Autoimmune Diseases as Stem Cell Disorders


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Using an animal model for insulin-dependent diabetes mellitus (IDDM), the NOD mouse, we have demonstrated that allogeneic bone marrow transplantation (BMT) has a preventative effect on IDDM, and that a combined transplantation of pancreas and bone marrow can be used to treat IDDM. We have also shown that BMT has a curative effect on systemic autoimmune diseases in (NZB × NZW)F₁, BXSB, and (NZW × BXSB)F₁ mice but not in MRL/lpr mice. Since MRL/lpr mice possess abnormal radioresistant hemopoietic stem cells (HSCs), they suffer a relapse 5 months after BMT. Recently, we have found that major histocompatibility complex (MHC)-matched stromal cells in the bone marrow are essential to the support of HSCs in the Go phase. We therefore attempted to treat autoimmune diseases in MRL/lpr mice by the transplantation of stromal cells with HSCs. Transplantation of HSCs with bone to recruit stromal cells was indeed found to have a curative effect on autoimmune diseases in the mice. These results indicate that BMT with bone graft will become a valuable strategy for the treatment of patients with both systemic and organ-specific autoimmune diseases. To prove that autoimmune diseases originate from defects in HSCs, we transferred the HSCs of autoimmune-prone mice to normal mice. BMT or transplantation of stem-cell concentrates induced organ-specific and/or systemic autoimmune diseases in [NOD → C3H/HeN] and [(NZW × BXSB)F₁ → C3H/HeN or C57BL/6J] chimeric mice. These results provide direct evidence that the etiopathogenesis of autoimmune diseases, including both organ-specific and systemic autoimmune

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diseases, is attributable to defects in the HSCs themselves. We further provide that various intractable diseases such as non-insulin-dependent diabetes mellitus and chronic nephritis (focal glomerulosclerosis) are also organ-specific autoimmune diseases, and that BMT can be used to treat them. ——— bone marrow transplantation; systemic autoimmune diseases; organ-specific autoimmune diseases; hematopoietic stem cells

Various mouse strains that spontaneously develop autoimmune diseases have contributed not only to better understanding of the fundamental nature of autoimmune diseases but also to the analysis of their etiopathogenesis. The etiopathogenesis of systemic autoimmune diseases has been attributed previously to T cell deficiencies, polyclonal B-cell activation, macrophage dysfunction and environmental factors such as hormonal disturbances (Theofilopoulos and Dixon 1985). However, there has recently been an increase in information suggesting that autoimmune diseases originate from defects in HSCs (Morton and Siegel 1974; Akizuki et al. 1978; Jyonouchi et al. 1981; Ikehara et al. 1985a, b, 1989, 1990; Yasumizu et al. 1987; Oyaizu et al. 1988).

In this paper, we review our findings indicating that autoimmune diseases are stem cell disorders, and provide evidence that BMT may become a useful tool for deciding if a certain intractable disease is a stem cell disorder.

Analyses of thymic abnormalities by transplantation of thymus or bone marrow between autoimmune-prone and normal mice

We previously found that autoimmune-prone mice such as (NZB × NZW)F₁ (B/WF₁), MRL/lpr and BXSB mice show thymic abnormalities including plasma cell infiltration (Ikehara et al. 1985c).

To clarify why thymic abnormalities develop in systemic autoimmune diseases, we transplanted the thymus or bone marrow from normal mice to autoimmune-prone mice, and vice versa (Nakamura et al. 1985). The data are summarized in Fig. 1; thymic abnormalities originate from defects in the bone marrow of autoimmune-prone mice, and the transplantation of bone marrow cells from normal mice to autoimmune-prone mice prevents both thymic abnormalities and autoimmune diseases.

Treatment of systemic autoimmune diseases by BMT

The next step was to examine whether BMT can be used to treat systemic autoimmune diseases. The protocol is shown in Fig. 2. After BMT, abnormal immunological functions (T cell deficiency, etc.), serological abnormalities (high levels of anti-DNA antibodies and circulating immune complexes, etc.), and histopathological findings (lupus nephritis, etc.) are normalized in B/WF₁ and BXSB mice (Ikehara et al. 1989).
Prevention and treatment of an organ-specific autoimmune disease (IDDM)

Based on the above observations, we attempted to determine whether organ-specific autoimmune disease could be treated using BMT.

First, we attempted to prevent insulinitis and overt diabetes by BMT. NOD mice (>4 months) were lethally irradiated and reconstituted with T cell-depleted BALB/c bone marrow cells. The mice were sacrificed more than 3 months after BMT. No lymphocyte infiltration was observed in the islets of BMT-treated NOD mice. Immunohistochemical studies revealed the presence of intact beta cells as well as alpha and delta cells. Glucose tolerance tests (GTTs) indicated that BMT-treated NOD mice exhibit a normal glucose response. Diabetic nephropathy was also corrected by BMT. Thus, BMT can prevent insulinitis and overt diabetes (Ikehara et al. 1985b). However, we could not treat overt diabetes in NOD mice by BMT, because mice with overt diabetes have no beta cells.
We next performed a combined transplantation of fetal or newborn pancreas and allogeneic bone marrow, since we know that organ allografts were not rejected if the organ was transplanted at the same time with bone marrow from the same donor (Nakamura et al. 1986). NOD mice that had already developed overt diabetes were then lethally irradiated and reconstituted with BALB/c bone marrow cells. An allogeneic pancreatic transplant from a fetal or newborn BALB/c mouse was then placed under the renal capsule of the NOD diabetic mouse. Three months after the transplantation, the mice exhibited a normal GTT pattern, and insulin levels in the sera were also normalized. Immunohistochemical studies revealed the presence of beta cells in the islets grafted under the renal capsule of NOD mice (Fig. 3). Thus, we succeeded in treating diabetes by a combined transplantation of pancreas and bone marrow (Yasumizu et al. 1987).

Prevention and treatment of both organ-specific (immune thrombocytopenic purpura) and systemic autoimmune diseases

We have recently found that (NZW × BXSB)F₁ (W/BF₁) mice, which develop lupus nephritis with myocardial infarction (Hang et al. 1981), show thrombocytopenia with age, and that the thrombocytopenia is attributable to the presence of both platelet-associated and circulating anti-platelet antibodies (Oyaizu et al. 1988). Thus, we conclude that the W/BF₁ mouse serves as a useful animal model of immune thrombocytopenic purpura (ITP) as well as systemic autoimmune diseases (SLE, etc.).

Transplantation of normal bone marrow cells from BALB/c-nu/nu mice to W/BF₁ mice was found to exert preventative and curative effects on the thrombocytopenia; the platelet counts were normalized, and circulating anti-platelet
Fig. 3. Histology of engrafted pancreas. Clusters of islet cells are observed under the renal capsule by hematoxylin/eosin staining (A). These cells are shown to contain insulin by means of immunohistological staining (B).
Transfer of insulitis and diabetes into C3H/HeN mice by transplantation of bone marrow cells from NOD mice

We attempted to transfer IDDM to normal mice by transplantation of NOD bone marrow cells. C3H/HeN mice were used as recipients. Mice of this strain express I-E^c^ molecules and have an aspartic acid at residue 57 (Asp-57) of the I-A^f^ chain (Estess et al. 1986; Koide and Yoshida 1989). We selected this strain because it has been postulated that failure to express the E^a^ gene is the abnormality that permits NOD mice to develop insulitis, leading to diabetes (Nishimoto et al. 1989; Reich et al. 1989). Also, it is thought that replacement of Asp-57 with Ser (non-Asp) in NOD mice (Acha-Ordea and McDeitt 1987) and with non-Asp in humans (Todd et al. 1987) may be the molecular anomaly responsible for the development of IDDM.

Female C3H/HeN (H-2^k^) mice were lethally irradiated (9.5Gy) at the age of 8 weeks and then reconstituted with T cell-depleted bone marrow cells of young (<8 weeks) female NOD (K^d^, I-A^NOD^, D^b^) mice. As controls, more than 50 C3H/

<table>
<thead>
<tr>
<th>1st antibody (plasma from)</th>
<th>Age (months)</th>
<th>Platelet count (Mean±s.d.)</th>
<th>2nd antibody (anti-Ig)^b^</th>
<th>Percent positive (mean±s.d.)</th>
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<tr>
<td>C3H/HeN</td>
<td>1.5</td>
<td>35.6</td>
<td>+</td>
<td>19.2±7.6</td>
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<td>49.6</td>
<td>+</td>
<td>33.9</td>
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<td>44.8</td>
<td>+</td>
<td>30.6</td>
</tr>
<tr>
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<td>+</td>
<td>35.3</td>
</tr>
<tr>
<td>W/BF_1</td>
<td>5.0 to 8.5</td>
<td>15.5±1.6</td>
<td>+</td>
<td>27.5±8.8</td>
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<tr>
<td>[BALB/c→W/B F_1]</td>
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<td>33.2</td>
<td>+</td>
<td>35.6</td>
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<td></td>
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<td>26.0</td>
<td>+</td>
<td>43.3</td>
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<tr>
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<td>+</td>
<td>44.1</td>
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<td>50.0</td>
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<tr>
<td></td>
<td>10.5</td>
<td>43.2</td>
<td>+</td>
<td>34.8</td>
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</table>

Platelets were obtained from 2-mo-old BALB/c mice.

^a^FITC-labeled goat anti-mouse Ig.

^b^The W/B F_1_ mice at the age of 3–5 mo were exposed to 9.5 Gy from a ^60^Co source and then reconstituted with 1.4×10^10^ bone marrow cells of BALB/c-^nu^-^nu_ mice. The mice were killed 5.5 mo after bone marrow transplantation.

**p<0.01 vs. data in old (5–8.5 mo) W/B F_1_ mice without BMT.

*p<0.02 vs. data in old (5–8.5 mo) W/B F_1_ mice without BMT.
HeN (H-2\textsuperscript{k}) mice were lethally irradiated and then reconstituted with T cell-depleted bone marrow cells of C3H/HeN, C57BL/6J (H-2\textsuperscript{b}), or BALB/c (H-2\textsuperscript{d}) mice. Even though these survived more than 1 year (survival rate, >90\%), neither insulitis nor overt diabetes developed. However, two of four \([\text{NOD-C3H/HeN}]\) chimeric mice developed both insulitis and overt diabetes 40 weeks after BMT. These mice exhibited elevated glucose levels and abnormal glucose tolerance curves (Fig. 4a).

It has been reported that cyclosporin A (CsA) given to neonatal mice perturbs T cell functions, resulting in the development of organ-specific autoimmune diseases (Sakaguchi and Sakaguchi 1989). \([\text{NOD-C3H/HeN}]\) mice were treated one month after BMT with CsA (10 mg/kg of body weight) daily for one month. Two of five mice in this group developed insulitis and overt diabetes as early as 20 weeks following BMT (Fig. 4b). In these mice, the beta cells of the pancreas were specifically destroyed by lymphocytes (Ikehara et al. 1990).

**Transfer of ITP and lupus nephritis into C3H/HeN or C57BL/6J mice by transplantation of bone marrow cells from W/BF\textsubscript{1} mice**

The next step was to investigate whether both systemic and organ-specific autoimmune diseases could be transferred to normal mice by BMT. Since the male W/BF\textsubscript{1} mouse, which develops lupus nephritis and myocardial infarction, is an impressive animal model of ITP, we used W/BF\textsubscript{1} (H-2\textsuperscript{k}/H-2\textsuperscript{b}) mice as donors and C3H/HeN (H-2\textsuperscript{k}) or C57BL/6J (H-2\textsuperscript{b}) mice as recipients.

C3/HeN or C57BL/6J mice were lethally irradiated (9.5Gy) and then reconstituted with T cell-depleted bone marrow cells of young (<8 weeks) male W/BF\textsubscript{1} mice. \([W/BF\textsubscript{1} \rightarrow C57BL/6J]\) mice showed thrombocytopenia (<10\textsuperscript{5} platelets per mm\textsuperscript{3}; normal mice, >10 \times 10\textsuperscript{5}) in 5 of 11 mice (45\%) 3 months after BMT, and in 5 more of the same 11 mice (total 10/11: 91\%) by 5 months after BMT. \([W/
BF₁→C3H/HeN] mice also developed thrombocytopenia in 4 of 8 mice (50\%) within 3 months of BMT and in 6 of 8 mice (75\%) within 6 months of BMT.

Cytofluorometric analyses demonstrated the presence of both platelet-associated antibodies and circulating anti-platelet antibodies in the thrombocytopenic mice (Fig. 5). Immunohistopathological analyses revealed typical wire-loop lesions in the glomeruli of the [W/BF₁→C57BL/6J] or [W/BF₁→C3H/HeN] mice.

To confirm that defective HSCs were indeed the elements responsible for the development of the autoimmune diseases, we transferred W/BF₁ bone marrow cells from a HSC-enriched fraction (fraction II) to C3H/HeN mice, since both Visser et al. (1984) and we (Miyama-Inaba et al. 1987) have reported that, after T cells, B cells and macrophages have been depleted from bone marrow cells, spleen colony-forming units are enriched in a low-density fraction obtained by a Percoll discontinuous-density centrifugation method. Lethally irradiated (9.5Gy) C3H/HeN mice that had been injected with W/BF₁ HSC-enriched bone marrow cells were also found to develop thrombocytopenia and lupus nephritis (Ikehara et al. 1990). We conclude from these experiments that the etiopathogenesis of both systemic and organ-specific autoimmune diseases can be attributed to abnormal-

![Fig. 5. Analyses of anti-platelet antibodies. (a) Control, C3H/HeN platelets treated with BALB/c serum. (b) Platelet-associated antibodies of [W/BF₁→C3H/HeN] mouse (3 months after BMT; platelet count 23×10⁴ per mm³). (C) Circulating anti-platelet antibodies shown by treatment of C3H/HeN platelets with serum from [W/BF₁→C3H/HeN] mouse (3 months after BMT).](#)
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Treatment of autoimmune diseases in MRL/lpr mice by BMT with bone grafts

Since MRL/lpr mice possess abnormal radioresistant HSCs, they suffer a relapse 5 months after conventional BMT (Ikehara et al. 1989). Recently, we have found that MHC-matched stromal cells in the bone marrow are essential to the support of HSCs in the G0 phase (manuscript in preparation). We therefore attempted to treat autoimmune diseases in MRL/lpr mice by the transplantation of stromal cells with HSCs. As shown in Fig. 6, transplantation of HSCs with bones to recruit stromal cells was indeed found to have a curative effect on autoimmune diseases in MRL/lpr mice (Ikehara et al. 1991; Ishida et al. 1994). These results indicate that BMT with bone grafts will become a valuable strategy for the treatment of patients with autoimmune diseases.

Treatment of non-insulin-dependent diabetes mellitus (NIDDM) by BMT

There have recently been several reports indicating that autoimmune mechanisms are involved in the development of NIDDM (Flier et al. 1975; Yokokawa et al. 1989). These findings prompted us to examine the involvement of the immune system in the etiopathogenesis of NIDDM using KK-Ay mice. The KK mouse is an inbred strain established by Kondo et al. from native Japanese mice in 1957 (Kondo et al. 1957). Several abnormalities have been found in this mouse strain, including impaired glucose tolerance, hyperglycemia, insulin resistance of peripheral tissue, hyperinsulinemia, and glomerular changes (Danforth 1927; Carpenter and Mayer 1958; Nakamura 1962; Tsuchida 1966; Nakamura and Yamada 1967). In 1969, Nishimura et al. introduced the yellow obese gene from Ay mice into the KK mice by repeatedly crossing these two strains. The congenic mice established by this procedure have been termed "yellow KK" or "KK-Ay" mice. These mice

![Fig. 6. Survival rate of MRL/lpr mice with BMT and bone graft.](image-url)
show no sexual or seasonal variation in the appearance of glycosuria, but show hyperglycemia and hyperinsulinemia at the age of 5 weeks.

We performed allogeneic BMT on 19-week-old KK-Ay (H-2b) mice when they showed glycosuria (*); T cell-depleted BALB/c bone marrow cells (2×10⁷) were injected intravenously through the tail veins of lethally (9 Gy) irradiated KK-Ay mice one day after irradiation. As a control group, urine sugar-negative 8-week-old female KK-Ay mice were lethally (9 Gy) irradiated and then reconstituted with 2×10⁷ bone marrow cells of age- and sex-matched KK-Ay mice. Body weight, food and water consumption, and urine sugar were monitored daily. All mice in both groups that received BMT showed negative urine sugar one week after BMT. Food and water consumption were similar, and weight loss of about 10% was observed in both groups 2 weeks after BMT. [KK-Ay→KK-Ay] mice showed a gradual appearance of glycosuria from 4 weeks after BMT and developed urine sugar (†) 8 weeks after BMT (16 weeks of age), whereas [BALB/c→KK-Ay] mice continued to show negative urine sugar. We performed intraperitoneal GTTs monthly on both groups, starting one month after BMT in [BALB/c→KK-Ay] mice, and 2 months after BMT in [KK-Ay→KK-Ay] mice. Fig. 7 shows the significant difference between these two groups in the glucose tolerance curve. [BALB/c→KK-Ay] mice showed negative urine sugar and a normal glucose tolerance curve even 4 months after BMT. FACS analyses using the spleen cells of [BALB/c→KK-Ay] mice revealed that donor (H-2a)-derived cells had become dominant (>95%). In contrast, glucose tolerance was abnormal and urine sugar positive in the [KK-Ay→KK-Ay] mice 2 months after BMT.

Serum lipid and insulin levels were examined before and after BMT. As

![Fig. 7. GTTs in [KK-Ay→KK-Ay] and [BALB/c→KK-Ay] mice: syngeneic group (○) and allogeneic group; 1 month (△), 2 months (○), 3 months (□) and 4 months (●) after BMT. [KK-Ay→KK-Ay] mice showed glycosuria, while [BACB/c→KK-Ay] mice did not even at 4 months after BMT.](image-url)
<table>
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<tr>
<th>Mice</th>
<th>Treatment (age)</th>
<th>Insulin (ng/ml)</th>
<th>Cholesterol (mg/100 ml)</th>
<th>Triglyceride (mg/100 ml)</th>
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<tbody>
<tr>
<td>BALB/c</td>
<td>Non-treated (16-wk-old)</td>
<td>0.463 ± 0.133</td>
<td>72.33 ± 18.29</td>
<td>17.33 ± 16.9</td>
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<td></td>
<td>Non-treated (4-wk-old)</td>
<td>3.40 ± 0.53</td>
<td>85.00 ± 11.41</td>
<td>8.25 ± 28.97</td>
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<td>Non-treated (6-wk-old)</td>
<td>15.76 ± 14.67</td>
<td>90.00 ± 11.53</td>
<td>257.00 ± 58.95</td>
</tr>
<tr>
<td>KK-Ay</td>
<td>Before BMT (6-wk-old)</td>
<td>43.20 ± 13.10</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Before BMT (16-mo-old)</td>
<td>111.0 ± 12.72</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
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<td>4 months after BMT (6-wk-old)</td>
<td>81.197 ± 10.695</td>
<td>169.25 ± 26.26</td>
<td>273.5 ± 26.75</td>
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<tr>
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<td>4 months after BMT (19-mo-old)</td>
<td>50.924 ± 25.972</td>
<td>277.0 ± 17.66</td>
<td>160.75 ± 19.01*</td>
</tr>
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</table>

**p < 0.01 vs. the data of [BALB/c→KK-Ay] mice before BMT.**

**Immune response insulin was measured using a radioimmunoassay with the polyethylene glycol method. The data are expressed as the mean ± standard deviation of 10 mice.**

Table 2: Serum insulin and lipid levels
shown in Table 2, significant differences were noted between the two groups; serum lipid and insulin levels decreased after allogeneic BMT. The levels were between 4-wk-old and 6-wk-old non-treated KK-Ay mice, but not as low as those of normal BALB/c mice, since the islets still show hyperplasia. This suggests that hyperlipidemia and hyperinsulinemia in NIDDM are secondary to insulin resistance.

Glomerular changes before and after BMT were also examined in the two groups. Before BMT, the kidneys of KK-Ay mice showed a global and diffuse increase in the mesangial matrix (Fig. 8a). A linear distribution of IgG along the basement membranes of glomerular capillaries was noted (Fig. 8b). However, after BMT, the [BALB/c→KK-Ay] mice showed a normal appearance in H-E staining (Fig. 8c), and the IgG deposits had completely disappeared (Fig. 8d). The [KK-Ay→KK-Ay] mice, however, showed no change (data not shown).
Treatment of focal glomerulosclerosis (FGS) by BMT

An animal model for FGS, the FGS mouse, has recently been established by Kondo et al. The mice develop a high degree of proteinuria from the age of 3 months and show sclerotic change in their glomeruli. Unlike lupus mice, they show neither high levels of anti-DNA antibodies and CICs nor thymic abnormalities.

The etiopathogenesis of human FGS is unknown, and steroid therapy has no effect. Since we have found that glomerular damage induced by SLE is reversible and repaired by BMT, we attempted to treat FGS by BMT. FGS (H-2\(^k\)) mice with proteinuria (\#) were lethally irradiated and then reconstituted with T cell-depleted bone marrow cells of BALB/c (H-2\(^d\)) mice. The mice showed decreased proteinuria from 8 weeks after BMT and finally showed negative proteinuria 11 weeks after BMT. We carried out serial renal biopsies to analyze the repairing mechanism and found that donor-derived T cells with normal functions play a crucial role in the repair of glomerular damage (manuscript in preparation).

Furthermore, the transplantation of bone marrow cells from FGS mice to normal mice was found to induce FGS in the normal mice (Nishimura 1994). We therefore conclude that FGS is an organ (kidney)-specific autoimmune disease.

In conclusions, we have demonstrated that both systemic and organ-specific autoimmune diseases are stem cell disorders, and that BMT provides a useful tool for analyzing the etiopathogenesis of various intractable diseases.

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References


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