Using various animal models for autoimmune diseases, we have found that bone marrow transplantation (BMT) can be used to treat not only systemic autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), but also organ-specific autoimmune diseases such as immune thrombocytopenic purpura (ITP) and insulin-dependent diabetes mellitus (IDDM). We have also found that the transplantation of hematopoietic stem cell (HSC)-enriched populations from autoimmune-prone mice to normal mice induces autoimmune diseases in the recipients. These findings have recently been confirmed in humans; BMT can be used to treat autoimmune diseases such as RA, Crohn's disease and ulcerative colitis (UC), whereas autoimmune diseases such as Graves' disease and ITP have been transferred from donors to recipients by BMT. Based on these findings, we have proposed that autoimmune diseases are “stem cell disorders”. To elucidate the differences between normal and abnormal HSCs, we have very recently established a new method for purifying pluripotent HSCs (P-HSCs). Using P-HSCs purified by our method, we have compared normal and abnormal P-HSCs, and found that qualitative differences exist between them both in vivo and in vitro; although normal P-HSCs do not readily proliferate in major histocompatibility complex (MHC)-incompatible microenvironments, abnormal P-HSCs show a marked proliferative response even in the allogeneic environments. Abnormal P-HSCs are thus more “resilient” than normal P-HSCs.

Key words: Autoimmune disease, Stem cell disorder, Bone marrow transplantation

I. Introduction

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 previously been attributed to T cell deficiencies, polyclonal B-cell activation, macrophage dysfunction and environmental factors such as hormonal disturbances [39]. However, there has recently been an increase in information suggesting that autoimmune diseases originate from defects in HSCs [3, 14-17, 19, 30, 35, 44].

In this article, we show that autoimmune diseases are stem cell disorders, and provide evidence that BMT will become a valuable strategy for the treatment of various autoimmune diseases.

II. Treatment of Systemic Autoimmune Disease by BMT

When female (NZB x NZW)F1 (B/W F1) (>6 months), female MRL/lpr (>2 months), and male BXSB (>6 months) mice that had already shown clear evidence of lupus nephritis were lethally irradiated and then reconstituted with either allogeneic bone marrow cells of young (<2 months) BALB/c nu/nu (H-2b) mice or T cell-depleted bone marrow cells of BALB/c mice, the reci-
patients survived in good health for more than 3 months after BMT [14].

In BXSB and B/W F1 mice, BMT had completely curative effects. Glomerular damage was ameliorated, and the levels of autoantibodies (anti-DNA and anti-Sm antibodies [Abs]) and circulating immune complexes (CICs), particularly gp-70 anti-gp-70 CICs, were reduced. The repair of glomerular damage was assessed by performing renal biopsies before and after BMT, as shown in Fig. 1. In addition, immunological functions were normalized; T cell functions including IL-2 production were restored, and hyperfunctions of macrophages and B cells decreased. Assays for both mixed-lymphocyte reaction (MLR) and the generation of cytotoxic T lymphocytes (CTL) revealed that newly developed T cells from BMT-treated mice were tolerant of both bone marrow donor-type and host-type MHC determinants, but responded vigorously to third-party cells. In vitro primary anti-sheep red blood cell (SRBC) plaque-forming cell (PFC) assay also showed that some degree of co-operation was achieved among antigen-presenting cells (APC), helper T cells, and B cells.

III. Prevention and Treatment of Organ-Specific Autoimmune Disease by BMT Plus Organ Allografts

We next examined whether organ-specific autoimmune diseases could be treated by BMT using an animal model for IDDM, the NOD mouse.

First, we attempted to prevent insulitis and overt diabetes by BMT. NOD mice (>4 months) were lethally irradiated and then reconstituted with T cell-depleted BALB/c bone marrow cells. The mice were killed more than 3 months after BMT. No lymphocyte infiltration was observed in the islets of the BMT-treated NOD mice. Immunohistochemical studies revealed the presence of intact beta cells as well as alpha and delta cells. BMT-treated NOD mice showed a normal pattern in glucose tolerance tests (GTTs). Diabetic nephropathy was also corrected by BMT. Thus, BMT can prevent insulitis and overt diabetes [15]. 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 plus allogeneic bone marrow, since we know that organ allografts are accepted if the organ is transplanted from the same donor as the bone marrow at the same time [31]. NOD mice that had already developed overt diabetes were lethally irradiated and then reconstituted with allogeneic BALB/c bone marrow cells. The pancreatic tissues from fetal or newborn BALB/c mice were then engrafted under the renal capsules of NOD diabetic mice. Three months after the transplantation, the mice exhibited a normal GTT pattern, and insulin levels in the sera were also normalized.

Fig. 1. Histopathologic and immunofluorescent findings in the glomeruli of B/W F1 mice before and after BMT. Typical wire-loop lesions (top left) and IgG deposits (bottom left) are present in the glomeruli of the 8-month-old B/W F1 mouse before BMT. Five months after BMT, IgG deposits are markedly reduced (bottom right), and a glomerulus of the mouse exhibits a normal appearance on hematoxylin-eosin staining (top right).
Immunohistochemical studies revealed the presence of beta cells in the islets engrafted under the renal capsules of the NOD mice (Fig. 2). It should be noted that neither insulitis nor rejection occurred. Thus, we succeeded in treating diabetes by the combined transplantation of the pancreas and bone marrow [44].

IV. Prevention and Treatment of Both Organ-Specific and Systemic Autoimmune Diseases by BMT

We have recently found that (NZW × BXSB)F1 (W/BF1) mice, which develop lupus nephritis with myocardial infarction [11], show thrombocytopenia with age, and that the thrombocytopenia is attributable to the presence of both platelet-associated and circulating anti-platelet antibodies [35]. In addition, we have very recently found that myocardial infarction in W/BF1 mice (as shown in Fig. 3) is due to the presence of anti-cardiolipin Abs, and that the mouse is an animal model for anti-phospholipid Ab syndrome [2].

The transplantation of bone marrow cells from normal mice to W/BF1 mice was found to exert preventative and curative effects on lupus nephritis, thrombocytopenia and anti-phospholipid Ab syndrome; the platelet counts were normalized, and circulating anti-platelet Ab levels as

Fig. 2. Immunohistological findings of engrafted pancreas. Clusters of beta cells are observed under the renal capsule. Neither insulitis nor rejection occurs.

Fig. 3. Myocardial infarction due to the thrombus of a coronary artery in a W/B F1 mouse.
Ikehara

well as anti-phospholipid Ab levels were reduced [2, 35].

V. Repair Mechanism of Lupus Nephritis after BMT

To elucidate why and how glomerular damage is repaired by BMT, serial renal biopsies were carried out using B/WF1 mice before and after BMT. Donor-derived B cells and macrophages with normal functions developed two weeks (wks) after BMT. At this stage, the macrophages did not show immune complex (IC) clearance activity. Donor-derived T cells with normal functions were generated six wks after BMT. At this stage, visceral epithelial cells, macrophages and mesangial cells in the glomeruli were activated by T cells and showed marked phagocytic activity: macrophages and mesangial cells were found to be responsible for the clearance of ICs, whereas, to our surprise, epithelial cells were found to be responsible for the repair of injured basement membranes. These findings suggest that T cells with normal functions, which have the capacity to activate macrophages, mesangial cells and epithelial cells, play a crucial role in repairing IC-mediated glomerular damage (Fig. 4).

VI. Transfer of Organ-Specific Autoimmune Disease into Normal Mice by BMT

We attempted to transfer IDDM to normal mice by transplanting NOD bone marrow cells to C3H/HeN mice. C3H/HeN mice express I-Ea molecules and have an aspartic acid at residue 57 (Asp-57) of the 1-Aβ chain [8, 22]. We selected this strain because it has been postulated that the failure to express the Eα gene is the abnormality that permits NOD mice to develop insulinitis, leading to diabetes [32, 36]. Also, it is thought that replacement of Asp-57 with Ser (non-Asp) in NOD mice [1] and with non-Asp in humans [40] may be the molecular anomaly responsible for the development of IDDM.

Female C3H/HeN (H-2k) mice were lethally irradiated (9.5 Gy) at the age of 8 weeks and then reconstituted with T cell-depleted bone marrow cells of young (<8 weeks) female NOD (K₁, 1-Aγ, Dβ) mice. As controls, more than 50 C3H/HeN (H-2k) mice were lethally irradiated and then reconstituted with T cell-depleted bone marrow cells of C3H/HeN, C57BL/6J (H-2b), or BALB/c (H-2d) mice. Even though these survived more than 1 year (survival rate, >90%), neither insulinitis nor overt diabetes developed. However, two of four [NOD → C3H/HeN] chimeric mice developed both insulinitis and overt diabetes more than 40 weeks after BMT; beta cells were selectively destroyed by the infiltration of T cells (Fig. 5). These mice exhibited elevated glucose levels and abnormal glucose tolerance curves [17].

VII. Transfer of Both Organ-Specific and Systemic Autoimmune Diseases into Normal Mice by BMT

The next step was to investigate whether both systemic (SLE) and organ-specific (ITP) autoimmune diseases could be transferred to normal mice by BMT. Since the male W/BF1 mouse, which develops lupus nephritis and myocardial infarction, is an impressive animal model of ITP, we used W/BF1 (H-2β/H-2k) mice as donors and C3H/HeN (H-2k) or C57BL/6J (H-2b) mice as recipients. C3H/HeN or C57BL/6J mice were lethally irradiated (9.5 Gy) and then reconstituted with T cell-depleted bone marrow cells of young (<8 weeks) male W/BF1 mice. [W/BF1 → C57BL/6J] mice showed thrombocytopenia.
Autoimmune Diseases as "Stem Cell Disorders"

Fig. 5. Immunohistochemical findings in the islet of a [NOD → C3H/HeN] mouse with overt diabetes, 40 weeks after BMT. (a) Glucagon-producing cells (alpha cells). (b) Somatostatin-producing cells (delta cells). (c) Insulin-producing cells (beta cells). Note the selective destruction of beta cells by lymphoid cell infiltration.

(<10^5 platelets per mm^3; normal mice > 10^10^5) 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%) by 3 months after BMT and in 6 of 8 mice (75%) by 6 months after BMT.

Cytofluorometric analyses demonstrated the presence of both platelet-associated antibodies and circulating anti-platelet antibodies in the thrombocytopenic mice. Immunohistopathological analyses revealed typical wire-loop lesions in the glomeruli of the [W/Bf1 → C57Bl/6J] or [W/Bf1 → C3H/HeN] mice, as shown in Fig. 6.

To confirm that the defective HSCs were indeed the elements responsible for the development of the autoimmune diseases, we transferred cells in a HSC-enriched fraction (fraction II) of W/Bf1 bone marrow cells to C3H/HeN mice, since both Visser et al. [42] and we [29] have reported that, after T cells, B cells and macrophages have been depleted from bone marrow cells, spleen colony-forming units (CFU-S) are enriched in a low-density fraction (fraction II) obtained by a Percoll discontinuous-density centrifugation method. Lethally irradiated (9.5 Gy) C3H/HeN mice that had been injected with W/Bf1 HSC-enriched bone marrow cells were also found to develop thrombocytopenia and lupus nephritis [17].

We therefore conclude from these experiments that the etiopathogenesis of both systemic and organ-specific autoimmune diseases can be attributed to abnormalities in the HSC population.

VIII. Purification of Pluripotent Hemopoietic Stem Cells (P-HSCs)

To clarify the differences of P-HSCs between normal and autoimmune-prone mice, we have been purifying P-HSCs. P-HSCs are defined as cells with the capacity to perpetually self-renew eternally and to differentiate into cells in all lineages including lymphoid cells. We have previously demonstrated that P-HSCs can be purified by both in vivo and in vitro 5-fluorouracil (5-FU) treatments, followed by sorting wheat germ agglutinin-binding (WGA^+) cells [29]. However, the 5-FU treatments (both in vivo and in vitro) have cytotoxic effects even on P-HSCs. We have therefore modified the method to include only in vivo 5-FU treatment followed by sorting CD71-Class I^high^ cells from lineage-negative (Lin^-) cells. The sorted cells (only 4 cells) have the long-term repopulating ability in the assay of (male → female) chimeras [33]. It has been reported that HSCs are c-kit^+ or c-kit^low^ [20, 34]. We have, however, found that the P-HSCs are c-kit<low; only c-kit<low cells have the long-term (>1.5 year) reconstituting ability [6].

In vitro studies revealed that this population cannot proliferate in the presence of putative cytokines such as GM-CSF, stem cell factor (SCF) and IL-3, whereas it can do so by direct interaction with stromal cells without adding any cytokines (Fig. 7).
The morphology of P-HSCs was examined using an electron microscope. As shown in Fig. 8, the cells had a large nucleus with narrow cytoplasm. Their chromatin pattern was dispersed, but small aggregates appeared at nuclear margins. There were few cytoplasmic organelles but abundant free ribosomes. It should be noted that P-HSCs possess microvilli; they show active movement like neutrophils, as observed on video tape.

**IX. Qualitative Differences between Normal and Abnormal P-HSCs**

The next question was whether there are any qualitative differences between normal and abnormal P-HSCs. To answer this question, we first carried out BMT between normal and autoimmune-prone mice using partially purified P-HSCs. The transplantation of bones plus abnormal P-HSCs obtained from autoimmune-prone mice induced autoimmune diseases in normal mice, as did transplantation of T cell-depleted bone marrow cells (TCO-BMCs) (Fig. 9). However, the transplantation of bones plus normal P-HSCs could not reconstruct hemopoiesis in autoimmune-prone mice due to graft rejection (Fig. 10) [21], although the transplantation of T cell-depleted bone marrow cells from normal mice can be used to prevent and treat autoimmune diseases in autoimmune-
Autoimmune Diseases as "Stem Cell Disorders"

Fig. 9. Survival rate in (W/BF1→C3H) chimeric mice. C3H/HeN mice were irradiated (9.5 Gy) and then reconstituted with either 1-2×10^7 TCD-BMCs (●●●) or 1-2×10^5 WGA+ cells plus 40 Gy-irradiated bone grafts (○○○) or WGA+ cells alone from male W/BF1 mice (<2 mo) (■■■).

Fig. 10. Survival rate in (C3H→W/BF1) chimeric mice. Male W/F1 mice (<2 mo) were irradiated (9.0 Gy) and reconstituted with 1-2×10^7 TCD-BMCs (×××), 1-2×10^5 WGA+ cells with (○○○) or without (●●●) bone grafts from C3H mice.

prone mice [14-16], as described above. This finding suggests that abnormal P-HSCs are more resilient than normal P-HSCs; the former can proliferate in MHC-mismatched microenvironments, while the latter cannot. This was also confirmed in in vitro experiments. As shown in Fig. 11, abnormal P-HSCs can proliferate in collaboration with MHC-incompatible stromal cells, although normal P-HSCs can do so only in collaboration with MHC-compatible stromal cells, not MHC-incompatible stromal cells [21].

X. Evidence for Autoimmune Diseases as Stem Cell Disorders in Humans

Seven RA patients have received allogeneic BMT from HLA-identical siblings, all because of severe aplastic
anemia supervening after gold and/or D-penicillamine therapy [5, 18, 26]. Two patients are in complete remission with a follow-up of six years. It was also reported that two cases of psoriasis vulgaris were resolved after BMT: one was associated with AML [7], and the other with CML [25]. Stable remission of ulcerative colitis has also been reported in a young woman who received BMT because of AML [7]. Conversely, the adoptive transfer of autoimmune diseases after BMT has been reported. Grau et al. and others reported six cases of myasthenia gravis (MG) occurring after allogeneic BMT [9, 28]. Other adoptive, post-transplant autoimmune diseases include autoimmune thyroiditis [4, 43], IDDM [10, 23, 24, 41], and Graves' disease [12]. Recently, Marmont has reviewed these data in humans [27].

XI. Hypothetical Etiopathogenesis of Systemic and Organ-Specific Autoimmune Diseases

Based on the above observations (both in mice and humans), we have proposed a new concept of "stem cell disorders" including autoimmune diseases: i) stem cell aplasia (aplastic anemia), ii) monoclonal abnormal stem cell proliferative syndrome (leukemia and preleukemia), and iii) polyclonal abnormal stem cell proliferative syndrome (autoimmune diseases).

Regarding the etiopathogenesis of autoimmune diseases, our current hypothesis is as follows. As shown in Fig. 12, polygene abnormalities primarily exist at the gene level of P-HSCs. Probably endogenous or exogenous retroviruses are involved in the development of P-HSC abnormalities. Autoreactive immunocompetent cells including T cells differentiate from the abnormal P-HSCs. In systemic autoimmune diseases, autoreactive
T cells attack various organs as in chronic graft-versus-host reaction (GVHR), resulting in systemic tissue injury (Fig. 12A). In contrast, suppressor T cells play a crucial role in the development of organ-specific autoimmune diseases (Fig. 12B), since we have some data suggesting that there is a correlation between decreased CD8+ suppressor T cell counts and the development of organ-specific autoimmune diseases (unpublished data).

**XII. The Prospects for BMT**

In humans, BMT across MHC-barriers has had a low success rate as a consequence of i) GVHR due to contamination with T cells from the peripheral blood and ii) graft rejection. We have provided evidence that, in mice, no such problems are associated with BMT. GVHR can be prevented if T cell-depleted bone marrow cells are used. Graft rejection can be prevented by bone grafts and the transplantation of natural suppressor cells (NSCs) [37, 38]. It is certain that similar conditions to permit successful BMT in humans will be realized in the near future. When such conditions have been achieved, we can expect BMT to become a valuable strategy for the treatment of patients with autoimmune diseases. Furthermore, we would like to suggest that organ allografts of heart, kidney, pancreas, etc., may be accomplished without using long-term immunosuppressants if the organ is obtained from the same donor as the bone marrow and both are transplanted at the same time.

In humans, it is well known that the success rate of BMT in patients more than 45 years old is low. Recently, we have found that the low success rate is due to the aging of the thymus, and that BMT plus embryonal thymus grafts can be used to not only treat late-onset autoimmune diseases in MRL/+ mice [13] but also prevent age-related amyloidosis in SAMP 1 mice (manuscript in preparation). We believe that this is due to the atrophy of the thymus, and that transplantation of the embryonal thymus in conjunction with BMT should become a valuable strategy for older patients with various diseases.

**XIII. Acknowledgments**

These experiments were carried out in collaboration with researchers who appear in the references of this paper. I would like to express my deep appreciation to them.

I would also like to thank Ms. Yoshiko Shinno and Ms. Yuki Matsui for their skilful technical assistance, and Mr. Hilary Eastwick-Field and Ms. Keiko Ando for preparing this manuscript.

This work was supported by a grant from the Japanese Ministry of Health and Welfare; the Ministry of Education, Science, and Culture, Japan; the “Traditional Oriental Medical Science Program” of the Public Health Bureau of the Tokyo Metropolitan Government; the Mochida Memorial Foundation for Medical and Pharmaceutical Research (1986); the Naito Foundation (1986); the Mitsubishi Foundation (1986); the Suzuken Memorial Foundation (1987); the Takeda Science Foundation (1989); Yasuda Igaku Kinen Zaidan (1990); the Foundation for Rheumatic Diseases (1990), and the Science Research Promotion Fund of the Japan Private School Promotion Foundation, Japan.

**XIV. References**


