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TECHNICAL NOTE

Model of robust induction of glomerulosclerosis in mice: Importance of genetic background

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Model of robust induction of glomerulosclerosis in mice: Importance of genetic background.

Background. Increasing evidence suggests that genetic background plays an important role in the development of progressive glomerulosclerosis. The remnant kidney model (RKM) of progressive renal disease has been used extensively in rats. However, C57BL/6 mice are resistant to glomerulosclerosis with RKM induced by either pole amputation or renal artery ligation. A pole resection protocol, applied in 129/Sv mice, induced only mild glomerulosclerosis. We present here a highly reproducible, modified RKM approach to successfully establish a glomerulosclerosis model in mice.

Methods. Male C57BL/6 ($N = 17$), 129/Sv ($N = 20$) and Swiss-Webster ($N = 3$) mice underwent RKM as follows: the lower branch of the left renal artery was ligated to produce about one third infarct; the upper pole of the left kidney (about one third kidney size) was removed by cautery and the right kidney was nephrectomized to induce a total 5/6 nephrectomy (Nx). In some C57BL/6 mice, 7/8 nephrectomy was induced by removing additional renal mass from the upper pole of the left kidney by cautery. Systolic blood pressure (BP) was measured in conscious mice using a tail-cuff blood pressure monitor and animals were sacrificed at 9, 12, 18, and 24 weeks after nephrectomy. Kidneys were harvested for morphologic analysis.

Results. BP in C57BL/6 mice increased slightly after 5/6 nephrectomy over time without significant difference compared to baseline blood pressure except at 8 weeks (blood pressure at week 0, 98 ± 1 mm Hg; week 4, 105 ± 2 mm Hg; week 8, 113 ± 4 mm Hg; and week 12, 110 ± 3 mm Hg). Blood presssure remained normal in C57BL/6 mice at 18 weeks after 7/8 nephrectomy (103 ± 2 mm Hg). Blood pressure in 129/Sv mice increased significantly after 5/6 nephrectomy from 4 to 12 weeks (week 0, 112 ± 3 mm Hg; week 4, 161 ± 9 mm Hg; week 8, 166 ± 5 mm Hg; and week 12, 176 ± 5 mm Hg; $P <$ 0.01 weeks 4, 8, and 12 vs. week 0 blood pressure). Urine protein excretion in C57BL/6 mice increased only at 4 weeks after 5/6 nephrectomy, and was back to normal at 8 and 12 weeks (week 0, 13.2 ± 1.4 mg/24 hours; week 4, 20.5 ± 1.8 mg/24 hours; week 8, 18.8 ± 1.6 mg/24 hours; and week 12, 17.2 ± 1.2 mg/24 hours, $P < 0.05$ week 4 vs. week 0). 129/Sv mice developed significant proteinuria 12 weeks after 5/6 nephrectomy compared to their baseline and to levels achieved in C57BL/6 mice (week 0, 17.2 ± 1 mg/24 hours; week 4, 14.9 ± 1.8 mg/24 hours; week 8, 23.8 ± 6.7 mg/24 hours; and week 12, 36.3 ± 6.6 mg/24 hours, $P < 0.01$ week 12 vs. week 0; $P < 0.01$ 129/Sv vs. C57BL/6 at week 12). Mortality varied in response to nephrectomy injury in the different strains. Ten percent of C57BL/6 and 43% of 129/Sv died within 12 weeks after 5/6 nephrectomy. Although 50% of C57BL/6 mice died by 12 weeks after 7/8 nephrectomy, there was only mild glomerulosclerosis (<5%) in C57BL/6 mice even at 24 weeks after 5/6 nephrectomy or 18 weeks after 7/8 nephrectomy. In contrast, glomerulosclerosis was marked in both 129/Sv mice and Swiss-Webster mice as early as 9 weeks after 5/6 nephrectomy: 42% of glomeruli showed sclerosis in 129/Sv mice [average sclerosis index (SI), 0 to 4+ scale, 1.08] vs. 24% in Swiss-Webster mice (average SI, 0.57). Tubulointerstitial fibrosis developed in parallel with glomerulosclerosis in both 129/Sv and Swiss-Webster mice.

Conclusion. We conclude that genetic background is one of the important factors determining the susceptibility to the development of glomerulosclerosis in mice. We speculate that the superior effects of renal artery ligation plus cautery to produce glomerulosclerosis may result from higher blood pressure responses due to local ischemia activating the renin-angiotensin system.

Glomerulosclerosis, characterized by capillary obsolescence and increased extracellular matrix accumulation, is the final common pathway in a variety of kidney diseases leading to chronic renal failure [1]. The remnant kidney model (RKM), achieved usually by uninephrectomy combined with infarction of two thirds of the other kidney, has been extensively and successfully used in many different rat strains to study the pathogenesis of, as well as effectiveness of intervention on, glomerulosclerosis [2–5]. In contrast, mice have generally been resistant to various methods of inducing this model. The RKM was induced in albino mice (carrying a tyrosinase gene mutation) by uninephrectomy plus surgical amputation of the poles of the remaining kidney; however, significant glomerulosclerosis did not develop [6]. Ligation of the anterior renal artery branch to induce two thirds left kidney infarcts has been tried in male C57BLX Swiss-Webster mice. Only mild mesangial expansion and proliferation

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were observed over the short-term follow-up of 10 days [7]. Recently, Kren and Hostetter [8], using a nephrectomy with ligation protocol, studied the course of the remnant kidney model in C57BL/6 mice. There was no difference in systolic blood pressure and proteinuria compared to control mice that underwent sham operation. The percentage of glomerulosclerosis within the remnant kidney (12 ± 6%) was not significantly different compared to control kidneys (7 ± 2%) [8]. In this report, we present a modified, highly reproducible approach to successfully establish hypertension-associated glomerulosclerosis model in the susceptible 129/Sv mice.

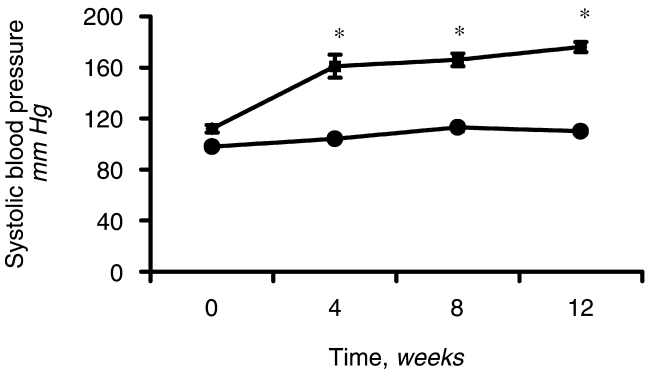

Fig. 1. Systolic blood pressure (BP) changes in remnant kidney model in mice. Blood pressure increased over time in 129/Sv (■) mice after 5/6 nephrectomy, contrasting normal blood pressure in C57BL/6 (●) mice after 5/6 nephrectomy. *$P < 0.01$.

METHODS

Animal preparations

Male C57BL/6 ($N = 17$), 129/Sv ($N = 20$) (Jackson Laboratories, Bar Harbor, ME, USA) and Swiss-Webster ($N = 3$) (Charles River Laboratory, Wilmington, MA, USA) mice, age 8 to 10 weeks, were housed in microisolator cages in a pathogen-free barrier facility under a 12-hour light/dark cycle. Food and water were supplied ad libitum. All animal protocols were approved by the Vanderbilt University Institutional Animal Care and Use Committee. Mice underwent RKM by a modified protocol as described below. The surgery was performed under anesthesia with sodium pentobarbital (50 mg/kg body weight, intraperitoneally). Bilateral dorsal, longitudinal incisions were made to expose both kidneys. The lower branch of the left renal artery was ligated by 6-0 silk suture to produce about one third area with visible renal ischemia; the upper pole of the left kidney was removed by cautery and the right kidney was decapsulated and nephrectomized to induce a total 5/6 nephrectomy. In some C57BL/6 mice, 7/8 nephrectomy was induced by removing additional renal mass from the upper pole of the left kidney by cautery. Animals were monitored (see below) and sacrificed at 9, 12, 18, and 24 weeks after RKM. Kidneys were harvested for morphologic analysis.

Analysis of kidney function

Systolic blood pressure and 24-hour urinary protein were assessed at weeks 0, 4, 8, and 12 for C57BL/6 and 129/Sv mice. Systolic blood pressure was measured using a tail-cuff Blood Pressure Monitor for Rats & Mice (Model 2000, Muromachi Kikai Co., LTD, Tokyo, Japan) in conscious, trained mice at room temperature. Mice were placed in metabolic cages for 24-hour urine collection. Urine protein was measured by Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA).

Structural analyses

Kidney tissue from mice was immersion-fixed in 4% paraformaldehyde/phosphate-buffered saline (PBS) solution, routinely processed, and 4 μm sections were stained with periodic acid-Schiff (PAS) and Masson's trichrome stain. The extent of glomerular sclerosis was assessed by examining all glomeruli on a kidney cross-section, and calculating the percent involved. A semiquantitative score [sclerosis index (SI)] was used to evaluate the degree of glomerulosclerosis. Severity of sclerosis for each glomerulus was graded from 0 to 4+ as follows: 0 represents no lesion, 1+ represents sclerosis of <25% of the glomerulus, while 2+, 3+, and 4+ represent sclerosis of 25% to 50%, >50% to 75%, and >75% of the glomerulus. A whole kidney average sclerosis index was obtained by averaging scores from all glomeruli on one section. Tubulointerstitial fibrosis was evaluated qualitatively on Masson's trichrome–stained section. All sections were examined without knowledge of the treatment protocol.

Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). Statistical difference was assessed by a single factor variance (ANOVA) followed by unpaired *t* test as appropriate. Nonparametric data were compared by Mann-Whitney U test. A P value <0.05 was considered to be significant.

RESULTS

Blood pressure in C57BL/6 mice increased slightly after 5/6 nephrectomy without significant difference compared to baseline blood pressure except at 8 weeks (blood pressure at week 0, 98 ± 1 mm Hg; week 4, 105 ± 2 mm Hg; week 8, 113 ± 4 mm Hg; and week 12, 110 ± 3 mm Hg) (Fig. 1). Blood pressure remained normal in C57BL/6 mice even after 7/8 nephrectomy at 18 weeks (103 ± 2 mm Hg). Blood pressure in 129/Sv mice increased significantly after 5/6 nephrectomy from 4 to 12 weeks (week 0, 112 ± 3 mm Hg; week 4, 161 ± 9 mm Hg;

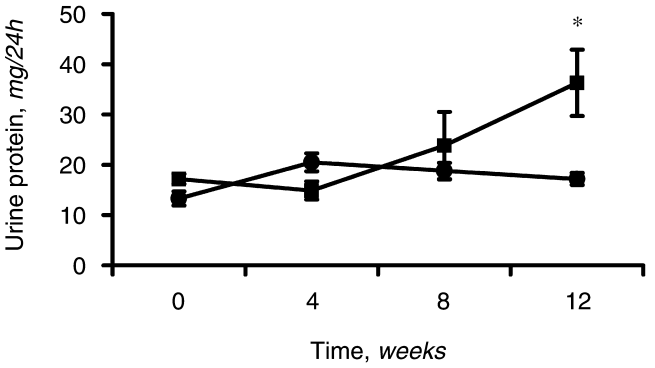

Fig. 2. Urine protein excretion in remnant kidney model in mice. Urine protein excretion in 129/Sv (■) and C57BL/6 (●) mice after 5/6 nephrectomy. *$P < 0.01$ week 12 129/Sv vs. week 0 129/Sv and week 12 C57BL/6.

week 8, 166 ± 5 mm Hg; and week 12, 176 ± 5 mm Hg, $P < 0.01$ weeks 4, 8, and 12 vs. week 0 blood pressure) (Fig. 1) and was higher than blood pressure in C57BL/6 after 5/6 nephrectomy from 4 to 12 weeks ($P < 0.01$ 129/Sv vs. C57BL/6 at weeks 4, 8, and 12). Urine protein excretion in C57BL/6 mice increased only at 4 weeks after 5/6 nephrectomy, and was back to normal at 8 and 12 weeks (week 0, 13.2 ± 1.4 mg/24 hours; week 4, 20.5 ± 1.8 mg/24 hours; week 8, 18.8 ± 1.6 mg/24 hours; and week 12, 17.2 ± 1.2 mg/24 hours, $P < 0.05$ week 4 vs. week 0) (Fig. 2). In contrast, 129/Sv mice developed significant proteinuria 12 weeks after 5/6 nephrectomy compared to baseline level of 129/Sv and the level achieved in C57BL/6 mice (week 0, 17.2 ± 1 mg/24 hours; week 4, 14.9 ± 1.8 mg/24 hours; week 8, 23.8 ± 6.7 mg/24 hours; and week12, 36.3 ± 6.6 mg/24hours, $P < 0.01$ week12 vs. week 0; $P < 0.01$ 129/Sv vs. C57BL/6 at week 12) (Fig. 2).

Mortality varied in response to 5/6 nephrectomy injury in the different strains. Ten percent of C57BL/6 and 43% of 129/Sv died within 12 weeks follow-up after 5/6 nephrectomy. Although 50% of C57BL/6 mice died by 12 weeks after 7/8 nephrectomy, there was only mild mesangial expansion present in some of these C57BL/6 mice. There was only mild glomerulosclerosis (<5%) in C57BL/6 mice even at 24 weeks after 5/6 nephrectomy (Fig. 3 A and B) or at 18 weeks after 7/8 nephrectomy (Fig. 3 C and D). Mild tubular dilation without tubulointerstitial fibrosis was observed in C57BL/6 mice after 5/6 nephrectomy or 7/8 nephrectomy (Fig. 3 A to D). In contrast, robust induction of glomerulosclerosis was present in both 129/Sv mice (Fig. 3 E and F) and Swiss-Webster mice (Fig. 3 G and H) as early as 9 weeks after 5/6 nephrectomy: 42% of glomeruli showed sclerosis at 9 weeks in 129/Sv mice (average SI, 0 to 4+ scale, 1.08), and there was sclerosis in 24% of glomeruli in Swiss-Webster mice (average SI, 0.57). Tubulointerstitial fibrosis developed in parallel with glomerulosclerosis in both 129/Sv and Swiss-Webster mice (Fig. 3 E to H).

DISCUSSION

Our study further confirmed, consistent with previous reports, that C57BL/6 mice are resistant to development of glomerulosclerosis after RKM. In contrast, a robust induction of marked glomerulosclerosis was successfully established in 129/Sv and Swiss-Webster mice by a modified protocol of renal artery branch ligation combined with cautery, which was linked to increased blood pressure.

Increasing evidence suggests that genetic background plays an important role in determining the response to the renal injuries. Genetic susceptibility to glomerulosclerosis in rats has been studied extensively. Nearly 100% of BUF/Mna strain rats spontaneously develop glomerulosclerosis with age. In contrast, two other rat strains, Wistar-Kyoto (WKY)/NCrj and ACI/NMs, are resistant to glomerulosclerosis [9]. Similarly, male Wistar rats are predisposed to spontaneous development of glomerulosclerosis, a process that can be accelerated by unilateral nephrectomy. On the other hand, male PVG/c rats are completely resistant to glomerulosclerosis, even at 1 year after uninephrectomy [10].

The radiation-induced Os mutation (Os/+) results in a 50% reduction in nephron number. However, the Os/+ mutation results in severe glomerulosclerosis only on the ROP strain, but not C57BL/6, background. The resistance to sclerosis in C57 Os/+ mutation occurred despite cell proliferation and glomerular hypertrophy responses [11]. Similar strain dependence in susceptibility to sclerosis was observed in response to diabetic injury and further nephron loss [12, 13].

C57BL/6 and 129/Sv are the most commonly used mice strains for disease studies. Differences in response to ischemia and vascular remodeling in C57BL/6 and 129/Sv mice have been reported [14, 15]. However, susceptibility to atherosclerosis is not parallel to the susceptibility to the glomerulosclerosis in these mice strains. C57BL/6 are highly susceptible and 129/Sv mice are less susceptible to atherosclerosis in response to both atherogenic diet– and apolipoprotein E (apoE) deficiency–induced atherosclerosis [16, 17]. Interestingly, in response to endothelial denudation–induced injury, the 129/Sv mice developed intermediate level of neointimal hyperplasia, whereas the atherosclerosis-susceptible C57BL/6 mice were resistant to neointimal hyperplasia [15].

Hypertension has important influence on the progression of the renal diseases and glomerulosclerosis. Variations of blood pressure response to RKM affect the outcome of development of glomerulosclerosis. WKY rats, which are genetically resistant to the development of hypertension, had no obvious glomerulosclerosis after 5/6 nephrectomy in the absence of hypertension [18]. In contrast, Dahl–salt sensitive rats with 5/6 nephrectomy displayed significantly more severe hypertension and focal glomerulosclerosis than salt-resistant rats [19]. Sponta-

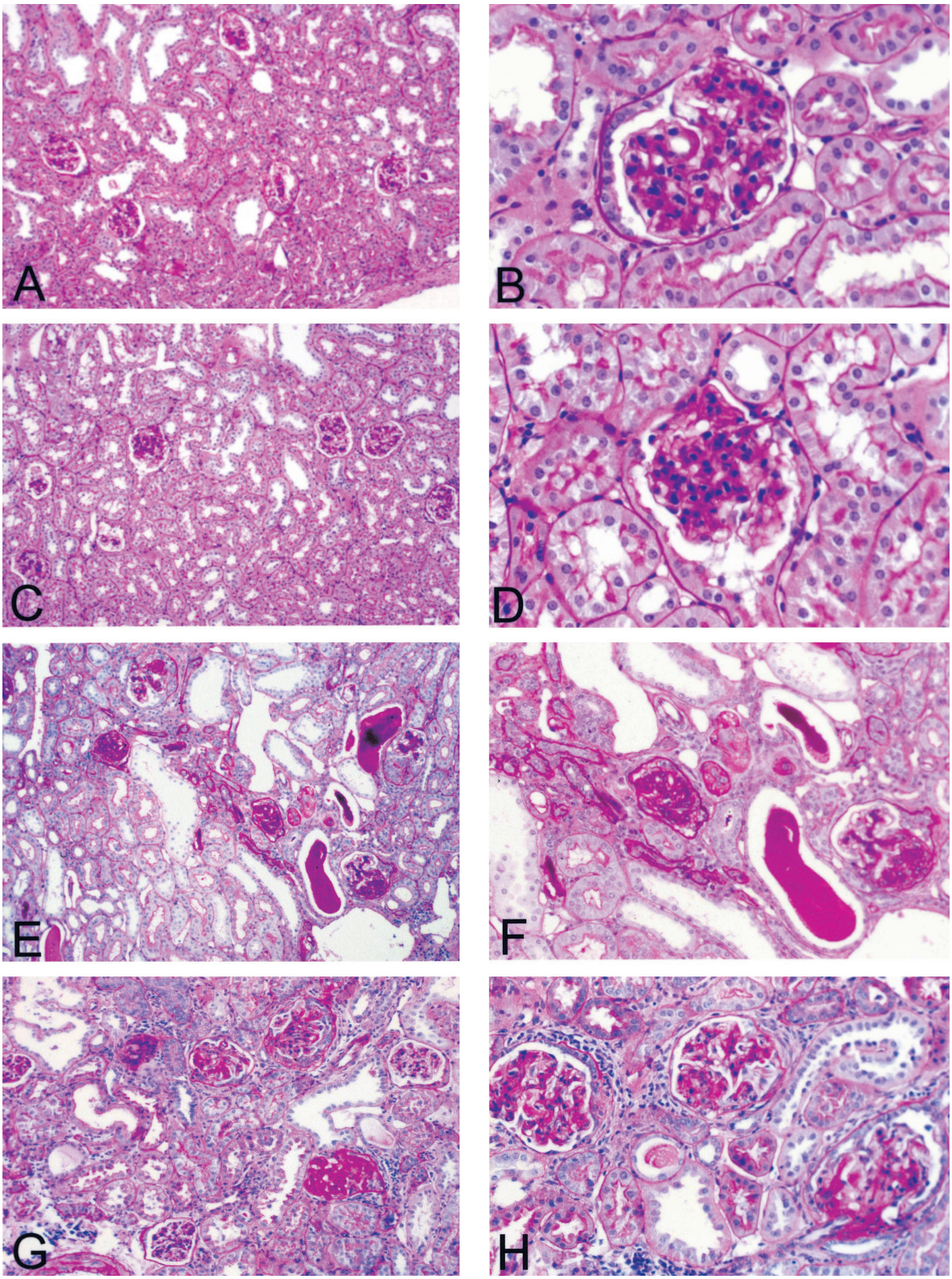

Fig. 3. Histologic changes in remnant kidney model in mice. Representative photographs from C57BL/6 mice 24 weeks after 5/6 nephrectomy (*A* and *B*); C57BL/6 mice 18 weeks after 7/8 nephrectomy (*C* and *D*); 129/Sv mice 9 weeks after 5/6 nephrectomy (*E* and *F*); and Swiss-Webster mice 9 weeks after 5/6 nephrectomy (*G* and *H*) (A, C, E, and G, ×100; B and D, ×400; F and H, ×200, periodic acid-Schiff stain).

neously hypertensive rats (SHR) develop much less renal damage than the stroke-prone strain of SHR (SHRsp) after salt-supplementation. It was suggested that SHRsp kidneys are intrinsically more susceptible than the SHR kidneys to renal damage when exposed to exactly the same blood pressure and metabolic environment, due to genetic differences in susceptibility to renal damage [20].

Lack of hypertension after overwhelming renal mass reduction after 7/8 nephrectomy in C57BL/6 mice suggests that severe reduction of renal mass per se is not responsible for the development of hypertension in RKM [4]. The significant variability in the hypertensive response in different genetic strains in rats or mice, even when the same RKM method was used, suggests that genetic predisposition may be an additional important determinant of the susceptibility to hypertension after severe loss of functional renal mass. Absence of hypertension in C57BL/6 mice, which was associated with more focal and milder glomerulosclerosis in response to 5/6 nephrectomy injury both in previous studies [8] and our current study, support the paramount role of genetic factors. In contrast, robust induction of glomerulosclerosis in 129/Sv mice after 5/6 nephrectomy in this study was associated with enhanced blood pressure response. Of note, the pole resection strategy used by Megyesi et al [21] in 129/Sv mice to induce 5/6 nephrectomy resulted in lower blood pressure (less than 135 mm Hg) than our ligation protocol, and milder glomerulosclerosis.

Recent studies suggest that susceptibility to the development of hypertension and further glomerulosclerosis in 129 and C57BL/6 strains may be influenced by renin gene polymorphisms. Some mouse strains (e.g., C57BL/6) have one gene (*Ren-1*^{c}), while other strains (e.g., 129) have two (*Ren-1*^{d} and *Ren-2*) [22]. The *Ren-1* genes govern expression of renin in the kidney in the juxtaglomerular apparatuses, while the *Ren-2* gene controls submaxillary gland renin expression and has very low renal expression. Mice with two renin genes (i.e., 129 strain) have tenfold higher plasma renin activity, angiotensin-dependent hypertension, and increased blood pressure and cardiac and renal hypertrophic responses to salt compared to one renin gene mice (i.e., C57BL/6). Renin gene status thus could have a major effect on susceptibility to injuries where the renin-angiotensin-aldosterone system (RAAS) plays a role [22]. The significance of the increased renin level in the development of hypertension and glomerulosclerosis was also documented in rats. In Sprague-Dawley rats, hypertension developed only in rats with reduced renal mass by pole infarction protocols, and correlated with increased renin level, contrasting normal blood pressure and normal renin level in rats with reduced renal mass by the surgical pole resection technique [4, 23]. Evidences from previous studies suggest increased renin expression and activity deriving from the ischemic edges of the infarcted area of the remnant kidney may be causal in the more pronounced hypertension [5, 24, 25]. The renin-angiotensin system has pronounced effects on glomerulosclerosis in humans and animal models, through both blood pressure–dependent and –independent effects [26, 27]. In addition, recent evidence supports a direct role for aldosterone in the progression of renal disease [5, 28]. Thus, augmentation of the RAAS may be a necessary or permissive step for development of glomerulosclerosis (Fig. 4). Conversely, inhibition of the RAAS is particularly effective in amelioration or even regression of progressive chronic renal diseases with effects that extend beyond antihypertensive actions [26, 29–31]. In addition, it is conceivable that additional non-RAAS genetic factors in 129 mice strain may contribute to the susceptibility to glomerulosclerosis through both blood pressure–dependent and –independent effects (Fig. 4).

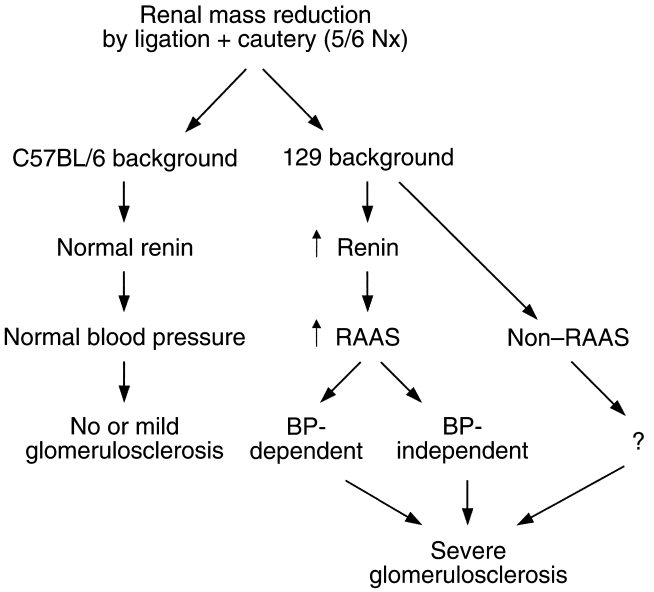

Fig. 4. Schema of proposed pathogenesis of glomerulosclerosis in C57BL/6 and 129/Sv mice. In response to renal mass reduction by ligation plus cautery, we propose that 129/Sv mice have increased renin level and activated renin-angiotensin-aldosterone system (RAAS), which accelerate glomerulosclerosis through both hemodynamic and nonhemodynamic effects. In addition, other non-RAAS genetic factors in 129/Sv mice may contribute to the development of glomerulosclerosis. On the other hand, C57BL/6 strain mice maintain normal renin levels and normal blood pressure (BP), which lead to resistance to the development of glomerulosclerosis.

We conclude that genetic background is a key factor determining the susceptibility to the development of glomerulosclerosis in mice. We speculate that the superior effects of renal artery ligation plus cautery to produce glomerulosclerosis may result from higher blood pressure and fibrotic responses due to local ischemia activating the RAAS.

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