Rationale for bone marrow transplantation in the treatment of autoimmune diseases

(bone marrow transplantation/BXSB mice/MRL/l mice/T-cell functions)

Susumu Ikehara*, Robert A. Good†, Takao Nakamura*, Ken’ichi Sekita*, Shuji Inoue*, Maung Maung Oo*, Eri Muso*, Katsuhiko Ogawa*, and Yoshihiro Hamashima*

*Department of Pathology, Faculty of Medicine, Kyoto University, Sakyoku, Kyoto 606, Japan; †Oklahoma Medical Research Foundation, 825 Northeast 13th Street, Oklahoma City, OK 73104

Contributed by Robert A. Good, December 3, 1984

ABSTRACT Transplantation of normal bone marrow from C3H/HeN nu/nu (H-2^d) mice into young MRL/MP-lpr/lpr (MRL/l; H-2^d) mice (<1.5 mo) prevented the development of autoimmune diseases and characteristic thymic abnormalities in the recipient mice. When female MRL/l (2>2 mo) or male BXSB (H-2^d) mice (9 mo) with autoimmune diseases and lymphadenopathy were lethally irradiated and then reconstituted with allogeneic bone marrow cells from young BALB/c nu/nu (H-2^d) mice (<2 mo), the recipients survived for more than 3 mo after the bone marrow transplantation and showed no graft-versus-host reaction. Histopathological study revealed that lymphadenopathy disappeared and that all evidence of autoimmune disease either was prevented from developing or was completely corrected even after its developing in such mice. All abnormal T-cell functions were restored to normal. The newly developed T cells were found to be tolerant of both bone marrow donor-type (BALB/c) and host-type (MRL/l or BXSB) major histocompatibility complex (MHC) determinants. Therefore, T-cell dysfunction in autoimmune-prone mice can be associated with both the involutionary changes that occur in the thymus of the autoimmune-prone mice and also to abnormalities that reside in the stem cells. However, normal stem cells from BALB/c nu/nu donors can differentiate into normal functional T cells even in mice whose thymus had undergone considerable involution, as in the case of BXSB or MRL/l mice in the present studies. These findings suggest that marrow transplantation may be a strategy ultimately to be considered as an approach to treatment of life-threatening autoimmune diseases in humans. T-cell dysfunction in autoimmune-prone mice previously attributed to involutionary changes that occur in the thymus of these mice may instead be attributed to abnormalities that basically reside in the stem cells of the autoimmune-prone mice.

The availability of several murine strains that develop systemic lupus erythematosus-like disease has prompted efforts to gain better understanding of the fundamental nature of autoimmune diseases through extensive studies of the immunological abnormalities of these mice. MRL/MP-lpr/lpr (MRL/l) and BXSB mice as well as NZB mice and NZB x NZW F_1 hybrids spontaneously develop autoimmune diseases characterized by anti-double-stranded (ds) DNA antibodies, immune-complex glomerulonephritis, and death from renal failure (1). Abnormalities have been found in or attributed to T cells, thymic epithelium, B cells, and/or macrophages in these mice (2–8). Recently, several groups have shown that the proneness to develop autoimmune diseases actually resides in defects at the lymphoid stem-cell level and that defects of function are not directly attributable to environmental factors such as hormones or viruses (9–11). Therefore, we examined whether or not transfer of normal stem cells into autoimmune-prone mice can be used to both prevent and treat autoimmune diseases.

In the present study, we show that allogeneic bone marrow transplantation from donors carrying the nu/nu genes permits both prevention and effective treatment of autoimmune diseases in both MRL/l and BXSB mice and that normal T cells, B cells, and macrophages developed in such mice transplanted with allogeneic bone marrow 3 mo after the bone marrow transplantation.

MATERIALS AND METHODS

Mice. Inbred MRL/l, MRL/MP—+/+ (MRL/l), and BXSB mice were obtained from The Jackson Laboratory. BALB/c nu/nu, BALB/c, C3H/HeN nu/nu, C3H/HeN, and DBA/2 mice were obtained from the Central Institute for Experimental Animals (Tokyo). These mice were maintained under specific pathogen-free conditions in our facilities.

Bone Marrow Transplantation. MRL/l and BXSB mice were irradiated (850 rads for MRL/l and 950 rads for BXSB from a cobalt-60 source) and reconstituted by intravenous injection of 2 x 10^7 bone marrow cells from young BALB/c nu/nu mice (<2 mo). These MRL/l and BXSB mice were usually sacrificed more than 3 months after bone marrow transplantation. Testing with anti-H-2 antisera and complement indicated that more than 90% of spleen cells were of donor type.

Cell Separation. Mice were sacrificed by cervical dislocation. The spleens were removed aseptically, minced, and gently passed through a fine mesh stainless-steel sieve into phosphate-buffered saline.

Cytotoxic Test. Spleen cells that were suspended in TC-199 (GIBCO) with 5% fetal calf serum (Microbiological Associates) were adjusted to 5 x 10^6 cells per ml and divided into two aliquots of 50 μl. The cells were incubated with 50 μl of a 1:10 dilution of anti-Thy-1.2 antibody for 30 min at 4°C. The cells were then washed once and resuspended in 100 μl of a 1:10 dilution of rabbit complement (Cedarlane Laboratories, Hornby, ON, Canada) previously absorbed with mouse spleen cells. After a 30-min incubation at 37°C, the viability of the cells was determined by trypan blue dye-exclusion test. The counts were converted to a cytotoxic index by the formula:

CI = \frac{\% ~V ~cells ~(C ~alone) - \% ~V ~cells ~(Ab + C)}{\% ~V ~cells ~(C ~alone)} \times 100

in which C represents complement; Ab, antibody; CI, cytotoxic index; and V cells, viable cells.

Abbreviations: MRL/l, MRL/MP-lpr/lpr; CICs, circulating immune complexes; MHC, major histocompatibility complex; Con A, concanavalin A; ds, double-stranded.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.
Mitogen Response. The mitogenic reactivity was determined by measuring incorporation of $[^{3}H]$/thymidine into DNA. Triplicate cultures were set up in wells of flat-bottom microtiter plates (Corning Glass Works 25860, Corning, NY), each containing $5 \times 10^5$ cells in 0.2 ml of RPMI-1640 medium (Nissui Seiyaku Co., Tokyo) that was supplemented with 2 mM l-glutamine (Wako Pure Chemical, Tokyo), penicillin (100 units/ml), streptomycin (100 $\mu$g/ml), and 5% human plasma. The cells were cultured in the presence of phytohemagglutinin P (Difco) at 25 $\mu$g/ml or of concanavalin A (Con A; Calbiochem) at 5.0 $\mu$g/ml. (The mitogens had been tested at various doses, and the dose selected was that giving the optimal responses.) The cultures were incubated for 72 hr at 37°C in a humidified atmosphere of 5% CO$_2$/95% air. $[^{3}H]$/Thymidine (0.5 $\mu$Ci in 20 $\mu$l; New England Nuclear; 1 Ci = 37 GBq) was present during the last 4 hr of the culturing period. The $^{3}H$ radioactivity incorporated into trichloroacetic acid-insoluble material was measured by a liquid scintillation counter.

Generation of Cytotoxic T Lymphocytes. Responder cells ($7.5 \times 10^5$) and mitomycin C (50 $\mu$g/ml)-treated stimulator cells ($2.5 \times 10^5$) were cocultured in RPMI-1640 medium containing 10% heat-inactivated human serum supplemented with streptomycin (100 $\mu$g/ml), penicillin (100 units/ml) and 2-mercaptoethanol (50 $\mu$M). After 5 days of coculture in a humidified CO$_2$ incubator, the cells were collected, and their cytotoxic activity was determined by $^{51}$Cr-release assay as described (12).

Measurement of Circulating Immune Complex (CIC). CICs were measured by using microtiter plates coated with complement component C1q as described (13).

Antibodies to dsDNA. Antibodies to dsDNA were measured by solid-phase binding assay as described (14).

RESULTS AND DISCUSSION
Autoimmune-prone mice, such as mice of (NZB × NZW) F$_1$, MRL/l, and BXSB strains, show premature thymic involution, including morphological and functional abnormalities (15-17). In order to prove why thymic abnormalities develop in autoimmune-prone mice, transplantation of the thymus and bone marrow was performed. When thymuses of newborn MRL/l (H-2$^b$) mice were grafted into C3H/HeN nu/nu (H-2$^d$) mice, the thymuses of the recipients did not show premature involution even 5 mo after grafting (0/11). By contrast, when thymuses of newborn normal C3H/HeN mice were grafted into MRL/l mice, the thymuses showed premature involution (8/9). Thus, premature thymic involution in autoimmune-prone mice is not intrinsically determined within the thymus but is secondary to other events. When normal bone marrow cells ($2 \times 10^7$) of young C3H/HeN nu/nu mice were transferred to irradiated young MRL/l mice (<1.5 mo),...
thymic abnormalities and autoimmune diseases did not develop up to 5 mo after the marrow transplant (0/5). It seems reasonable to conclude from these findings that abnormal stem cells of the autoimmune-prone mice are involved in a major way in the premature thymic involution and development of autoimmune diseases in these mice. These findings are in agreement with published observations of Yonouchi et al., who showed that transplants within the same major histocompatibility complex (MHC) corrected fully the immunologic and hematologic abnormalities that characterized B/W F1 mice (11).

The next step was to verify whether or not transfer of normal stem cells into autoimmune-prone mice could be used to treat autoimmune diseases in these animals. When female MRL/1 (H-2^d) (>2 mo) or male BXSB (H-2^b) (>9 mo) mice that had already shown clear evidence of autoimmune diseases and lymphadenopathy were lethally irradiated and reconstituted with allogeneic bone marrow cells from young BALB/c nu/nu (H-2^d) donors (<2 mo), the recipients survived in good health for more than 3 mo after bone marrow transplantation without showing any evidence of graft-versus-host reaction either under specific pathogen-free conditions or in a conventional environment. In contrast, when MRL/1 (>2 mo) mice were lethally irradiated and reconstituted with bone marrow cells of MRL/1 (<1.5 mo) mice, the recipients regularly died of progressive renal failure within 2 mo.

Histopathological study demonstrated that lymphadenopathy disappeared and that characteristic lymphoid cell infiltrations into the kidney and liver were markedly ameliorated in both MRL/1 and BXSB mice that had been reconstituted with bone marrow cells of BALB/c nu/nu mice (Fig. 1). An immunofluorescence study revealed that glomerular deposits of IgG, IgA, C3, and glycoprotein 70 (gp70) were all markedly reduced in the mice reconstituted with BALB/c nu/nu bone marrow cells; in particular, deposits of complement component C3 were almost completely absent from the kidneys of these mice.

It is well known that autoimmune-prone mice show high levels of CICs and anti-dsDNA antibodies with age (18, 19). We measured antibodies to dsDNA and CICs as previously described. As shown in Table 1, BXSB and MRL/1 mice reconstituted with normal bone marrow cells of BALB/c nu/nu mice and compared this number with that of monocytosis present in blood of untreated BXSB mice. The number of monocytosis in the BXSB mice was reduced to the normal range (<10%), whereas untreated BXSB mice (6 mo old) showed a marked monocytosis (>60%) in the peripheral blood. In addition, polyclonal B-cell hyperactivity in MRL/1 and BXSB mice, which has been reported (10, 18), also was reduced to normal levels after reconstitution with normal bone marrow across the MHC barriers.

In contrast, T-cell functions were increased as shown in Fig. 2: untreated MRL/1 mice at the age of 3 mo exhibited a high number of Thy-1.2-positive cells in their spleen but a low responsiveness to Con A. However, MRL/1 and BXSB mice reconstituted with normal bone marrow cells showed a significantly higher responsiveness to Con A than did untreated controls. Using anti-H-2^d, anti-H-2^b, and anti-H-2^k antibodies plus complement, we confirmed that the newly developed T cells in MRL/1 and BXSB mice were all derived from stem cells of BALB/c nu/nu mice. The assay for induction of cytotoxic T lymphocytes revealed that the T cells were tolerant to both bone marrow donor-type (BALB/c) and host-type (MRL/1 or BXSB) MHC determinants (Table 2). These results are consistent with findings (21–24) in radiation bone marrow chimeras, where MHC barriers were breached by using nonautoimmune mice. In the untreated

<table>
<thead>
<tr>
<th>Table 1. Correlation between circulating immune complex (CIC) levels and glomerular damages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>BALB/c</td>
</tr>
<tr>
<td>BXSB</td>
</tr>
<tr>
<td>MRL/1</td>
</tr>
</tbody>
</table>

CICs were measured by using microtiter plates coated with complement component Clq as described (13). Antibodies to dsDNA were measured by solid-phase binding assay (14).

*9—11, etc., means the BXSB mouse was irradiated at the age of 9 mo (or more as indicated), reconstituted with bone marrow cells of BALB/c nu/nu mice, and sacrificed at the age of 11 mo (or more as indicated), and the serum was used for the assays.

**MRL/1 mice were used as positive controls.

![Fig. 2](https://example.com/fig2)
Table 2. Newly-developed T cells are tolerant to both bone marrow (BM) donor-type (BALB/c) and host-type (MRL/l or BXSB) MHC determinants

<table>
<thead>
<tr>
<th>Age, mo</th>
<th>Mice</th>
<th>E/T ratio*</th>
<th>PR15(H-2b)</th>
<th>EL-4(H-2b)</th>
<th>X5563(H-2b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>BALB/c (H-2b)</td>
<td>2/1</td>
<td>4.2 ± 0.6</td>
<td>79.6 ± 9.4</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>C3H/HeN(H-2b)</td>
<td>2/1</td>
<td>51.4 ± 16.3</td>
<td>59.7 ± 0.5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>MRL/l (H-2b)</td>
<td>4/1(NW)</td>
<td>2.0 ± 0.5</td>
<td>0</td>
<td>1.2 ± 0.7</td>
</tr>
<tr>
<td>6</td>
<td>MRL/l with BALB/c nu/nu BM</td>
<td>4/1(NW)</td>
<td>0</td>
<td>46.2 ± 0.6</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>BXSB(H-2b)</td>
<td>4/1(NW)</td>
<td>1.4 ± 0.6</td>
<td>0</td>
<td>1.0 ± 0.5</td>
</tr>
<tr>
<td>12</td>
<td>BXSB with BALB/c nu/nu BM</td>
<td>4/1(NW)</td>
<td>0</td>
<td>0.5 ± 2.2</td>
<td>85.7 ± 3.4</td>
</tr>
</tbody>
</table>

Responder cells (7.5 × 10⁶) and mitomycin C (50 μg/ml)-treated stimulator cells (2.5 × 10⁶) were cocultured in RPMI-1640 medium containing 10% heat-inactivated human serum, supplemented with streptomycin (100 μg/ml), penicillin (100 units/ml), and 2-mercaptoethanol (50 μM). After 5 days of coculture in a humidified CO₂ incubator, the cells were collected and their cytotoxic activity was determined by ³¹Cr-release assay.

*Effector/target cell ratio.
†Spleen cells were passed through a nylon-wool column and used as responder cells.

MRL/l (3 mo) and BXSB (>9 mo) mice, cytotoxic T lymphocytes were not generated, even when nylon-wool nonadherent spleen cells were used as responder cells. It should be noted that T-cell dysfunction in autoimmune-prone mice is attributable not only to the involution of the thymus but also to abnormal stem cells, because normal stem cells of BALB/c nu/nu mice can differentiate into functional T cells even after the thymus of the autoimmune-prone recipient has already undergone considerable involution in BXSB or MRL/l strains.

Thus, normal T cells, B cells, and macrophages have developed 3 mo after bone marrow transplantation of BALB/c nu/nu marrow cells into lethally irradiated MRL/l or BXSB mice. Based on these findings, we can conclude that the etiopathogenesis of autoimmune diseases lies in a primary defect of stem cells and that abnormal stem cells induce premature thymic involution and T-cell dysfunction. The abnormal stem cells also differentiate into abnormal macrophages and abnormal B cells; the latter then produce autoantibodies with the help of abnormal macrophages and T cells. Thus, a wide range of abnormalities consequent to these abnormalities in various compartments of the immune system appears to induce the immunological imbalance that results in autoimmune diseases in mice of these autoimmune-prone strains.

In the present study, we used bone marrow cells of young BALB/c nu/nu mice (<2 mo) because we have found that a small number of functional T cells can be detected in spleen of nu/nu mice (12) and that these functional T cells increase with age (25). The results described here suggest that allogeneic bone marrow transplantation can be used without serious reactions, even when MHC barriers are crossed, provided that the T cells normally present in bone marrow are completely removed. Candidates for treatments for autoimmune diseases include total lymphoid irradiation (26) and employment of immunosuppressive agents such as cyclosporin A (27), azathioprine (28), cyclophosphamide (29), and/or steroid hormones. These approaches, however, exert suppressive effects on T-cell and other immunological functions.

With the recent progress of therapy, most patients with autoimmune diseases do not die of renal failure but of opportunistic infection attributable to immunosuppression. Since we provided evidence that T-cell functions increase after bone marrow transplantation in mice, it seems likely with further improvement in bone marrow transplantation technology, which will lead to reducing the mortality rate of the basic procedure and selecting appropriate resistance genes for haplo-identical donors, bone marrow transplantation ultimately might become a viable strategy for the treatment of severe life-threatening autoimmune diseases in humans.

We thank Dr. T. Tanisaka for performing irradiation studies. We also thank Mr. K. Tsuchida, Mr. T. Obata, Ms. K. Kitamura, Ms. M. Asano, and Ms. Myint Myint Than for their expert technical assistance and Ms. S. Kurimoto and Ms. K. Shimizu for their help in the preparation of the manuscript. This work was supported in part by the following grants: a grant from the Japanese Ministry of Welfare and Health, the Research Grant for 1984 from the Dr. Shimizu Foundation for the Promotion of Immunology, the National March of Dimes—Birth Defects Foundation Grant 1-789, and grants AI-03592 and AI-19495 from the U.S. National Institutes of Health.