-BX) $\rightarrow$ BX] mice responded only to DBA/2 cells and not to either BXSB or CBA/H spleen cells. [BX $\rightarrow$ BX] showed normal responses to allogeneic CBA/H and DBA/2 spleen cells. Thus, the chimeric mice prepared by this method were tolerant of both donors and recipients and were responsive by proliferation to allogeneic cells of a third party.

Mitogen Responses. Studies of proliferative response to Con A, PHA, PWM, or LPS carried out on the chimeric mice and control mice are summarized in Fig. 3. In 34-wk-old BXSB mice, spleen cell mitogenic responses to PHA, Con A, and PWM were profoundly depressed as compared to the responses of spleen cells from age-matched CBA/H mice. On the contrary, the response to LPS was augmented. Spleen cells from [CBA(pre-BX) $\rightarrow$ BX] mice responded well to Con A, PHA, LPA, and PWM, and these responses were at the same level as was seen with CBA/H controls.

***In Vitro* Anti-SRBC PFC (Direct).** Direct PFC assays were carried out after 5 days of culture (Table 2). Spleen cells from BXSB male and [BX $\rightarrow$ BX] mice did not produce significant anti-SRBC plaque-forming cell responses. This immunologically deficient state was an improvement in BXSB male mice that had been supralethally irradiated and reconstituted with CBA/H bone marrow cells [CBA(pre-BX) $\rightarrow$ BX].

FIG. 2. MLC in chimeric mice. Responding cells (4×10^5) and stimulator spleen cells (2×10^5) in 200 μ l of medium were cultured for 72 hr and pulsed with 0.4 μ Ci of [3 H]thymidine for the final 8 hr. Values are the mean $\pm$ SEM from four mice in each group.

FIG. 3. Mitogen response of spleen cells from chimeric mice. Spleen cells (4×10^5) were cultured with $0.4 \mu\text{g}$ of PHA, $0.4 \mu\text{g}$ of Con A, $20 \mu\text{g}$ of LPS, or 1:250 diluted PWM in $200 \mu\text{l}$ of medium. Eight hours prior to harvest, $0.4 \mu\text{Ci}$ of ^3H thymidine was added to each well. Net ^3H thymidine uptake levels after subtraction of uptake in control and unstimulated cultures (<4500 cpm) are shown. Values are the mean $\pm$ SEM from four mice in each group.

Autoantibodies in Chimeric Mice. BXSB male mice regularly showed high titers of ANA and anti-dsDNA antibodies, whereas CBA and B6 mice lacked these antibodies (Table 3). In two separate experiments, supralethally irradiated [CBA-(tol-BX) → BX] mice had no demonstrable ANAs and greatly reduced anti-dsDNA antibodies.

DISCUSSION

Clinical approaches to treatment of autoimmune disease have often been insufficient and inadequate. New approaches to manipulate ongoing autoimmune disease or to prevent expression of the immunologic abnormalities that lead to severe tissue destruction in autoimmune disease seem much in order.

Thus far in the animal model, few efforts have been made to produce long-term correction of autoimmunity. Total

Table 2. *In vitro* anti-SRBC PFC in spleen cells from chimeric mice

Mice	Age, wk	Anti-SRBC PFC (direct) per 4×10^5 cultured cells	
		Experiment 1	Experiment 2*
CBA	34	724 $\pm$ 244 (4)	597 $\pm$ 83 (4)
BX	34	16 $\pm$ 11 (4)	0 (1)
[CBA(pre-BX) → BX]	34	503 $\pm$ 183 (4)	329 $\pm$ 165 (4)
[BX → BX]	34	33 $\pm$ 16 (4)	12 $\pm$ 9 (4)
CBA	48	448 $\pm$ 52 (4)	329 $\pm$ 48 (4)
BX	23	18 $\pm$ 9 (4)	25 $\pm$ 11 (4)
[CBA(pre-BX) → CBA]	48	367 $\pm$ 89 (4)	266 $\pm$ 65 (4)
[CBA(pre-BX) → BX]	48	287 $\pm$ 48 (4)	198 $\pm$ 23 (4)
[CBA → CBA]	48	198 $\pm$ 92 (4)	245 $\pm$ 19 (4)

Recipient mice at 8 wk of age were transplanted with bone marrow cells after a 950-rad irradiation. Values are the mean $\pm$ SEM. Parentheses show the number of mice used for assays.

*Experiment 2 was carried out 3 days after experiment 1.

Table 3. Autoantibodies in chimeric mice

Mice	Age, wk	n	ANA*	Anti-dsDNA†
CBA	34	8	20	0.078 $\pm$ 0.005
BX	34	5	320–1620	0.381 $\pm$ 0.058
[CBA(pre-BX) → BX]	34	8	20	0.111 $\pm$ 0.015
[BX → BX]	34	8	180–540	0.632 $\pm$ 0.233
CBA	48	8	NT	0.063 $\pm$ 0.015
BX	23	8	NT	0.406 $\pm$ 0.142
[CBA(pre-BX) → BX]	48	8	NT	0.091 $\pm$ 0.023
[CBA → CBA]	48	8	NT	—

Recipient mice at 8 wk of age were transplanted with bone marrow cells 24 hr after a 950-rad irradiation. n, Number of mice used for assays. NT, not tested.

*ANA values are expressed as dilution.

†OD at 405 nm.

lymphoid irradiation (13), whole-body irradiation (14), radiomimetic cytotoxic drug therapy (27, 28), and anti-lymphocyte or anti- θ treatments have been used with some success (29, 30). Most efforts to apply marrow transplantation have employed marrow from H-2-matched donors or congenic donors that permit introduction of cells bearing only a few genes from autoimmune-resistant donors that differ from the genes of the recipients.

In present experiments we introduced a method that has permitted regular establishment of very long-lived chimeras in autoimmune-prone mice across MHC barriers. This approach has been used to prevent development of autoimmune disease and to correct immunologic abnormalities and autoimmune phenomena in autoimmune-prone BXSB male mice. Basically, prior to taking marrow, the donors were first made tolerant by repeated injection of lymphoid cells from the respective recipient strain during the period when the respective donor was being treated also with CY. These tolerant marrow T cells were then purged by treatment *in vitro* with anti-Thy-1⁺C. This T-cell-purged marrow from tolerant donors was used for transplantation to autoimmune-prone mice. When this was done, the majority of fully allogeneic chimeras was found not to develop autoimmune phenomena or evidence of autoimmune disease. Such mice were fully immunocompetent and could even make antibodies to a thymus-dependent antigen, SRBC. This cannot generally be accomplished in allogeneic chimeras across MHC barriers. Further, these animals did not develop the late wasting disease without the usual overt manifestations of GVH reaction described by Rayfield and Brent (13) and also encountered in our laboratory (11).

By contrast, our findings are consonant with the observations of Ikehara *et al.* (31), who used bone marrow from *nu/nu* athymic donors on several different genetic backgrounds to prevent and treat effectively autoimmune disease in autoimmune-prone mice when donor and recipient differed at the MHC barriers. In their experiments, even the thymus involution and aberrant cellular infiltrations, as well as all other immunologic abnormalities, were corrected in autoimmune-prone mice by transplantation of marrow cells from donors resistant to expression of autoimmune disease.

It thus seems clear that with the manipulation of transplantation of bone marrow cells from donors made tolerant to recipient and also purged of postthymic θ -positive cells it has been possible to correct most abnormalities in autoimmune-prone mice. Whether this approach can be developed for application to treatment of life-threatening autoimmune disease in humans is still questionable because it will be necessary to learn how to prepare the cells from autoimmune-resistant donors, as has been done in our mice, so that they do not produce acute and chronic GVH disease or

induce the peculiar wasting syndrome described by Rayfield and Brent (13).

The fully allogeneic chimeras we have produced surely bear in their hematopoietic tissue genes that render them resistant to the development of autoimmune disease. How these resistance genes work needs to be ascertained. It is important to know whether this treatment inhibits viral development or viral antigenic expression. Such an influence may be especially important, since Izui *et al.* (32) showed that gp70 antigen coded in the virus genomes and antibody formed against it may play a crucial role in development of autoimmune renal injury in mice.

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