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

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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.

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-2k), BALB/c (H-2a), and B6 (H-2s) mice were lethally irradiated from a 60Co 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 10⁷ 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 (10⁷) or an HSC-enriched population (10⁶ Fc.2 cells or 10⁵ 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 (Poly-science 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).

Cytfluorometric Analyses. H-2 typing was carried out using a FACScan® (Becton Dickinson & Co.), as previously described (19).
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 (x600). (B) IgG deposits are noted in the segmental sclerotic mesangial areas of two glomeruli in the mouse (x300). (C) Electron-dense materials are seen in the mesangial 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 (x600). (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) (x6,000).
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 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 Clq-binding assay using ELISA. Immunoplates were coated with human purified Clq (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 Clq-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\(^b\)) 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\(^b\)) mice reconstituted with BALB/c (H-2\(^d\)) BMCs were H-2\(^d\) positive (Fig. 2).

Thus, we succeeded in treating FGS by BMT.

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

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).
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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