Genes Expressed by the Kidney, but Not by Bone Marrow–Derived Cells, Underlie the Genetic Predisposition to Progressive Glomerulosclerosis after Mesangial Injury

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Abstract. Progressive renal failure is accompanied by uncontrolled accumulation of extracellular matrix in glomeruli and tubulointerstitium, eventually resulting in glomerulosclerosis. Although glomerulosclerosis occurs secondary to various renal diseases, the fact that not all patients develop progressive glomerulosclerosis suggests that genetic factors may underlie the tendency to progress, or not to progress. Identified were two Lewis rat substrains with small genetic differences but with considerable difference in resolution of glomerulonephritis after anti–Thy-1 administration. In the second study, kidneys were exchanged between the substrains. After transplantation, anti–Thy-1 nephritis was induced and glomerular damage assessed at day 21. Severe damage was observed in Lewis/Maastricht glomeruli independent of whether the kidney had been transplanted or not. Similarly, Lewis/Møllegard glomeruli, whether transplanted or not, revealed no residual histopathologic abnormalities. The inherited differences between the two substrains with regard to their insusceptibility to develop progressive glomerulosclerosis after mesangial injury are governed by genes expressed by the kidney, but not by bone marrow–derived cells.

Progressive renal failure is accompanied by uncontrolled accumulation of extracellular matrix in glomeruli and tubulointerstitium, eventually resulting in glomerulosclerosis and interstitial fibrosis. Glomerulosclerosis is a process that occurs secondary to various renal diseases such as diabetes mellitus, systemic lupus erythematosus, Wegener granulomatosis, IgA nephropathy, and essential hypertension. However, not all patients with these diseases develop progressive glomerulosclerosis and loss of renal function, which suggests that patients can be subdivided into progressors and nonprogressors (1). No predictive markers for progressive glomerulosclerosis or repair after acute immune-mediated injury (9). The injury is induced by the injection of antibodies against Thy1.1, a glycosylphosphatidylinositol-anchored protein present on mesangial cells (10). Antibody injection results in binding of the antibody on mesangial cells, classical pathway complement activation, platelet aggregation, influx of polymorph nuclear neutrophils and monocytes, and mesangial lysis and apoptosis. This is followed by mesangial cell proliferation and expansion of the mesangial ma-
trix (11). In most rat strains, including Lew/Moll, the mesangio-proliferative lesion spontaneously resolves within 4 wk after induction of the disease. In contrast, Lew/Maa rats do not show spontaneous repair of glomerular injury after induction of anti-Thy-1 nephritis; instead, they develop progressive glomerulosclerosis (12). F1 hybrids of Lew/Moll and Lew/Maa rats show repair within 3 wk, revealing a dominantly inherited trait (E. De Heer, unpublished observation). Both strains were derived from Lewis inbred rats and have small genetic differences, thus providing a unique tool with which to identify genes involved in development of progressive glomerulosclerosis or repair of glomerular damage.

The development of glomerulosclerosis in this model is the result of a dynamic interaction of the immune system and the kidney responding to inflammatory damage. The goal of this study was to determine whether in this model disease progression is mediated by genes intrinsic to the kidney or by extrinsic factors determined by bone marrow–derived cells. Bone marrow and renal transplantation were performed between the two substrains. The data presented indicate that the inherited ability of Lew/Maa rats to develop progressive glomerulosclerosis in the course of anti-Thy-1 nephritis is governed by the phenotype of the kidney and not by the phenotype of the bone marrow.

Materials and Methods

Animals

Female Lew/Maa rats were provided by Frans Weekers from the University of Maastricht, Maastricht, The Netherlands. Female Lew/Moll rats were obtained from Taconic M&B Breeding Center, Ry, Denmark. Animal care and experimentation were in accordance with legislation on animal experiments as determined by the Dutch Veterinary Inspection.

Antibodies

Monoclonal anti-Thy-1 antibody was affinity purified from culture supernatants from hybridoma ER4 (9) on protein A–Sepharose 4B (Pharmacia, Uppsala, Sweden). It was subsequently depleted from possible contamination with endotoxin by running it batchwise over Detoxy-Gel (Pierce, Rockford, IL).

Generation of Bone Marrow Chimeras

The generation of bone marrow chimeras was based on a previously described protocol (13). Briefly, all animals, weighing between 160 and 200 g were killed by irradiation (8 Gy per animal in 50 fractions) by the use of an x-ray generator. No anesthesia was administered. We based the dose of irradiation on pilot dose-response experiments and on preceding reports in the literature. In our hands, an increased of the irradiation dose up to 8.5 Gy followed by bone marrow reconstitution was lethal to all recipient Lewis rats within 5 d (data not shown). Twenty-four hours after total-body irradiation, rats received adult bone marrow transplants. Bone marrow cells were collected by flushing bone shafts of femurs with Hanks buffered medium. Cells were sieved through 50-μm sieves and washed twice with ice-cold Hanks buffered medium. Subsequently, cells were re-suspended in ice-cold Hanks buffered medium at a concentration of 5 × 10^7 cells/mL. Rats received 5 × 10^7 bone marrow cells by intravenous injection directly after isolation of the bone marrow cells.

To exclude the effect of radiation and bone marrow transplantation, bone marrow chimeric rats were generated and examined during an equivalent period of time as the experimental group but without inducing anti-Thy-1 nephritis. No additional histopathologic abnormalities were observed in the glomeruli of the control rats. It would be desirable to confirm successful bone marrow transplantation because an incomplete ablation of recipient bone marrow cells could influence the experiment. Confirmation of complete ablation of all recipient bone marrow cells for example with allotypic markers for major histocompatibility complex (MHC) class I is technically impossible in this situation because the MHC haplotypes of these Lewis substrains are identical.

Kidney Transplantation

Kidney transplantation was performed heterotopically as described previously (14). Briefly, the left kidney without the adrenal gland, together with a patch of the aorta, a cuff of the inferior vena cava, the ureter, and the bladder dome, were removed and transplanted in the heterotopic position. The donor ureter, together with a small piece of the donor bladder, was anastomosed to the dome of the recipient bladder. To exclude an additional role of substrain-dependent rejection on glomerular morphology, kidney transplantations were performed between the Lewis substrains without inducing anti-Thy-1 nephritis. Immunosuppressive therapy after transplantation was omitted in this study. Because the two Lewis substrains have identical MHC haplotypes, rejection was not expected, nor was it observed after transplantation. However, some transient tubulointerstitial infiltrates were observed, even in syngeneic transplants. Because no additional effect was observed from the transient tubulointerstitial infiltrate in the course of anti-Thy-1 nephritis, the presence of this infiltrate was not considered further.

Histological Examination

Kidney tissue fixed in 4% neutral-buffered formalin and embedded in paraffin were cut into 4-μm sections and stained with periodic acid–Schiff. Glomerular injury was quantified according to a protocol based on the method described by Bidani et al. (15). Briefly, normal glomeruli were defined by the appearance of the glomeruli in the kidney of the control group. Abnormal glomeruli were defined by the appearance of microaneurysms, mesangial cell proliferation, extracellular matrix expansion, glomerular crescent formation, collapsed capillary loops with mesangial expansion, and hyaline deposits. Forty glomeruli in each section were scored in a blinded fashion by two independent observers. Data are expressed as the mean per group animals of the percentage of abnormal glomeruli in 40 analyzed glomeruli.

Statistical Analyses

All data are expressed as means ± SD. Statistical analysis was performed using two-tailed unpaired t tests. Statistical significance was defined as P < 0.05.

Experimental Design

The natural course of anti-Thy-1 nephritis was monitored in both substrains. Therefore, anti-Thy-1 nephritis was induced in the rats by intravenous injection of 2 mg/kg ER4 antibody; subsequently, animals (seven per group) were killed 3, 7, 21, and 90 d after induction of the disease, followed by the removal of the kidneys. Sections of the removed kidneys were histologically analyzed. Tissue samples taken from rats with anti-Thy-1 nephritis served as a control.

To determine whether the bone marrow expresses genes that are related to progressive glomerulosclerosis, bone marrow chimeric rats were generated by lethal irradiation of each substrain, followed by reconstitution with bone marrow cells derived from the other substrain.
(seven animals per group). Four weeks after recovery, chimeric rats have reconstituted their immune system (16). Four weeks after bone marrow transplantations, anti-Thy-1 nephritis was induced and monitored in these bone marrow–reconstituted rats by histologic examination of renal biopsy material and renal tissue taken at days 7 and 21, respectively.

To determine whether cells from the kidney express genes that are related to progressive glomerulosclerosis, kidneys were exchanged between the two substrains by heterotopic transplantation of one kidney; the other kidney was left in situ (four animals per group). Anti-Thy-1 nephritis was induced in the transplanted rats 1 wk after transplantation by intravenous injection of 2 mg/kg ER4 antibody. After 3 wk, the rats were killed, and the autologous and the transplanted kidneys were removed and histologically examined. In both experiments, syngeneically transplanted rats and transplanted rats without induction of anti-Thy-1 nephritis served as a control. All experiments were terminated at 3 wk after disease induction because by this time, disease was such that whether animals would recover completely or progress to chronic renal disease could be determined.

Results
Anti–Thy-1 Nephritis Time Course in Lew/Maa and Lew/Moll Rats

Both substrains showed severe mesangial cell lysis, apoptosis, and an influx of polymorph nuclear neutrophils and monocytes 3 d after induction of anti-Thy-1 nephritis (data not shown). Increased numbers of mesangial cells and extracellular matrix expansion were observed in both substrains at 7 d. After 21 d, no histopathologic abnormalities were seen in Lew/Moll rats kidneys. At 21 d, however, glomeruli in Lew/Maa rats demonstrated mesangial proliferation, matrix expansion and glomerular crescent formation (Figure 1). The numbers of abnormal glomeruli were quantified in kidney sections obtained 7 and 21 d after induction of anti-Thy-1 nephritis. The number of abnormal glomeruli was higher in Lew/Maa rats compared with Lew/Moll rats at day 7 (69% ± 15% versus 40% ± 8%). But the damage per glomerulus was identical in both substrains after 7 d. Because of the scoring technique no discrimination could be made in the severity of the damage between individual glomeruli. Significant, less abnormal glomeruli were observed in Lew/Moll in comparison with Lew/Maa glomeruli at day 21 (57% ± 3% versus 9% ± 1%; P < 0.001; Figure 2). After 3 mo, Lew/Maa rats developed focal segmental glomerulosclerosis (Figure 3). Preliminary experiments have shown that even after 6 mo, progressive glomerulosclerosis persists (data not shown).

Bone Marrow Transplantation

To investigate whether the substrain-related differences are due to genes expressed by bone marrow–derived cells, anti-Thy-1 nephritis was monitored in bone marrow–reconstituted rats. Lew/Moll kidneys, irrespective of whether or not the rats received Lew/Maa or Lew/Moll bone marrow, recovered completely within 3 wk, whereas Lew/Maa recipients of bone marrow from Lew/Maa showed persistent and severe glomerular damage. Glomerular damage scores for the both control groups and both experimental groups receiving bone marrow transplant after total-body irradiation and induction of anti-Thy-1 nephritis are shown in Figure 4.

Kidney Transplantation

As shown in Figure 5, disease progression in response to anti-Thy-1–induced glomerulonephritis followed the genotype of the kidney and not of the recipient animal. When anti-Thy-1
nephritis was induced, glomerular damage appeared in Lewis/Maa rat kidneys whether they were transplanted into Lew/Maa or into Lew/Moll recipients. Glomerular damage also persisted in the nontransplanted autologous Lew/Maa kidneys irrespective of whether or not the rats received a Lew/Maa or a Lew/Moll transplant. Glomeruli of Lew/Moll kidneys, either transplanted or autologous, revealed no residual histopathologic abnormalities 21 d after induction of anti–Thy-1 nephritis (Figure 5).

To examine whether the transplantation procedure itself interfered with the development of anti–Thy-1 nephritis, autologous kidney transplantations were performed, followed by induction of anti–Thy-1 nephritis. In Lew/Maa rats, both the transplanted and the nontransplanted kidney showed glomerular damage. Lew/Moll rats showed no histopathologic abnormalities in the glomeruli, both in the transplanted and in the nontransplanted kidney 21 d after induction of anti–Thy-1 nephritis (Figure 5). Graphical representation of glomerular damage scores is presented in Figure 6 for the autologous and transplanted kidneys in the four transplant groups.

Discussion

The aim of this study was to investigate whether genes involved in the development of progressive glomerulosclerosis are expressed by the kidney or by bone marrow–derived cells. Therefore, we studied the development of glomerulosclerosis in the anti–Thy-1 nephritis model in two Lewis substrains. The development of glomerulosclerosis in this model is the result of a dynamic interaction between factors intrinsic to the kidney and extrarenal factors. In addition to the immune system, other factors extrinsic to the kidney are related to the development of chronic renal disease. In humans, these include smoking, age, systemic hypertension, and diabetes. The study presented here indicates that, at least in these two Lewis substrains, factors intrinsic to the kidney and not extrinsic factors determine whether progressive glomerulosclerosis develops in the course of anti–Thy-1 nephritis.

This finding represents an important step in identification of genetic factors, which predispose rats to progressive renal disease. We did not identify the genes responsible for the development of progressive glomerulosclerosis or the specific cells within the kidney, which are expressing those progression-related genes. There is, however, increasing evidence for
a pivotal role of glomerular mesangial cells, and the biomolecules they produce, in the development of anti-Thy-1 nephritis and glomerulosclerosis as summarized by Floege et al. (17). Mesangial cells produce factors involved in the mesangial cell proliferation and mesangial matrix expansion that follows mesangial cell injury during anti-Thy-1 nephritis. Among them are the growth factors PDGF, basic fibroblast growth factor (bFGF), and TGF-β. Administration of anti-bFGF antibodies resulted in an overall reduction in mesangial cell proliferation (18). Others showed that administration of an aptamer-based antagonist against PDGF in anti-Thy-1 nephritis resulted in an almost complete inhibition of matrix protein accumulation (19).

Blockade of TGF-β reduces the disease severity of anti-Thy-1 nephritis whether neutralizing antibodies are injected. Soluble TGF-β receptors are produced by gene transfection or when the natural antagonist, decorin, is administered intravenously or by gene therapy (20–23). In addition, constitutive overexpression of growth hormone has been shown to promote the development of glomerulosclerosis (24). These data reveal that growth hormones such as bFGF, PDGF, and TGF-β are involved not only in the development of anti-Thy-1 nephritis, but also in glomerulosclerosis after this disease is experimentally induced.

In addition to growth factors, the chemokine monocyte chemoattractant protein 1 (MCP-1), which is among those produced by mesangial cells, also plays an important role in the development of anti-Thy-1 nephritis. This is confirmed by neutralization studies of MCP-1 in Wistar rats during anti-Thy-1 nephritis (25). Furthermore, MCP-1 has a fibrogenic effect through the stimulation of TGF-β (26). This indicates that MCP-1, at least in part, mediates glomerular matrix accumulation. However, in previous experiments that used a kidney transplant approach similar to the one used here, we have shown that the MCP-1 expression after induction of anti-Thy-1 nephritis was not significantly different between Lewis and Fisher 344 rats, a strain that does not develop progressive
sclerosis (27). Thus, factors other than MCP-1 clearly modulate the fibrotic response in this model.

In addition to the pivotal role of mesangial cells in anti-Thy-1 nephritis, the infiltration of macrophages in the glomerulus is also one of the hallmarks of anti-Thy-1 nephritis. Ketteler and coworkers (12) found that LPS-stimulated macrophages isolated from Lew/Maa rats expressed more inducible nitric oxide synthase mRNA and NO activity per cell than those from Lew/Moll rats, whereas the numbers of infiltrating macrophages in Lew/Maa and Lew/Moll rats were equal 6 h after induction of anti-Thy-1 nephritis. This study suggests a possible involvement of increased activity of inducible nitric oxide synthase produced by macrophages in the development of glomerulosclerosis. The bone marrow experiments in our study rule out the notion that a substrain-dependent differential activation of bone marrow–derived macrophages underlies the differential course of disease. This should indicate a major role for resident macrophages of the kidney in the development of glomerulosclerosis after mesangial injury. A different NO responsiveness, which should reside in these resident macrophages of the kidney, could explain the differential course of disease after activation of these enzymes.

Apart from the role of mesangial cells and macrophages in anti-Thy-1 nephritis, there is increasing evidence for a pathogenic involvement of glomerular endothelial cells. Iruela-Arispe and colleagues (28) demonstrated that endothelial cell proliferation is markedly increased during the first week after induction of anti-Thy-1 nephritis. In addition, glomerulosclerosis appears to be strongly associated with impairment of vascular regeneration (29). It has recently been suggested that the glomerular epithelial cell also plays a role in the progression of anti-Thy-1 nephritis (30).

Regarding our results, the following considerations seem to be of special importance. First, it could be suggested that the lack of recovery of mesangial injury in Lew/Maa rats after is due to failure of recovery or due to severe initial injury. The initial injury during of anti-Thy-1 nephritis is caused by complement-dependent mesangiolysis. Identical glomerular C3 and C5b-9 protein levels were observed in both Lewis substrains (12). Because there are no significant differences in the severity of mesangiolysis, we conclude that the lack of recovery in Lew/Maa rats is due to a failure of recovery. Theoretically, we agree that the severity of damage may underlie the capacity of glomeruli to either fail to recover from immune injury, or to remodel. However, at day 7, a broad overlap was observed in the degree of severity within glomeruli of Lew/Moll and Lew/Maa rats, whereas all glomeruli from Lew/Moll rats remodeled within 4 wk. In contrast, glomerular pathologic changes of Lew/Maa rats persisted even when transplanted into Lew/Moll recipients. This, however, does not exclude the notion that only particular lesions in the glomeruli from the Lew/Maa rat strain lack the capacity to recover. This aspect is currently under investigation.

Second, in our study, we exchanged the bone marrow of the two substrains by subjecting the rats to a total-body irradiation followed by bone marrow transplantation. Although it has been reported that total-body irradiation followed by bone marrow transplantation by itself can cause renal toxicity and glomerulosclerosis, the earliest signs of renal toxicity after total-body irradiation appear after 3 mo (31), much later than the observation period in our study. Therefore, we believe that an additional effect of total-body irradiation on the glomerular damage in our experiments can be excluded.

Third, after induction of anti-Thy-1 nephritis, all rats developed a full-blown disease at day 7 that was indistinguishable between the substrains, but only Lew/Maa rats developed progressive glomerulosclerosis. These results indicate that progressive disease can develop when the severity of disease at day 7 is similar.

Finally, the kidney exchange experiment described in this study shows that progressive glomerulosclerosis in the kidney of the recipient was not transferred systemically to the transplanted kidney. Thus, kidney transplantation would be expected to be a successful therapy for the kind of genetic defect existing in the Lew/Maa rats. Finally, although we demonstrated that glomerulosclerosis will only develop in the kidney able to express genes that are responsible for the development of glomerulosclerosis, it is still possible that after transplantation and induction of anti-Thy-1, nephritis recipient–derived cells infiltrate the donor kidney and express these progression genes. This is supported by previous experiments describing bone marrow transplantation in combination with glomerulosclerosis, revealing that glomerular mesangial cell progenitors are derived from the bone marrow (32). However, only 7% to 8% of mesangial cells are reported to be replaced by bone marrow cells after induction of anti-Thy-1 nephritis in rats. Because of this low number, it has been suggested that most of the glomerular regeneration should be provided by locally proliferating mesangial cells (18,33).

In conclusion, although the literature suggests that both intra- and extrarenal processes contribute to the development of anti-Thy-1 nephritis and glomerulosclerosis, our study demonstrates that at least in Lew/Maa rats, the genetic predisposition to progressive glomerulosclerosis is governed by genes expressed by the kidney, but not by those expressed in bone marrow–derived cells. Identification of the genes responsible for the development of glomerulosclerosis by differential gene expression analysis is under way in our laboratory. It is hoped that this approach will identify genes of relevance to progression in humans.

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References


