Prevention of Crescentic Glomerulonephritis in SCG/Kj Mice by Bone Marrow Transplantation (44290)

CHERRY, R. W. ENGELMAN, B. Y. WANG, K. KINJOH, N. S. EL-BADRI, R. A. GOOD[1]
Department of Pediatrics, All Children's Hospital, University of South Florida, St. Petersburg, Florida 33701

Abstract. **Transplantation of MHC-compatible, T-cell-depleted, bone marrow cells has successfully treated autoimmunities, immunodeficiencies, malignancies, and developmental deficiencies of the hematopoietic system. Recombinant inbred SCG/Kj mice develop spontaneous crescentic glomerulonephritis, systemic vasculitis, and a lymphoproliferative disorder early in life. To determine whether the precipitous autoimmune disease of SCG/Kj mice could be treated by bone marrow transplantation, 30 SCG/Kj mice were engrafted with T-cell-depleted, bone marrow (TCDM) from allogeneic, MHC-compatible, autoimmune-resistant C3H/He donors, and 30 SCG/Kj mice served as controls and received TCDM from syngeneic, SCG/Kj donors. A significant survival advantage was evident from SCG/Kj mice engrafted with C3H/He TCDM ($p < 0.005$), and an 89% extension of median survival compared to recipients of SCG/Kj TCDM. Within 28 weeks post-transplantation, 62% of mice engrafted with SCG/Kj TCDM had died with clinical signs of fatal crescentic glomerulonephritis. This result compared with only 10% of mice engrafted with C3H/He TCDM. Mice engrafted with SCG/Kj TCDM developed significantly greater titers of autoantibodies to ss-DNA, ds-DNA, and myeloperoxidase (ANCA) ($p < 0.001$), had shorter latencies to the development of, and a greater incidence of proteinuria, hematuria, and peripheral lymphadenopathy, and a greater mean grade of glomerular lesion ($p < 0.001$), than mice engrafted with C3H/He TCDM. These findings indicate that the genetic defect of the SCG/Kj strain of mice resides within the hematopoietic stem cells and provokes the speculation that bone marrow transplantation might be a useful means of treating progressive crescentic glomerulonephritis in humans.** [P.S.E.B.M. 1998, Vol 218]

Rapidly progressive crescentic glomerulonephritis is a clinical and histopathological entity often linked to forms of systemic vasculitis, such as Wegener's granulomatosis and polyarteritis nodosa (1–3). The characteristic glomerular lesion consists of the accumulation of multiple layers of fusiform cells within Bowman's space forming a distinct crescent of cells. Although the clinical presentation of crescentic glomerulonephritis can be insidious, and often consists only of hematuria, the fulminating nature and poor prognosis of the glomerular lesions makes this disease most intractable to therapy (1–3). Anticoagulation therapy, steroids, immunosuppressive drugs, and combinations of these treatments with plasma exchange therapy may be of some benefit, but most patients exhibit progressive renal failure, and prognosis remains dismal (4).

The recombinant inbred SCG/Kj strain of mice serves as a model of rapidly progressive crescentic glomerulonephritis, and was recently established by selectively mating siblings of (BXSB × MRL/Mp *lpr/lpr*)F_1 mice that had developed crescentic glomerulonephritis, and then inbreeding the progeny for over 40 generations (5). Weanling 4–6-week-old SCG/Kj mice spontaneously develop rapidly progressive glomerulonephritis, necrotizing vasculitis, and massive lymphoproliferation, which leads to premature death, and 50% mortality when mice are only 16 weeks of age.

Renal lesions include fine granular immune deposits along the glomerular basement membrane, hypertrophy of

[1] To whom requests for reprints should be addressed at Department of Pediatrics, All Children's Hospital, 801 Sixth Street South, St. Petersburg, FL 33701. E-mail: goodr@allkids.org
Aided by grants from USPHS-NIH Institute on Aging #AGO5628-13, the Pediatric Cancer Foundation to Children's Research Institute, and the Eleanor Naylor Dana Charitable Trust.

Received July 16, 1997. [P.S.E.B.M. 1998, Vol 218]
Accepted February 5, 1998.

0037-9727/98/2183-0223$10.50/0

the glomerular epithelium, fibrin depositions, and extra glomerular cellular proliferations and hemorrhage within Bowman's space (5). Marked extra glomerular cellular hyperplasia leads to the accumulation of two or more distinct layers of cells within Bowman's space, forming a characteristic crescent of cells.

Although the pathogenesis of autoimmunity in SCG/Kj mice is not fully understood, there is a positive association between the severity of glomerular lesions, the degree of systemic vasculitis, and the circulating autoantibody titer of antineutrophilic cytoplasmic antibody (ANCA), which binds to myeloperoxidase in a peripheral pattern (5). Increased titers of anti-ss-DNA and anti-ds-DNA autoantibodies, and a polyclonal hypergammaglobulinemia also develop. Lymphoproliferation in SCG/Kj mice is attributable to the accumulation of Thy-1^+, Ly-1^+, $CD4^-$, $CD8^-$, and $B220^+$ T cells.

Transplantation of MHC-compatible, T-cell-depleted, bone marrow cells has successfully treated patients with immunodeficiency (6), aplastic anemia (7), leukemia (8), and a wide spectrum of genetically determined diseases (9, 10). In the experimental setting, systemic and organ-specific autoimmune diseases have been treated and frequently cured by bone marrow transplantation (11, 12). The dramatic action of bone marrow transplantation to treat these diseases suggests that they develop as a consequence of genetic defects residing within the hematopoietic stem cells (11, 12).

In this study, we describe how by engrafting SCG/Kj mice with T-cell-depleted bone marrow, the progression to severe renal clinical pathology in SCG/Kj mice is abrogated. That development of this precipitous autoimmune disease, which expresses itself in nursling and weanling mice, can be interrupted by the transplantation of bone marrow cells, indicates that the genetic origin of this disease resides in the hematopoietic stem cells.

Material and Methods

Animals. Mice of the SCG/Kj strain were derived by inbreeding progeny of (BXSB × MRL/Mp *lpr/lpr*)F_1 mice, as previously described (5). Sixty female SCG/Kj mice were divided into two experimental groups and served as recipients of grafts. One group of thirty mice were engrafted with T-cell-depleted bone marrow cells from MHC-compatible male C3H/He donors. The other thirty mice were engrafted with syngeneic T-cell-depleted bone marrow cells from male SCG/Kj donors. Twenty additional SCG/Kj mice were untreated, and evaluated for the development of renal clinical and anatomical pathology. These studies using animals were conducted in AAALAC-accredited facilities, in compliance with the principles of the *Guide for the Care and Use of Laboratory Animals.*

Bone Marrow Transplantation. Bone marrow cells were harvested from the femurs and tibias of donors and depleted of T cells by complement-dependent cytotoxicity using purified anti-Thy 1.2 monoclonal antibody (Pharmigen, San Diego, CA) and rabbit complement (Cedarlane, Ontario). Six-week-old SCG/Kj mice served as recipients of marrow grafts and were exposed to 950 rads of total body irradiation (^{137}Cs irradiation, 75 rads/min). T-cell-depleted bone marrow cells (2×10^7) from either SCG/Kj or C3H/He male donors were injected intravenously into female SCG/Kj recipients one day after lethal irradiation.

Chimeric Analysis. For chimeric analysis, genomic DNA from splenocytes and circulating leukocytes was extracted and purified (Micro-Turbo-Gen, Invitrogen, San Diego, CA) and analyzed for the percentage of male DNA using PCR amplification and a male-specific pY2 primer. The percentage of male DNA was determined by plotting the optical density of the sample on a curve drawn using optical density readings of known standards.

Renal Clinical Pathology. Proteinuria was detected using tetra-bromphenol paper, and hematuria was detected by hemoglobin-catalyzing reaction of di-iso-propyl benzene di-hydro-peroxide and 3,3′,5,5′-tetra-methyl benzidine (Labstix, Miles, Elkhart, IN). Proteinuria was graded 1^+ to 4^+. Grades of 2^+ indicated 100 mg/dl of urinary protein. Grades 2^+ or greater were considered indicative of heavy proteinuria.

Glomerular Histopathology. Tissues were fixed in 10% formalin and stained with hematoxylin and eosin (H&E), or Periodic Acid Schiff (PAS). Lesions of crescentic glomerulonephritis were graded using the World Health Organization's (WHO) classification of glomerular disease (13). Grade I consisted of mild fibrin deposits and increased cellularity. Grade II consisted of mild hypertrophy of the glomerular lining epithelium, mild to moderate fibrin deposition, and increased glomerular cellularity. Grade III consisted of moderate to severe hypertrophy of glomerular lining epithelium, fibrin deposition, and glomerular hypercellularity. Grade IV consisted of marked glomerular hypercellularity with more than two layers of fusiform cells within Bowman's space forming a distinct crescent of cells, and an associated lymphocytic infiltration. Twenty glomeruli were graded according to this classification and used to calculate the mean glomerular histopathology score for each mouse. Those scores were used to calculate mean scores for each dietary cohort.

Autoantibody Titers. Autoantibodies to ds-DNA, ss-DNA, and ANCA (antimyeloperoxidase) were quantified using an enzyme-linked immunosorbent assay (ELISA) as described elsewhere (14).

Flow Cytometry. Suspensions of spleen and lymph node cells from five mice of each experimental cohort were incubated with phycoerythrin-conjugated or fluorescein-conjugated anti-Thy-1.2, anti-CD3, anti-CD8, anti-CD4, anti-L3T4, anti-Gr1, anti-Mac1, or anti-B220 (RA3-6b2) antibodies (Pharmingen, San Diego), and subpopulations of cells were quantified using flow cytometric analysis.

Statistical Analysis. A log-rank mantel-Haenszel test was used to compare the survival of each experimental cohort and differences in the rate of development of pro-

teinuria and hematuria. Student's t tests were used to compare mean glomerular histopathology scores.

Results

Chimeric Analysis. Syngeneic or allogeneic TCDM was transplanted from male SCG/Kj or C3H/He donor mice, respectively, to recipient female SCG/Kj mice. As expected, chimeric analysis of mice showed that approximately 87% of the splenic and circulating leukocytic cellular DNA in SCG/Kj recipients of C3H/He donor cells, and 92% of the splenic and circulating leukocytic cellular DNA in SCG/Kj recipients of SCG/Kj donor cells, amplified using the pY2 primer and indicated successful marrow engraftments.

Survival Analysis. Within 28 weeks post-transplantation, 62% of the SCG/Kj recipients of TCDM from autoimmune-prone SCG/Kj donor mice had died with clinical signs of renal dysfunction and histological findings of crescentic glomerulonephritis (Fig. 1). In contrast, only 10% of the SCG/Kj recipients of TCDM from autoimmune-resistant C3H/He donor mice had died with evidence of renal disease at this interval. Median survival age of recipients of SCG/Kj TCDM was 27 weeks, whereas that of mice engrafted with C3H/He TCDM was greater than 50 weeks of age, at which point the study was terminated. Engraftment of autoimmune-prone SCG/Kj mice with autoimmune-resistant C3H/He TCDM resulted in more than a 89% extension in median survival of the recipients. Survival analysis using a log-rank Mantel-Haenszel test found a substantial survival advantage for recipients of C3H/He TCDM in comparison to those receiving SCG/Kj TCDM ($p < 0.005$). It is noteworthy that, at the termination of the study, the remaining 50% of mice that had received grafts of C3H/He TCDM, remained free of substantive renal clinical and anatomical pathology (see below).

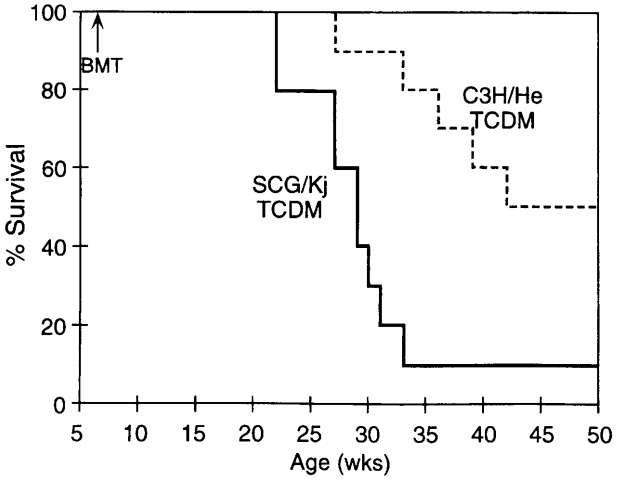

Figure 1. Survival curves of female SCG/Kj mice, exposed to 950 rads of total body irradiation (^{137}Cs irradiation, 75 rads/min), and intravenously administered 2×10^7 T-cell-depleted bone marrow cells from either SCG/Kj ($n = 30$, dotted line, closed circles), or C3H/He ($n = 30$, closed line, open circles), male donors, when recipients were 6 weeks old (BMT indicates bone marrow transplantation).

Renal pathology. Clinical onset of renal pathology as evidenced by heavy proteinuria (>100 mg/dl) and hematuria occurred early in mice transplanted with SCG/Kj TCDM. Within 10 weeks post-transplantation, 50% of these mice had developed both hematuria and heavy proteinuria (>100 mg/dl), and by 20 weeks post-transplantation all mice of this group had exhibited these signs of renal dysfunction.

In contrast, by 20 weeks post-transplantation only 12% of recipient mice of autoimmune-resistant C3H/He TCDM had developed heavy proteinuria, and 10% had developed hematuria. Further, 50% of the recipients of C3H/He TCDM remained free of any signs of renal clinical pathology when the study was terminated, 52 weeks post-transplantation. This abrogation in the onset of clinical renal pathology among mice engrafted with C3H/He TCDM was reflected in lower mean scores of glomerular lesions among these mice compared to mice engrafted with SCG/Kj TCDM (see below).

Autoantibodies. Serum levels of anti-ss-DNA, anti-ds-DNA, and antimyeloperoxidase (ANCA) autoantibodies were determined for mice from each group of recipients of either SCG/Kj or C3H/He TCDM when mice were 12, 16, and 20 weeks of age. Serum from either 20-week-old C3H/He or untreated SCG/Kj mice served as controls. Mice engrafted with autoimmune-prone SCG/Kj TCDM showed an increase in all three of the autoantibody titers with age, and titers of autoantibodies were statistically greater than in mice engrafted with C3H/He TCDM when mice were 16 and 20 weeks of age ($p < 0.001$) (Fig. 2). When SCG/Kj recipients of SCG/Kj TCDM were 20 weeks of age, titers of anti-ss-DNA, anti-ds-DNA, and anti-myeloperoxidase (ANCA) autoantibodies approached levels measured in SCG/Kj untreated controls.

Histopathology. Twenty glomeruli within each kidney of untreated SCG/Kj mice, or SCG/Kj recipients of either SCG/Kj TCDM, or C3H/He TCDM were graded according to the WHO classification of glomerular disease. Mean glomerular lesion scores for each mouse were determined, and mean glomerular lesion scores for each experimental cohort were calculated and compared (Fig. 3). Glomerular lesions of mice engrafted with C3H/He TCDM were significantly less severe compared to those of recipients of SCG/Kj TCDM ($p < 0.001$), or SCG/Kj untreated controls ($p < 0.001$). Mean glomerular lesion scores of SCG/Kj control mice and those of the recipients of SCG/Kj TCDM were comparable. All of the surviving SCG/Kj mice that were engrafted with C3H/He TCDM and that lacked clinical signs of renal disease at the termination of the study when mice were 52 weeks of age (50% of the original 30 mice) had either no significant glomerular lesions or only mild fibrin deposition within some glomeruli. In contrast, recipients of SCG/Kj TCDM had moderate to severe hypertrophy of glomerular lining epithelium, fibrin deposition, and glomerular hypercellularity, some with a distinct crescent of cells within Bowman's space (Grade III–IV lesions).

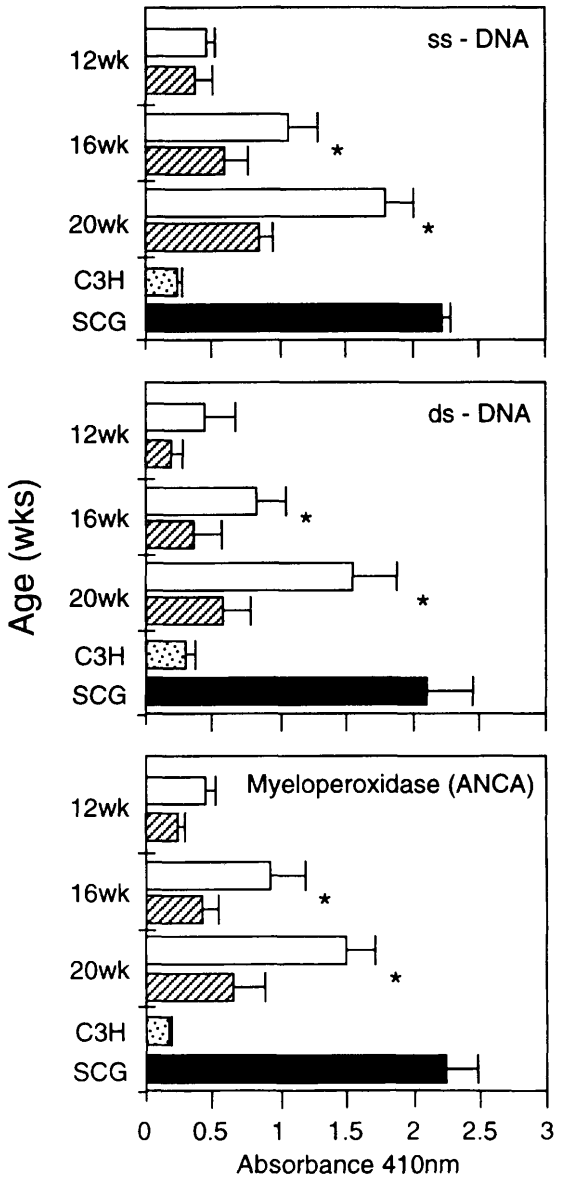

Figure 2. Serum levels of anti-ss-DNA, anti-ds-DNA, and antimyeloperoxidase (ANCA) autoantibodies of TCDM recipient SCG/Kj female mice when 12, 16, and 20 weeks of age. Solid bar indicates SCG/Kj donor TCDM. Striped bar indicates C3H/He donor TCDM. *indicates a significant difference between age-matched experimental groups, where $p < 0.001$. Serum of 20-week-old C3H/He, or untreated SCG/Kj mice, served as references.

Lymphoid Hyperplasia. Lymphoid hyperplasia as evidenced by a peripheral leukocytosis and an increase in splenic and lymph node weights was apparent only among SCG/Kj mice engrafted with SCG/Kj TCDM and the SCG/Kj untreated controls. Lymphoid organ weights, organ-to-body weight ratios, and circulating leukocyte counts of these mice were grater than those of SCG/Kj mice engrafted with C3H/He TCDM ($p < 0.001$). Splenic and lymph node leukocytes were evaluated using flow cytometric analysis. A preponderance of CD4/CD8-double-negative, Thy 1.2-positive, B220-positive leukocytes was present in the lymphoid organs and peripheral blood of SCG/Kj recipients of SCG/Kj TCDM, and even more prevalent in SCG/Kj untreated controls, compared to SCG/Kj recipients of C3H/He TCDM ($p < 0.001$).

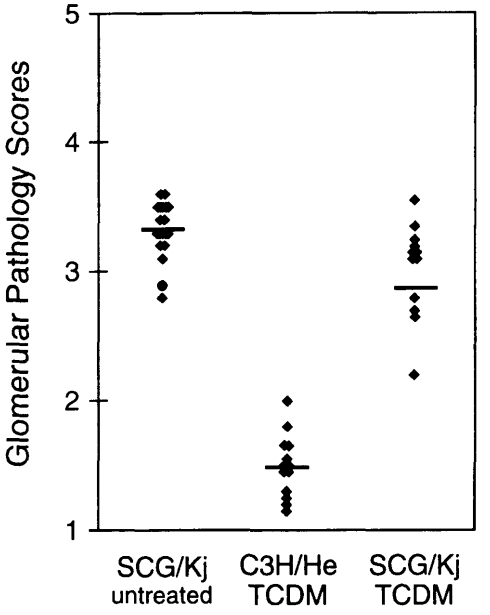

Figure 3. Glomerular lesion scores of untreated SCG/Kj mice ($n = 20$), or SCG/Kj recipient mice of either C3H/He TCDM ($n = 14$), or SCG/Kj TCDM ($n = 14$), graded according to the WHO classification of glomerular disease. Overall mean glomerular lesion scores for each experimental cohort are indicated by horizontal bars. *indicates a significant difference between groups, where $p < 0.001$.

Discussion

Transplantation of autoimmune-resistant C3H/He TCDM to SCG/Kj recipients abrogated the development of renal clinical pathology in 50% of the recipients, significantly reduced the proportion of CD4-negative, CD8-negative, Thy 1.2^+, $B220^+$ T cells present in lymphoid organs and the peripheral blood, prevented lymphoproliferation, blocked the production of autoantibodies, prevented the development of substantive renal histopathological lesions in 50% of the recipients, and extended median survival by more than 89% compared to recipients of SCG/Kj TCDM. These findings suggest that the molecular genetic lesions responsible for this immunologically based disease reside in the hematopoietic stem cell compartment.

Untreated SCG/Kj mice exhibited a more precipitous pathogenesis, a shorter latency to renal clinical pathology, a more rapid rise in autoantibody titers, and a 10-week earlier median mortality than did SCG/Kj recipients of SCG/Kj TCDM. This delay in the full development of autoimmune disease in SCG/Kj recipients of TCDM may be attributed to the effects of total body irradiation and TCDM engraftment. Neonatal SCG/Kj mice do not develop detectable autoantibodies until approximately 4 weeks of age (5). Total body irradiation alone may eliminate autoimmune leukocytic clones, and it has been used to prevent the systemic autoimmunity of one of the founder strains (MRL/Mp *lpr/lpr*) of the original parental hybrid of this recombinant inbred strain (15). In addition, in the present study, T cells were purged

by complement fixation from the bone marrow graft. Consequently, a delay in the full expression of autoimmunity in the SCG/Kj recipients of SCG/Kj TCDM compared to SCG/Kj untreated controls may be attributed to the time required for redevelopment of autoimmune clones of leukocytes in the engrafted marrow. Regardless, full expression of autoimmunity did occur in the recipients of SCG/Kj TCDM.

Reinforcement of bone marrow transplantation with co-transplantation of either bone or stromal cells has been beneficial in the treatment of experimental autoimmune diseases (16). Stromal or bone co-grafts appear to produce chemokines, cytokines, and growth factors that facilitate the establishment of the transplanted hematopoietic cells, and result in elimination of the autoimmune disease (17, 18). Conversely, co-engraftment of SCG/Kj mice with both SCG/Kj stromal or bone cells and TCDM may reaccelerate the pathogenesis of the engrafted mice so that it approaches that of untreated SCG/Kj controls.

The reciprocal transplantation of autoimmune-prone SCG/Kj TCDM to C3H/He recipients was attempted (data not shown), however, recipients died acutely or developed chronic graft-versus-host disease, findings which are similar to those observed when bone marrow or spleen cells of autoimmune-prone MRL/Mp *lpr/lpr* mice are transplanted to other autoimmune-resistant strains (19–21).

The presence of antineutrophilic cytoplasmic autoantibody (ANCA) has been demonstrated in humans in a variety of systemic vasculitides, such as Wegener's granulomatosis, classic polyarteritis nodosa, the Churg Strauss syndrome or Takayasu's arteritis, or in other diseases such as ulcerative colitis, Crohn's disease, and primary sclerosing cholangitis, and has been associated with crescentic glomerulonephritis especially that which develops in autoimmune disease (22–27). The underlying mechanism of the influence of ANCA on the pathogenesis of these varied diseases has not been clarified. ANCA reacts specifically with the constituents of neutrophil azurophilic granules and monocyte lysosomes (28). The two patterns of ANCA binding to neutrophils and monocytes are either a cytoplasmic pattern (designated c-ANCA), or a perinuclear pattern (p-ANCA). The c-ANCA reacts with the azurophilic granule constituent proteinase-3, and has been associated with diseases of the respiratory tract, whereas p-ANCA reacts with myeloperoxidase and has been associated with certain renal diseases, especially those resulting in the histopathologic presence of crescentic glomerulonephritis (28).

Hematopoietic stem cells derived from the bone marrow, peripheral lymphoid organs, circulating blood, or umbilical cord blood have been transplanted as a means to treat a number of genetic and acquired diseases (29, 30). Future studies using hematopoietic stem cell transplantation from each of these compartments would permit further definition of the cellular origin of the autoimmunity of SCG/Kj mice, and in other experimental models would test the efficacy of stem cell transplants in the treatment of other autoimmunities, immunodeficiencies, and malignancies.

We thank C. Thompson for assistance with the care, feeding, and monitoring of mice, H. Brown, Ph.D., Department of Epidemiology and Biostatistics, for assistance with statistical analyses, and Ms. Tazim Verjee for assistance with the preparation of this manuscript.

1. Glassock RJ. A clinical and immunopathological dissection of rapidly progressive glomerulonephritis. Nephron **22:**253–264, 1978.
2. Couser WG. Rapidly progressive glomerulonephritis: Classification, pathogenetic mechanisms, and therapy. Am J Kidney Dis **11:**449–464, 1988.
3. Kincaid-Smith P, Whitworth JA. Clinical and morphological features in rapidly progressive glomerulonephritis. In: The Kidney, A Clinico-Pathological Study. Cambridge, MA: Blackwell Scientific, p105, 1987.
4. Beryne GM, Baker SB. Acute anuric glomerulonephritis. QJM **33:**105–115, 1964.
5. Kinjoh K, Kyogoku M, Good RA. Genetic selection for crescent formation yields mouse strain with rapidly progressive glomerulonephritis and small vessel vasculitis. Proc Natl Acad Sci USA **90:**3413–3417, 1993.
6. Gatti RA, Meuwissen HF, Allen HD, Hong R, Good RA. Immunological reconstitution of sex-linked lymphopenic immunological deficiency. Lancet **2:**1366–1369, 1968.
7. Good RA, Gatti RA, Hong R, Meuwissen HF. Successful marrow transplantation for correction of immunological deficit in lymphopenic agammaglobulinemia and treatment of immunologically induced pancytopenia. Exp Hematol **19:**4–10, 1969.
8. Thomas ED, Buckner CD, Banaji M. One hundred patients with acute leukemia treated by chemotherapy, total body irradiation, and allogeneic marrow transplantation. Blood **49:**511–533, 1977.
9. Good RA. Organization and development of the immune system: Relation to its reconstruction. In: Bone Marrow Transplantation: Foundation for the 21st Century. Ann NY Acad Sci **770:**8–33, 1995.
10. Good RA, Kapoor N, Reisner Y. Bone marrow transplantation, an expanding approach to treatment of many diseases. Cell Immunol **82:**36–54, 1983.
11. Nakamura T, Ikehara S, Good RA, Inoue S, Sekita A, Furukawa F, Tanaka H, Maung O, Hamashima Y. Abnormal stem cells in autoimmune-prone mice are responsible for premature thymic involution. Thymus **7:**151–157, 1985.
12. Ikehara S, Kawamura M, Takao F, Inaba M, Yasumizu R, Than S, Hisha H, Sugiura K, Koide Y, Yoshida TO, Ida T, Imura H, Good RA. Organ-specific and systemic autoimmune diseases originate from defects in hematopoietic stem cells. Proc Natl Acad Sci USA **87:**8341–8344, 1990.
13. Churg J, Sobin LH. Renal Disease: Classification and Atlas of Glomerular Disease. Tokyo: Igaku-Shoin, 1982.
14. Sasaki T, Muryoi T, Sekiguchi Y, Tamate E, Yoshinaga K, Kitagawa Y. Monoclonal human anti-DNA antibodies from EB virus-transformed lymphocytes of systemic lupus erythematosus (SLE) patients. J Clin Immunol **5:**246–253, 1985.
15. Theofilopoulos AN, Balderas R, Shawler DL, Izui S, Kotzin BL, Strober S, Dixon FJ. Inhibition of T-cell proliferation and SLE-like syndrome of MRL/lpr mice by whole body or total lymphoid irradiation. J Immunol **125:**2137–2142, 1980.
16. Ikehara S, Inaba M, Ishida T, Ogata H, Hisha H, Yasumizu R, Oyaizu N, Sugiura K, Toki J, Nakao F, Soe T, Kawamura M, Nishioka M, Nagata N, Good RA. Rationale for transplantation of both allogeneic bone marrow and stromal cells in the treatment of autoimmune diseases. In: Chaplin RE, Gale RP, Eds. New Strategies in Bone Marrow Transplantation. New York: Wiley-Liss, Inc., pp151–157, 1991.
17. Cherry, Yasumizu R, Toki J, Asou H, Nishino T, Komatsu Y, Ikehara S. Production of hemopoietic stem cell chemotactic factor by bone marrow stromal cells. Blood **83:**964–971, 1994.

18. Kaufmann CL, Colson YL, Wren SM, Watkins S, Simmons R, Ildstad ST. Phenotypic characterization of a novel bone marrow–derived cell that facilitates engraftment of allogeneic bone marrow stem cells. Blood **84:**2436–2446, 1994.
19. Theofilopoulos AN, Dixon FJ. Murine model of systemic lupus erythematosus. Adv Immunol **37:**269–390, 1985.
20. Fujiwara M, Kariyone A. One-way occurrence of graft-versus-host disease in bone marrow chimera between congeneic MRL mice. Immunology **53:**251–258, 1984.
21. Theofilopoulos AN, Balderas RS, Gozes Y, Aguado MT, Hang LH, Marrow PR, Dixon FJ. Association of *lpr* gene with graft-versus-host disease-like syndrome. J Exp Med **162:**1–12, 1985.
22. Ogata H, Bradley WG, Inaba M, Ogata N, Ikehara S, Good RA. Long-term repopulation of hematolymphoid cells with only a few hematopoietic stem cells in mice. Proc Natl Acad Sci USA **92:**5945–5949, 1995.
23. Nassbeerger L, Sjoholm AG, Bygren P, Thysell H, Hojer-Madsen M, Rasmussen N. Circulating antineutrophil cytoplasm antibodies in patients with rapidly progressive glomerulonephritis and extracapillary proliferation. J Int Med Res **225:**191–196, 1989.
24. Andrassy K, Koderisch J, Rufer M, Erd A, Waldherr R, Ritz E. Detection and clinical implication of antineutrophil cytoplasm antibodies in Wegener's granulomatosis and rapidly progressive glomerulonephritis. Clin Nephrol **32:**159–167, 1989.
25. Addis BJ. Wegener's granulomatosis, ANCA, and microscopic polyarteritis. J Pathol **164:**189–190, 1991.
26. Field M. ANCA: Their role in diagnosis and pathogenesis of vasculitis. Br J Rheumatol **30:**229–231, 1991.
27. Halbwachs-Mecarelli L, Nusbaum P, Noel LH, Reumaux D, Erlinger S, Grunfeld JP, Lesavre P. ANCA directed against cathepsin G in ulcerative colitis, Crohn's disease, and primary sclerosing cholangitis. Clin Exp Immunol **90:**79–84, 1992.
28. Kallenberg CGM, Cohen TJW, van der Woude FJ, Goldschmeding R, von dem Borne AEG, Weening JJ. Autoimmunity to lysosomal enzymes: New clues to vasculitis and glomerulonephritis? Immunol Today **12:**61–64, 1991.
29. Good RA. Marrow transplantation and stem cell transplantation in 1996: The developmental perspective. In: Ikehara S, Takaku F, Good RA, Eds. Bone Marrow Transplantation: Basic and Clinical Studies. New York: Springer-Verlag, pp277–301, 1996.
30. Ikehara S. The prospects for bone marrow transplantation from mouse to human. In: Ikehara S, Takaku F, Good RA, Eds. Bone Marrow Transplantation: Basic and Clinical Studies. Springer-Verlag, pp302–331, 1996.