

# Organ-specific and systemic autoimmune diseases originate from defects in hematopoietic stem cells

(mouse model/bone marrow transplantation/diabetes mellitus/idiopathic thrombocytopenic purpura)

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**ABSTRACT** Transplantation of bone marrow cells from nonobese diabetic (NOD) mice, a model for type 1 diabetes mellitus, to C3H/HeN mice, which express I-E $\alpha$  molecules and have aspartic acid at residue 57 of the I-A $\beta$  chain, induced insulinitis followed by overt diabetes in the recipient C3H/HeN mice more than 40 weeks after bone marrow transplantation. When cyclosporin A, which perturbs T-cell functions, was injected intraperitoneally into [NOD  $\rightarrow$  C3H/HeN] chimeric mice daily for 1 month, the chimeric mice developed insulinitis and overt diabetes within 20 weeks following bone marrow transplantation. Transplantation of bone marrow cells from (NZW  $\times$  BXSB)F<sub>1</sub> mice, which develop lupus nephritis, myocardial infarction, and idiopathic thrombocytopenic purpura, into C3H/HeN or C57BL/6J mice induced in the recipient strains both lupus nephritis and idiopathic thrombocytopenic purpura more than 3 months after transplantation. Transplantation of a stem-cell-enriched population from (NZW  $\times$  BXSB)F<sub>1</sub> mice into normal mice also induced autoimmune disease in the recipients. These results indicate that both systemic autoimmune disease and organ-specific autoimmune disease originate from defects that reside within the stem cells; the thymus and environmental factors such as sex hormones appear to act only as accelerating factors.

Since the thymus plays a crucial role in positive and negative selection of T cells and is engaged in deletion of autoreactive clones (1), it has been thought that the etiopathogenesis of both systemic and organ-specific autoimmune diseases could be attributed to defects in the thymus. However, considerable evidence indicates that autoimmune diseases originate from defects that reside in the hematopoietic stem cells (2-7). Previous studies (7-11) using autoimmune-prone mice demonstrated that allogeneic bone marrow transplantation (BMT) can be used to prevent and treat both systemic and organ-specific autoimmune diseases. In this context, we decided that it would be important to determine whether transplantation of particular bone marrow cells, especially hematopoietic stem cells from bone marrow of autoimmune-prone mice, would lead to the development of autoimmune diseases in normal mice, an environment that includes the normal thymus.

We demonstrate herein that BMT or transplantation of stem-cell concentrates induces organ-specific and/or systemic autoimmune diseases in [NOD  $\rightarrow$  C3H/HeN] and [(NZW  $\times$  BXSB)F<sub>1</sub>  $\rightarrow$  C3H/HeN] or [(NZW  $\times$  BXSB)F<sub>1</sub>  $\rightarrow$  C57BL/6J] chimeric mice. These results provide direct evidence that the etiopathogenesis of autoimmune diseases, including both organ-specific and systemic autoimmune dis-

eases, is attributable to defects that reside in the stem cells themselves.

## MATERIAL AND METHODS

**Mice.** Nonobese diabetic (NOD) mice were obtained from Shionogi Aburahi Laboratories (Shiga, Japan) and maintained under specific pathogen-free conditions in the animal facility at Kansai Medical University. NZW and BXSB mice (The Jackson Laboratory) and (NZW  $\times$  BXSB)F<sub>1</sub> (W/BF<sub>1</sub>) mice were maintained under specific pathogen-free conditions at Kiwa Laboratory Animals (Wakayama, Japan). C57BL/6J and C3H/HeN mice were obtained from CLEA Japan (Osaka).

**BMT.** Mice were exposed to 9.5 Gy from a <sup>60</sup>Co source and then reconstituted by intravenous injection of  $1.0 \times 10^7$  T-cell-depleted bone marrow cells or  $1.0 \times 10^6$  cells of a stem-cell-enriched population from young autoimmune-prone mice, as described (10).

**Cytofluorometric Analyses.** Analyses of H-2 determinants, platelet-associated antibodies, and circulating anti-platelet antibodies were carried out using a FACStar (Becton Dickinson), as described (11, 12). Spleen cells suspended in phosphate-buffered saline containing 2% fetal bovine serum and 0.05% sodium azide were stained with fluorescein isothiocyanate-conjugated monoclonal antibodies against H-2D<sup>d</sup> (030-20F), H-2D<sup>b</sup> (030-14F), H-2K<sup>d</sup> (030-21F), or H-2K<sup>b</sup> (030-11F). Monoclonal antibodies were purchased from the Meiji Institute of Health Science (Odawara, Japan).

**Glucose Tolerance Tests.** These were carried out as described (9).

**Preparation of a Stem-Cell-Enriched Population.** To obtain a stem-cell-enriched population, bone marrow cells that had been depleted of T cells, B cells, and macrophages were fractionated by centrifugation in a Percoll discontinuous density gradient (13).

**Histological Study.** Major organs were obtained at autopsy, and sections were stained with hematoxylin/eosin. For immunohistochemical studies, specimens were immediately embedded in optimal-cutting-temperature compound and frozen in dry ice/acetone. Two-micrometer cryostat sections were used for immunohistochemical study (9, 10).

## RESULTS

**Transfer of Insulinitis and Diabetes into C3H/HeN Mice by Transplantation of Bone Marrow Cells from NOD Mice.** The first step was to examine whether an organ-specific autoim-

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Abbreviations: BMT, bone marrow transplantation; CsA, cyclosporin A; W/BF<sub>1</sub>, (NZW  $\times$  BXSB)F<sub>1</sub>; NOD, nonobese diabetic.  
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mune disease, insulin-dependent diabetes mellitus, which occurs in NOD mice, could be transferred to normal mice by transplantation of NOD bone marrow cells. C3H/HeN mice were used as recipients. Mice of this strain express I-E $\alpha$  molecules and have an aspartic acid at residue 57 (Asp-57) of the I-A $\beta$  chain (14, 15). We selected this strain because it has been postulated that failure to express the E $\alpha$  gene is the abnormality that permits NOD mice to develop insulinitis, which leads to diabetes (16, 17). Also, it is thought that replacement of Asp-57 with Ser (non-Asp) in NOD mice (18) and with non-Asp in humans (19) may be the molecular anomaly responsible for the development of insulin-dependent diabetes.

Female C3H/HeN (H-2<sup>k</sup>) 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 (K<sup>d</sup>, I-A<sup>NOD</sup>, D<sup>b</sup>) mice. As controls, more than 50 C3H/HeN (H-2<sup>k</sup>) mice were lethally irradiated and then reconstituted with T-cell-depleted bone marrow cells of C3H/HeN, C57BL/6J (H-2<sup>b</sup>), or BALB/c (H-2<sup>d</sup>) mice. Even though these survived more than 1 year (survival rate, >90%), neither insulinitis nor overt diabetes developed. However, two of four [NOD  $\rightarrow$  C3H/HeN] chimeric mice developed both insulinitis and overt diabetes 40 weeks after BMT (Fig. 1a). These mice exhibited elevated glucose levels and abnormal glucose tolerance curves.

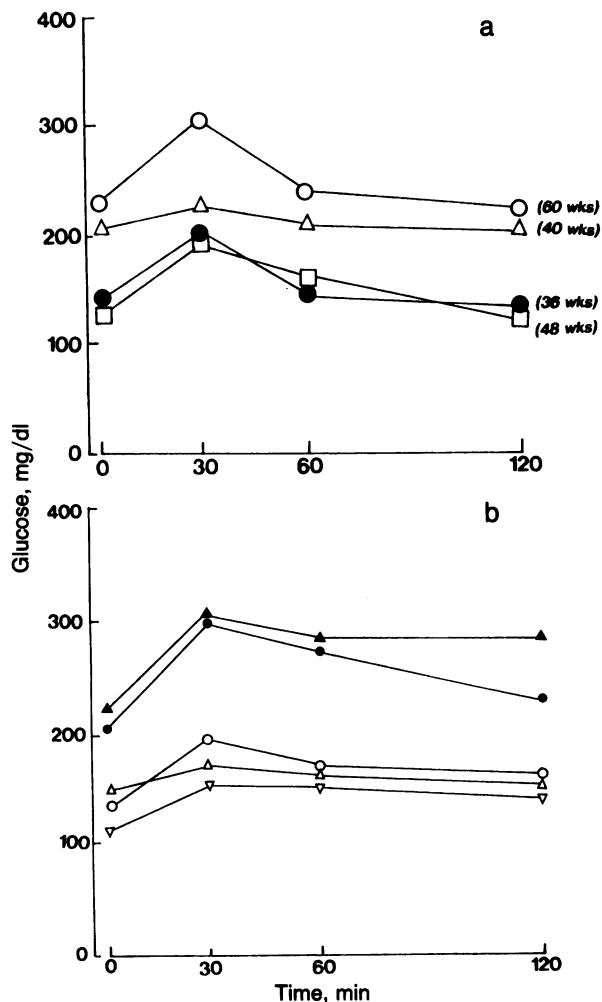


FIG. 1. Glucose tolerance tests. (a) [NOD  $\rightarrow$  C3H/HeN] mice 36 weeks (●), 40 weeks (Δ), 48 weeks (□), and 60 weeks (○) after BMT. (b) [NOD  $\rightarrow$  C3H/HeN] mice, 20 weeks after BMT, that had been treated with CsA (10 mg/kg of body weight) daily for 1 month.

CsA was found to accelerate the development of diabetes in such recipient mice, perhaps because CsA given to neonatal mice can inhibit and perturb T-cell functions and lead to the development of organ-specific autoimmune diseases (20). [NOD  $\rightarrow$  C3H/HeN] mice were treated 1 month after BMT with CsA (10 mg/kg of body weight) daily for 1 month. Two of five mice in this group developed insulinitis and overt diabetes as early as 20 weeks following BMT (Fig. 1b). In these two mice, the beta cells of the pancreas were specifically destroyed by lymphocytes that had infiltrated the pancreatic islets (Fig. 2), indicating that the insulinitis was not due to other influences of graft-versus-host reaction. Cytofluorometric analyses of spleen cells from [NOD  $\rightarrow$  C3H/HeN] mice revealed that hematolymphoid cells of the recipients had been replaced with donor (NOD)-derived cells (Fig. 3).

**Transfer of Idiopathic Thrombocytopenic Purpura and Lupus Nephritis into C3H/HeN or C57BL/6J Mice by Transplantation of Bone Marrow Cells from W/BF<sub>1</sub> 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<sub>1</sub> mouse, which develops lupus nephritis and myocardial infarction, is an impressive animal model of idiopathic thrombocytopenic purpura (11, 12), we used W/BF<sub>1</sub> (H-2<sup>z</sup>/H-2<sup>b</sup>) mice as donors and C3H/HeN (H-2<sup>k</sup>) or C57BL/6J (H-2<sup>b</sup>) 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/BF<sub>1</sub> mice. [W/BF<sub>1</sub>  $\rightarrow$  C57BL/6J] mice showed thrombocytopenia (<10<sup>5</sup> platelets

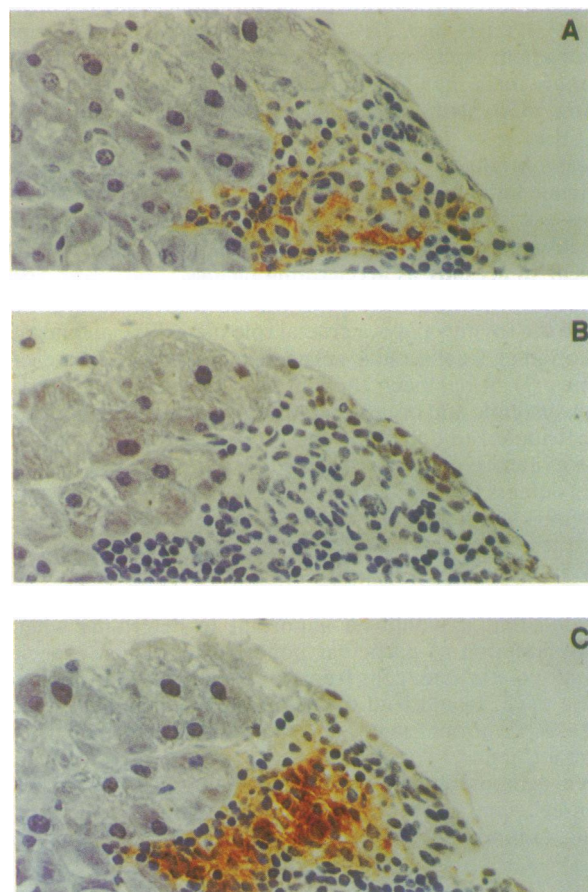


FIG. 2. Immunohistochemical findings in the islet of a [NOD  $\rightarrow$  C3H/HeN] mouse with overt diabetes, 40 weeks after BMT. (A) Glucagon-producing cells (alpha cells). (B) Insulin-producing cells (beta cells). (C) Somatostatin-producing cells (delta cells). Immunohistochemical examinations were carried out as described (8, 9). ( $\times 600$ .)

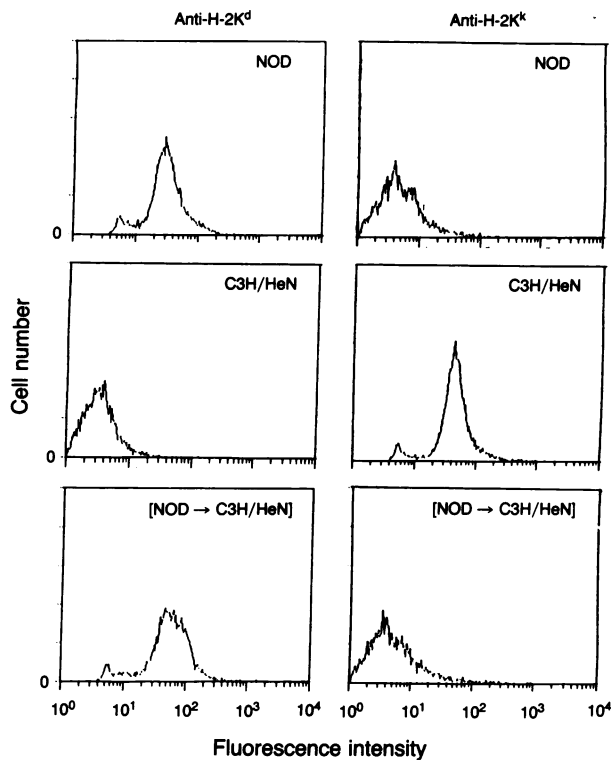


FIG. 3. Cytofluorometric analyses of spleen cells of a NOD mouse (Top), a C3H/HeN mouse (Middle), and a [NOD → C3H/HeN] mouse 60 weeks after BMT (Bottom). Cells were stained with anti-H-2K<sup>d</sup> (Left) or anti-H-2K<sup>k</sup> (Right) monoclonal antibody.

per mm<sup>3</sup>; normal mice, >10 × 10<sup>5</sup> in 5 of 11 mice (45%) studied 3 months after BMT, and in 5 more of the same 11 mice (total 10/11 or 91%) by 5 months after BMT. [W/BF<sub>1</sub> → C3H/HeN] mice also developed thrombocytopenia in 4 of 8 instances (50%) within 3 months after BMT and in 6 of 8 mice within 6 months after BMT.

Cytofluorometric analyses demonstrated the presence of both platelet-associated antibodies and circulating anti-platelet antibodies in the thrombocytopenic mice (Fig. 4). Immunohistopathological analyses revealed typical wire-loop lesions in the glomeruli of the [W/BF<sub>1</sub> → C57BL/6J] or [W/BF<sub>1</sub> → C3H/HeN] mice (Fig. 5).

To confirm that the defective stem cells were indeed the elements responsible for the development of the autoimmune diseases, we transferred W/BF<sub>1</sub> bone marrow cells from a stem-cell-enriched fraction (fraction II) to C3H/HeN mice, since both Visser *et al.* (21) and Miyama-Inaba *et al.* (13) have reported that, after T cells, B cells and macrophages are 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 (9.5 Gy) C3H/HeN mice that had been injected with W/BF<sub>1</sub> stem-cell-enriched bone marrow cells were also found to develop thrombocytopenia and lupus nephritis. We conclude from these experiments that the etiopathogenesis of both systemic and organ-specific autoimmune diseases can be attributed to abnormalities in the stem-cell population.

### DISCUSSION

It should be emphasized that the thymus and environmental factors such as sex hormones must act only as accelerating factors in the development of the autoimmune diseases. Further, although the thymus plays a crucial role in positive and negative selection of T cells and is engaged in deletion of

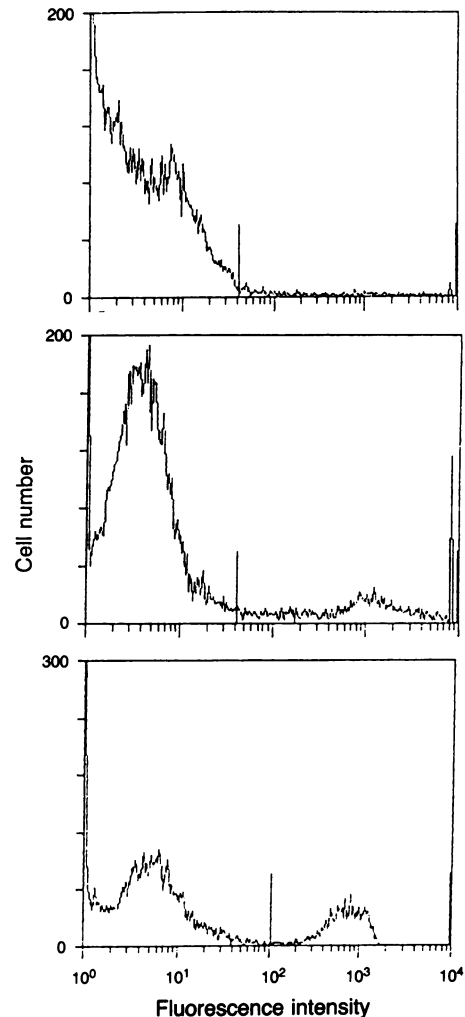


FIG. 4. Analyses of anti-platelet antibodies. (Top) Control, C3H/HeN platelets treated with BALB/c serum. (Middle) Platelet-associated antibodies of [W/BF<sub>1</sub> → C3H/HeN] mouse (3 months after BMT; platelet count, 23 × 10<sup>4</sup> per mm<sup>3</sup>). (Bottom) Circulating anti-platelet antibodies shown by treatment of C3H/HeN platelets with serum from [W/BF<sub>1</sub> → C3H/HeN] mouse (3 months after BMT). Analyses were carried out as described (11, 12).

autoreactive clones (1, 22–25), it is the stem cells (upon which the thymus acts) that contain the abnormalities that culminate

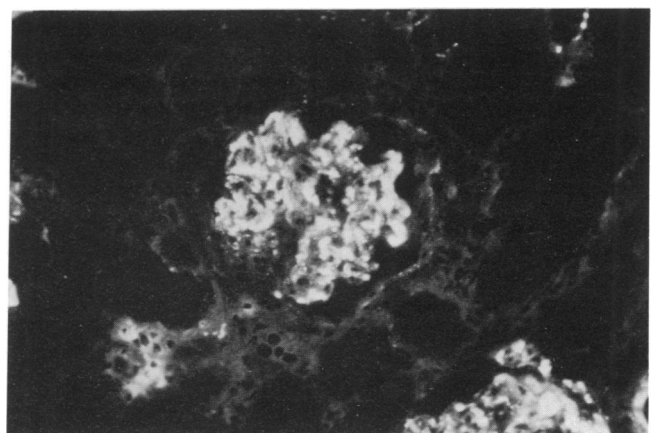


FIG. 5. Representative immunofluorescence micrograph of a glomerulus of a [W/BF<sub>1</sub> → C3H/HeN] mouse 5 months after BMT. Note the marked IgG deposits in both capillary and mesangial areas. (×320.)

in the expression of either organ-specific autoimmunities directed toward specific hematological cells or systemic autoimmune injuries resulting from immune complex-mediated autoimmunities. In addition, these findings suggest that genetically based susceptibility to most classes of autoimmune diseases, at least in mice, reside within the hematopoietic stem cells.

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