Brief Definitive Report

Focal Segmental Glomerular Sclerosis, a Type of Intractable Chronic Glomerulonephritis, Is a Stem Cell Disorder

By Masanori Nishimura,*‡ Junko Toki,* Kikuya Sugiura,* Futoshi Hashimoto,* Takeshi Tomita,§ Hiroshi Fujishima,‖ Yasuzo Hiramatsu,‖ Niro Nishioka,* Norikazu Nagata,* Yohichi Takahashi,‡ and Susumu Ikehara*

*From the *First Department of Pathology, Kansai Medical University, Moriguchi-City, Osaka 570; the ‡Osaka Red Cross Hospital, Tennouji-ku, Osaka 560; §Nagoya University, Shouwa-ku, Nagoya 466; and ‖Setsunan University, Hirakata-City, Osaka 573-01, Japan*

Summary

The etiopathogenesis of focal and segmental glomerular sclerosis (FGS) remains unknown. Using a new animal model for FGS (FGS mouse), we demonstrate here that bone marrow transplantation from normal mice to FGS mice with a high grade of proteinuria (+ + +) ameliorates FGS, and that the transplantation of bone marrow cells or purified hemopoietic stem cells (HSCs) from FGS mice induces FGS in normal mice. These findings strongly suggest that FGS is a stem cell disorder; the abnormalities may be genetically programmed at the level of HSCs.

Focal and segmental glomerular sclerosis (FGS) is a condition characterized by the presence of localized or segmental sclerosis in some glomerular tufts (1). FGS is resistant to the therapy of steroid hormones or immunosuppressive agents.

Animal models of FGS have been induced by chemicals or other artificial means (2–6). Spontaneous animal models for FGS have also been reported, but most are found in rats (7–9). A model mouse of FGS has recently been established from the F_5 offspring of the crossing between CBA/Nga and RFM/Nga mice (10). The mice show high levels of proteinuria after 40 d of age. The abnormality appears to be controlled by two pairs of autosomal recessive genes.

We have recently found that bone marrow transplantation (BMT) or BMT plus bone grafts (to recruit donor-derived stromal cells) can be used to prevent and treat both systemic and organ-specific autoimmune diseases in NOD, (NZB × NZW)F_1, MRL/lpr, BXSB, (NZW × BXSB)F_1, NZB/KN, and KK-Ay mice (11–18), and that the transplantation of a hemopoietic stem cell (HSC)-enriched population from autoimmune-prone mice to normal mice induces autoimmune diseases in normal mice (19). These findings prompted us to examine whether BMT could be used to treat FGS and/or whether the transplantation of a HSC-enriched population from FGS mice to normal mice could induce FGS in normal mice.

In this paper, we provide evidence that FGS, one type of intractable chronic glomerulonephritis, is a stem cell disorder.

Materials and Methods

Mice. FGS mice were obtained from Nagoya University or Setsunan University. BALB/c and C57BL/6 (B6) mice were purchased from CLEA Japan (Osaka). These mice were maintained under specific pathogen-free conditions in the animal facility at Kansai Medical University.

BMT. FGS (H-2^k), BALB/c (H-2^d), and B6 (H-2^b) mice were lethally irradiated from a ^{60}Co source (8.5 Gy for FGS and BALB/c mice, and 9.5 Gy for B6 mice). FGS mice were reconstituted with intravenous injection of 10^7 T cell–depleted bone marrow cells (TCD-BMCs) from BALB/c or B6 mice to treat the FGS. To induce FGS in normal mice, TCD-BMC (10^7) or an HSC-enriched population (10^6 Fr.2 cells or 10^5 wheat germ agglutinin [WGA]$^+$ cells) from young FGS mice (6–9 wk old) were transferred to irradiated B6 or BALB/c mice. The HSC-enriched population was prepared, as described below.

Preparation of HSC-enriched Population. To obtain an HSC-enriched population, BMCs that had been depleted of T cells, B cells, and macrophages were fractionated by centrifugation using Percoll discontinuous density gradients to remove granulocytes. HSCs were enriched in Fr.2, as previously described (20). The Fr.2 cells (lineage-negative [Lin$^-$] cells) were then stained using FITC-WGA (Polyscience Inc., Warrington, PA), and the WGA$^+$ cells were sorted using a FACStar® (Becton Dickinson & Co., Mountain View, CA).

Bone Grafts. Femurs and tibias, from which bone marrow cells had been flushed out, were grafted in the subcutis to recruit donor-derived stromal cells, as previously described (16, 17).

Cytofluorometric Analyses. H-2 typing was carried out using a FACScan® (Becton Dickinson & Co.), as previously described (19).

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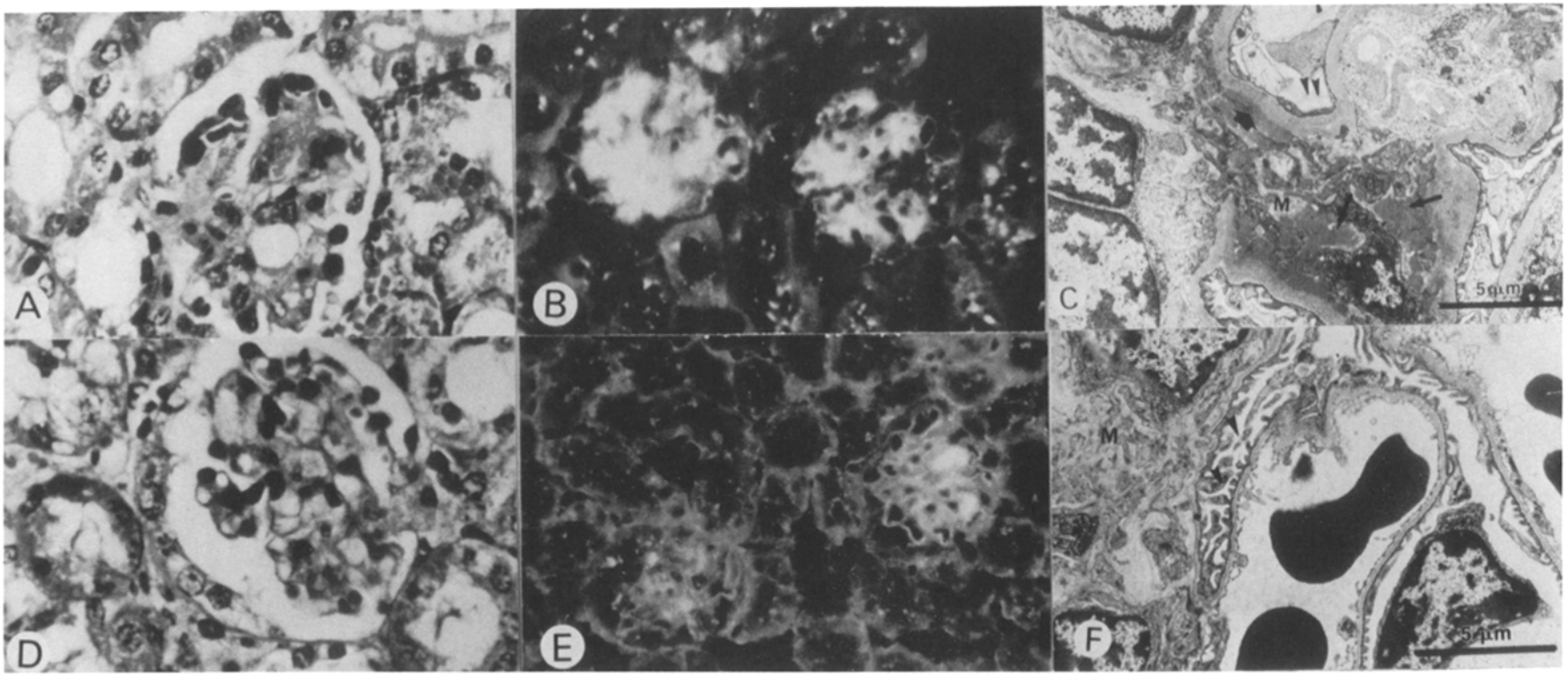

Figure 1. Histopathological findings in the kidney of a (BALB/c → FGS) chimeric mouse before and after BMT. (*A*) The glomerulus of a FGS mouse (3 mo old) before BMT shows solidification of one or more lobules of the tufts (×600). (*B*) IgG deposits are noted in the segmental sclerotic mesangial areas of two glomeruli in the mouse (×300). (*C*) Electron-dense materials are seen in the mesengial matrix (*thin arrows*) and the paramesangial subendothelium (*thick arrows*). Obliteration of a capillary lumen and the effacement of foot processes (*arrowhead*) are also noted. (*D*) The glomerulus of the FGS mouse (6 mo old) shows normal appearance 11 wk after BMT (×600). (*E*) IgG deposits are markedly reduced 11 wk after BMT. (*F*) Electron-dense materials disappear, and the interdigitating foot process (*arrowhead*) of the podocytes noted, although there is still some proliferation of the mesangial matrix (*M*) (×6,000).

Spleen cells suspended in PBS containing 2% FCS and 0.05% sodium azide were stained with FITC-conjugated mAbs against H-2D^b, H-2D^d, and H-2D^k. The mAbs were purchased from the Meiji Institute of Health Science (Odawara, Japan).

Histological Studies. The kidneys were obtained by biopsy or autopsy. Sections were stained with hematoxylin/eosin (H-E). For immunofluorescence (IF) studies, organs were frozen using OCT compound (Tissue-Tek; Miles Inc., Elkhart, IN). 3-μm sections were incubated at room temperature with FITC-conjugated rabbit anti-mouse IgG, IgM, IgA, or FITC-conjugated anti-mouse C3 (Medical and Biological Laboratories, Nagoya, Japan). The fluorescence intensity of the IgG, IgM, IgA, and C3 deposits in the mesangial areas was graded as: −, no visible deposits; ±, weak deposits; +, slight deposits; + +, moderate deposits; + + +, strong deposits.

Small blocks of the renal cortex were fixed in 2% glutaraldehyde and then in 1% osmic acid. After being embedded in Epon 812, ultra-thin sections were prepared for EM studies.

Autoantibodies. Anti-ssDNA antibodies and rheumatoid factors (RF) were measured by an ELISA. Immunoplates (Nunc, Roskilde, Denmark) were coated with heat-denatured calf thymus DNA (ssDNA) (Sigma Chemical Co., St. Louis, MO) at 50 μg/ml or goat IgG (Cappel Laboratories, West Chester, PA) at 10 μg/ml for 2–3 h at 37°C, and further coated with 2% egg albumin-PBS (EA-PBS) overnight at 4°C to block nonspecific binding. Mouse sera were diluted 1:35 in EA-PBS with 0.05% Tween 20 (EA-PBS-Tween). Diluted sera were added to wells and incubated for 1 h at room temperature. After washing in PBS-Tween, wells were incubated for 1 h with alkaline phosphatase–labeled Ab specific for mouse IgM or IgG (Cappel Laboratories). After the addition of substrate, OD was determined with an automated spectrophotometer, and Ab activity was expressed as mean OD ± SD. Immune complexes in the sera were measured by a C1q-binding assay using ELISA. Immunoplates were coated with human purified C1q (Sanko Junyaku Co., Ltd., Tokyo, Japan) at 10 μg/ml, and postcoated with EA-PBS. Sera (5 μl) were incubated with 25 μl 0.2 M EDTA for 30 min at 37°C, and 200 μl EA-PBS-Tween was added. Samples including aggregated mouse IgG as standards were added to C1q-coated plates, and the bound IgG was measured by alkaline phosphatase–labeled anti-mouse IgG. Results were expressed as micrograms per milliliter equivalent to aggregated mouse IgG.

Each experiment included more than five mice. All experiments were repeated more than three times. Reproducible results were obtained, and representative data are therefore shown in the figures.

Results and Discussion

We selected 3–5-mo-old FGS mice with severe proteinuria (+ + +). Before BMT, we performed renal biopsy. Light microscopic (LM) studies showed perivascular infiltration of lymphocytes and plasma cells. The glomeruli exhibited solidification of one or more lobules of the tufts (Fig. 1 *A*). IF studies revealed granular deposits of IgG (+ + +), IgM (+ +), IgA (+), and C3 (±) in the mesangial areas segmentally (Fig. 1 *B*). In humans, IgM, usually in combination with C3, is commonly demonstrated in the segmental sclerotic areas, although IgG has also been recorded in segmental sclerotic areas (1, 21). EM studies demonstrated electron-dense materials in the mesangial matrix and paramesangial subendothelium (Fig. 1 *C*). In addition, obliteration of capillary lumens and the effacement of foot processes were noted.

The FGS (H-2^k) mice with proteinuria (+ + +) were lethally (8.5 Gy) irradiated and then reconstituted with TCD-BMCs of BALB/c (H-2^d) mice with bone grafts (to recruit stromal cells), since we know that donor-derived stromal cells are necessary for reconstitution, particularly when the mice are radiosensitive, as previously reported in MRL/lpr and NZB/KN mice (16, 17). Proteinuria began to decrease 6 wk after the transplants and became almost undetectable after 11 wk. We killed these mice and carried out LM, IF, and EM studies. The glomeruli showed normal appearance in LM studies (Fig. 1 *D*). IF studies revealed markedly reduced deposits of immunoglobulins such as IgG and IgM (Fig. 1 *E*). Electron-dense materials disappeared, and the interdigitating foot processes of the podocytes were noted, although there was still some proliferation of the mesangial matrix at this stage (Fig. 1 *F*). Chimerism was evaluated using a FACScan®. Spleen cells of FGS (H-2^k) mice reconstituted with BALB/c (H-2^d) BMCs were H-2^d positive (Fig. 2). Thus, we succeeded in treating FGS by BMT.

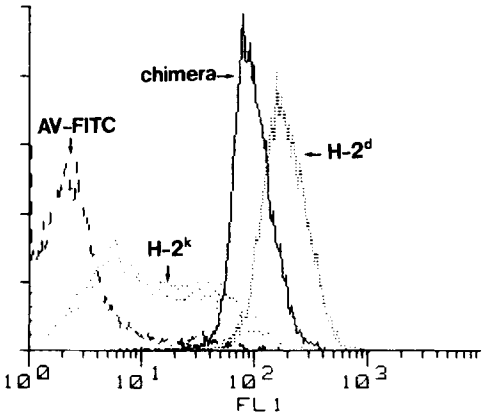

Figure 2. H-2 typing in a (BALB/c → FGS) mouse. Spleen cells in a (BALB/c → FGS) mouse are H-2^d positive.

The next step was to examine whether FGS could be in-

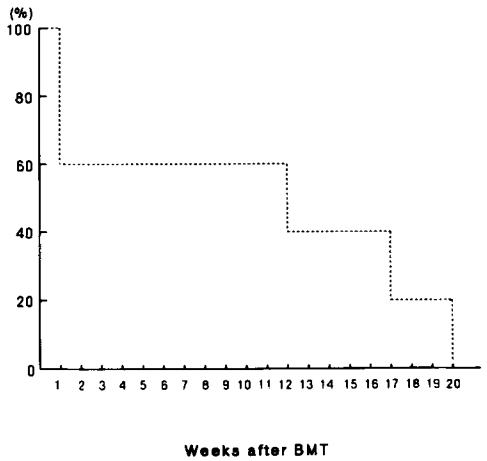

Figure 3. The fate of (FGS → B6) chimeric mice. (FGS → B6) mice begin to show proteinuria (+ +) from 7 wk after BMT and show severe proteniuria (+ + +) by 17 wk after BMT, followed by death from renal failure by 20 wk.

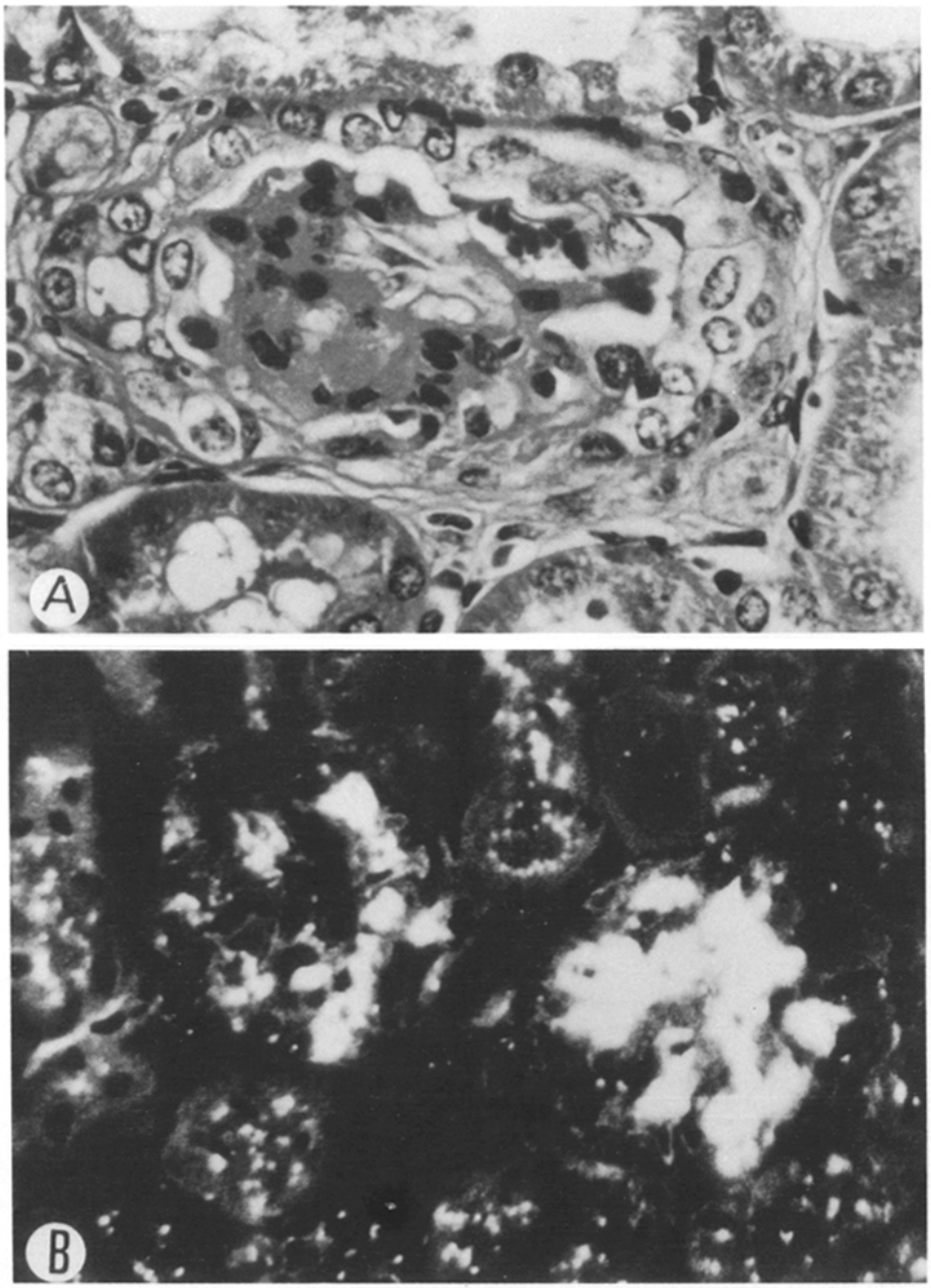

Figure 4. Histopathological findings in the kidney of a (FGS → B6) chimeric mouse. (*A*) Glomerulosclerosis is seen in a kidney of the (FGS → B6) mouse 17 wk after BMT (×600). (*B*) IgG deposits are noted in two glomeruli of the mouse (×300).

duced in normal mice by transplantation of BMCs from FGS mice. B6 mice were lethally irradiated (9.5 Gy) and reconstituted with 10^7 TCD-BMCs of FGS mice. The (FGS → B6) mice began to show proteinuria (++) from 7 wk after BMT, and showed severe proteinuria (+++) by 17 wk after BMT, followed by death from renal failure by 20 wk (Fig. 3). Fig. 4 shows the histopathological findings in the (FGS → B6) mice. Swelling of glomeruli was observed 9 wk after BMT (data not shown), and glomerulosclerosis developed 17 wk after BMT (Fig. 4 *A*). IgG deposits were found in the glomeruli (Fig. 4 *B*), and electron-dense materials were found in the basement membranes and also mesangial matrix 17 wk after BMT (data not shown). To provide evidence that FGS is, indeed, a stem cell disorder, we purified the HSCs of FGS mice. FGS developed also in the B6 mice reconstituted with an HSC-enriched population (10^6 Fr.2 cells or 10^5 WGA^+ cells) of the FGS mice.

Based on these findings, we conclude that FGS is a stem cell disorder. Since we have shown that both systemic and organ-specific autoimmune diseases are stem cell disorders (18), we propose that FGS is an organ (kidney)-specific autoimmune disease. However, it remains to be resolved which au-

toantibodies are involved in the development of FGS. We have measured anti-DNA antibodies and circulating immune complexes in the sera of FGS mice, but have been unable to detect any significant difference between normal and FGS mice (data not shown).

A particular form of FGS recently has been described in patients with AIDS (22–24). It is well known that patients with AIDS develop autoimmune diseases. It is therefore conceivable that exogenous or endogenous viruses are involved in the development of FGS. We are in the process of elucidating the abnormalities of HSCs in FGS at the molecular level.

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Address correspondence to Dr. Susumu Ikehara, First Department of Pathology, Kansai Medical University, 1 Fumizono-cho, Moriguchi-City, Osaka 570, Japan.

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