Role of Hypertension in Progressive Glomerular Immune Injury

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SUMMARY The relationship between hypertension, ferritin-antiferritin mesangial immune injury (FIC), and progressive glomerular damage was studied in hypertensive (8% NaCl chow) Dahl salt-sensitive rats (DS) and in spontaneously hypertensive rats (SHR). The glomeruli of SHR are protected from the increased perfusion pressure that accompanies systemic hypertension by preglomerular vasoconstriction, while the glomeruli of hypertensive DS are not. Blood pressure, serum creatinine levels, urinary protein excretion, and glomerular injury (assessed by semiquantitative morphometric analysis) were determined in 20-week-old SHR and DS with FIC. In addition, half of a group of 20-week-old SHR with FIC were uninephrectomized and progression of glomerular injury was assessed 12 weeks later. Control rats for each of the groups did not receive FIC. Our studies showed that more extensive mesangial expansion and glomerulosclerosis developed in hypertensive DS with FIC than in rats without FIC. Glomerular injury in DS with FIC affected cortical and deep glomeruli. Similarly, hypertensive SHR with FIC had minimal damage in cortical glomeruli. In deep glomeruli of SHR, mesangial expansion was similar to that of DS, but glomerulosclerosis was absent. In SHR, a 50% reduction in renal mass, a maneuver known to decrease preglomerular vasoconstriction, resulted in mesangial expansion similar to that in DS in cortical glomeruli while deep glomeruli developed mesangial expansion as well as glomerulosclerosis. Our results suggest that when hypertension and mesangial immune injury coexist with renal vasodilatation (as occurs in DS with 2 kidneys and in SHR after uninephrectomy), they act synergistically to induce progressive glomerular damage. Similar mechanisms may be operative in hypertensive humans with glomerulonephritis and may condition the rate of progression to renal insufficiency. (Hypertension 7: 398-404, 1985)

KEY WORDS • Dahl rats • spontaneously hypertensive rats • mesangium • glomerulosclerosis

In spite of a better understanding of the mechanisms that participate in immune injury, immunologically mediated glomerular disease continues to be the most common cause of end-stage renal failure. Eighty percent of patients with chronic glomerulopathies become hypertensive, which, in itself, may contribute to progression of the glomerular damage. Clinical studies have yielded conflicting results; while some studies have clearly shown that hypertension is a risk factor, other studies have been unable to confirm these findings. How can these divergent observations be explained? Are they mutually exclusive, or do they just represent a spectrum of the same phenomenon? A possible explanation is that similar levels of systemic hypertension in different individuals may be accompanied by different degrees of "glomerular hypertension." Progressive glomerular damage, however, may be dependent on the latter. Indeed, the relevance of "intrarenal hypertension" in the initiation and progression of various glomerulopathies has recently been stressed.

As this hypothesis cannot yet be tested in humans we have used experimental models. We studied the effects of glomerular ferritin-antiferritin immune complex disease (FIC), a model of discrete mesangial immune injury, in hypertensive Wistar-Okamoto spontaneously hypertensive rats (SHR) and in hypertensive Dahl salt-sensitive rats (DS). This model of immune-mediated renal disease was chosen because it is reproducible, it does not induce severe alterations in the glomerular architecture, and finally, because it resem-
bles certain human glomerulopathies such as IgA-IgG nephritis and early lupus nephritis.

Of extraordinary importance for our studies is that the hemodynamic determinants of glomerular filtration rate are markedly different in SHR and DS. Adult SHR have been shown to have normal whole kidney and single nephron glomerular filtration rate after hypertension has developed. Because of a marked increase in preglomerular arteriolar resistance, glomerular capillary pressure in cortical nephrons is normal in spite of severe systemic hypertension. Thus, glomeruli of SHR are protected from systemic hypertension. Hypertensive DS have normal whole kidney blood flow and glomerular filtration rate in spite of a 15% congenital decrease in the number of glomeruli. This is achieved by increments in glomerular blood flow and transglomerular hydraulic pressure that result in increased single nephron glomerular filtration rate. The glomerular hyperfiltration in DS results from decreased preglomerular arteriolar resistance.

Our results indicate that, at similar levels of systemic hypertension, the consequences of mesangial immune injury are different in SHR and DS. Severe glomerular injury developed in DS but not in SHR; however, uninephrectomy in SHR, a maneuver known to decrease preglomerular vasoconstriction, induced glomerular injury with characteristics similar to those observed in DS with two kidneys. Thus, these studies support the concept that systemic hypertension induces progressive glomerular damage primarily when accompanied by glomerular hypertension.

Methods

Male 6-week-old DS weighing approximately 130 g were purchased from Brookhaven National Laboratories, Brookhaven, New York. Male 6-week-old SHR weighing 120 to 140 g were purchased from Taconic Farms, Germantown, New York. All rats were housed two to a cage and had free access to water. The DS were fed standard rat chow that contained 8% NaCl according to the experimental design. The SHR were fed standard rat chow containing 0.3% NaCl. Rat chow was purchased from Ralston Purina Company (St. Louis, MO). The blood pressure was measured in unanesthetized rats by a tail cuff method with a physiograph MK IV (Narco Biosystems, Houston, TX). The blood pressure was measured during the morning in a quiet environment, and an average of three successive readings was recorded. The blood pressures were determined in all rats every 2 weeks.

Ferritin-Antiferritin Immune Complex Disease

We previously have described in detail the experimental model of FIC. Briefly, rats were immunized with twice crystallized cadmium-free equine spleen ferritin (Calbiochem-Behring Company, LaJolla, CA). The ferritin was administered intraperitoneally (i.p.) in a dose of 4 mg/100 g body weight, 5 days a week for 8 weeks. Control rats received 2 ml of distilled water i.p.

Experimental Design

Serum creatinine levels, 24-hour urinary protein excretion, and renal tissue were obtained from groups of rats at 20 and 32 weeks of age. Wedge-shaped renal tissue containing cortex and medulla was obtained at the time of uninephrectomy or death.

Twenty-six DS were divided into two groups (Figure 1). All rats were fed 8% NaCl from the sixth week of age until death. When the rats were 12 weeks of age, ferritin immunization was begun in 14 (Group II; Table 1) while the other 12 rats received distilled water i.p. (Group I; see Table 1). Immunization was completed at 20 weeks of age, when DS were killed.

Fifty-two SHR were included in the study (Figure 2). Twenty-eight SHR were divided into two groups; 14 received i.p. distilled water from the twelfth to the twentieth week (Group III; see Table 1), while the other 14 received ferritin (Group IV; see Table 1). At 20 weeks of age, 10 rats in each group were uninephrectomized and maintained up to 32 weeks of age, when survivors were killed (Groups VII and VIII; see Table 1). The remaining 24 rats were divided into two groups; 12 rats were immunized with ferritin from the twelfth to the twentieth week, while the other 12 rats received i.p. distilled water (Groups V and VI; see Table 1). Rats in these two groups were maintained up to 32 weeks of age, when survivors of both groups were killed. We chose 32 weeks as the end point to
study SHR because in pilot studies we found that we were able to appreciate changes in renal pathology (see Results). We did not want to use rats in which age-related changes could play a preponderant role in the glomerular pathology, and previous studies have shown that age-related changes are not present in 32-week-old SHR. 15

The initial number of rats in some of the groups differed from the number of rats studied at the completion of the study because (1) some rats died during immunization or operation and (2) rats were studied for 5 to 8 months. Mortality occurs over such a period for reasons that are not always clear as rats are found dead in their cages.

### Tissue Processing

The renal tissue obtained was processed for light and immunofluorescence microscopy according to techniques previously described. 11,14 All tissues were evaluated independently by two investigators without knowledge of the group to which the rat belonged. We defined glomerulosclerosis or hyalinization as the disappearance of cellular elements from the tuft, collapse of capillary lumens, and folding of the glomerular basement membrane with entrapment of amorphous material. The extent of increase in mesangial matrix and the degree of glomerulosclerosis were evaluated and scored separately. Mesangial matrix expansion was defined by the presence of increased amounts of periodic acid–Schiff positive material in the mesangial region. This change occasionally was accompanied by variable increases in cellularity. In some glomeruli the mesangial matrix expansion or the glomerulosclerosis affected only a small percentage of the glomerulus, while in others the process was diffuse. A semiquantitative score was developed to evaluate the degree of damage. 14 A minimum of 60 glomeruli (range, 60–100) in each specimen was examined, and the severity of the lesion was graded from 0 to 4+ according to the percentage of glomerular involvement. Thus, a 1+ lesion represented an involvement of 25% of the glomerulus, while a 4+ lesion indicated that 100% of the glomerulus was involved. An injury score was then obtained by multiplying the degree of damage (0–4+) by the percentage of the glomeruli with the same degree of injury (i.e., increase in mesangial matrix material or glomerulosclerosis). The extent of the injury for each tissue specimen was then obtained by adding these scores. Injury scores were evaluated independently for cortical and deep glomeruli. For that purpose, all glomeruli located up to 3 glomerular diameters below the capsule were considered to be cortical while the rest were considered deep glomeruli. As the injury score for individual tissue specimens derived by each investigator varied from 9 to 20%, the scores obtained by the two investigators were averaged.

### Analytical Methods and Statistical Analysis

The total urinary protein content was determined by the Biuret technique. 14 The serum creatinine level was determined colorimetrically by a modified Jaffe reaction. 11

Where applicable, data are expressed as mean ± sd. Significance of differences between all groups was determined by one-way analysis of variance; square root transformation was used as a variance-stabilizing device. Comparisons were assessed for significance by modified t statistics, and critical values were calculated by the method of Bonferroni. 16 Statistical significance was defined as p < 0.05.

### Results

#### Group 1

All rats in Group 1 (20-week-old DS fed 8% NaCl chow) were hypertensive and proteinuric (see Table 1). Histologically, their glomeruli demonstrated a dif-
fuse increase in mesangial matrix as well as evidence of glomerulosclerosis (see Figure 4). The mesangial injury score was similar in deep and in cortical glomeruli, 78.4 and 83.4 respectively; the deep to cortical glomeruli (D/C) ratio of mesangial injury was 1.09. Glomerulosclerosis affected cortical and deep glomeruli; however, it was slightly more accentuated in deep glomeruli, with a D/C ratio of sclerosis of 1.4. The intrarenal vessels showed increased thickening of the walls. There was scattered tubular dilatation that affected both cortical and juxtamedullary tubules, although it was more pronounced in the latter. Areas of fibrosis and cellular infiltrates were present, primarily surrounding areas of damaged tubules and glomeruli. Immunofluorescence microscopy revealed 1 to 2 + C3 in areas of glomerulosclerosis. Although C3 also was present in tubular basement membranes, it was particularly evident in the basement membranes of damaged tubules.

**Group II**

The mean blood pressure in Group II (20-week-old DS with FIC fed 8% NaCl chow) was similar to that of DS without FIC and of SHR (see Table 1). The urinary protein excretion and serum creatinine levels were higher than in any other group of rats studied (see Table 1). The morphological changes observed in these rats were characterized by a marked increment in mesangial matrix as well as widespread glomerulosclerosis (Figures 3 and 4). The mesangial injury score was 139 in cortical glomeruli and 164 in deep glomeruli; the D/C injury score was 1.06. A slightly greater degree of glomerulosclerosis was observed in deep glomeruli (D/C ratio of sclerosis 1.5). The intrarenal vasculature showed increased thickness of the vascular walls. Tubular dilatation and interstitial fibrosis were also marked. Immunofluorescence microscopy showed 2+ IgG and C3 in the mesangium with accentuation of C3 deposition in areas of sclerosis. Accen-

tuation in C3 deposition was also observed in tubular basement membranes of damaged tubules.

**Groups III and V**

The mean blood pressure in 20-week-old (Group III) and 32-week-old (Group V) SHR was similar. Urinary protein excretion of SHR was lower than in DS (see Table 1). In 20-week-old SHR superficial glomeruli had minimal mesangial alterations (injury score 38.3), which contrasted with mesangial injury in deep glomeruli (injury score 98.4; see Figure 4). The changes observed in 32-week-old SHR were similar to those observed in 20-week-old SHR except they were slightly accentuated (mesangial injury score, 41.3 in cortical and 110.5 in deep glomeruli). The D/C injury score was 2.6 in 20-week-old rats and 2.7 in 32-week-old rats. Glomerulosclerosis, however, was not observed in cortical or deep glomeruli in either 20-week-old or 32-week-old SHR. Tubulointerstitial changes were mild and present in both age groups. The intrarenal vasculature was thickened in all rats. Results of immunofluorescence microscopy were normal.

**Groups IV and VI**

The blood pressure in Groups IV (20-week-old SHR with FIC) and VI (32-week-old SHR with FIC) was similar to that observed in SHR without FIC (see Table 1). The morphological changes observed in Group IV were similar to those seen in SHR without FIC (Groups III and V), although they were more accentuated in cortical (injury score 48.8) as well as in deep glomeruli (injury score 136.0), which reflects the effects of mesangial FIC injury (see Figure 4). The D/C injury score was 2.8, similar to that of SHR without FIC (Groups III and V). The changes observed in 32-week-old rats (Group VI) were similar to those in Group IV. The injury score was 53.1 in cortical glomeruli and 149.0 in deep glomeruli. The D/C injury score was 2.8.
Group VII

Blood pressure in Group VII (1-kidney, 32-week-old SHR without FIC) was similar to that in two-kidney (2K) SHR (see Table 1). The morphological changes observed in these rats were markedly different in severity as well as in distribution (see Figure 4). Compared with 20-week-old and 32-week-old 2K SHR (Groups III and V respectively), the mesangial injury score in cortical glomeruli of one-kidney (1K) SHR increased by 77 and 64% respectively, while in deep glomeruli, the injury score increased 18 and 4% respectively. The D/C injury score was 1.7. In addition, glomerulosclerosis was not detected in 2K SHR, while it was evident in deep glomeruli of 1K SHR; injury score for sclerosis was 10.5. The intrarenal vasculature showed increased thickness of the vascular wall. Perivascular infiltrates of mononuclear cells were observed in some areas. Tubular dilatation and protein casts within the tubules were observed and associated with either areas of tubular atrophy or surrounding areas of glomerulosclerosis. Immunofluorescence microscopy showed 2 to 3 + IgG and C3 in the mesangium; C3 also was present in areas of focal sclerosis as well as in membranes of tubules, particularly atrophic ones.

Group VIII

The blood pressure in Group VIII (32-week-old, 1K SHR with FIC) was similar to that in the other groups of SHR and DS (see Table 1); however, these rats had marked mesangial expansion as well as increased glomerulosclerosis. Compared with 20-week-old and 32-week-old 2K SHR with FIC (Groups IV and VI respectively), the mesangial injury score increased by 130 and 110% in cortical glomeruli but only by 18 and 8% in deep glomeruli. The D/C injury score was 1.4 (see Figures 3 and 4). Proteinuria was also higher in Group VIII (see Table 1). Compared with 1K SHR without FIC (Group VII), the mesangial injury score was higher by 65% in cortical glomeruli and 38% in deep glomeruli. The incidence of glomerulosclerosis, which affected only deep glomeruli, was highest in this group of rats (injury score 31.5). The intrarenal vasculature showed widespread thickening of the vascular walls. Perivascular infiltrates of mononuclear cells also were observed scattered throughout. Tubulointerstitial changes were characterized by dilatation, casts within the tubules, and areas of peritubular fibrosis, particularly in juxtamedullary areas. Immunofluorescence microscopy showed 2 to 3 + IgG and C3 in the mesangium; C3 also was present in areas of focal sclerosis as well as in membranes of tubules, particularly atrophic ones.

Discussion

Hypertensive DS without FIC had mesangial expansion, progressive glomerulosclerosis, and proteinuria by 20 weeks of age. All these features were significantly magnified in hypertensive DS with FIC, which supports our previous studies that suggested that glomerular damage is fostered by the synergistic interaction between hypertension and mesangial immune injury. This synergism was evident whether hypertension occurred after mesangial immune injury or, as in the present study, preceded it. Furthermore, our previous study provided evidence that hypertension does not develop in Dahl salt-resistant rats with FIC and that they have strikingly less glomerular damage. The mean score of mesangial injury in cortical glomeruli of 20-week-old SHR with or without FIC was significantly lower than in DS, although the severity of hypertension was similar. Moreover, glomerulosclerosis was not observed in SHR but was present in cortical as well as deep glomeruli of DS with or without FIC. A propensity toward a greater degree of glomerulosclerosis in deep versus cortical glomeruli of DS was observed in the present experiments but not in our pre-
vious studies. The reasons for this observation are unclear but could be related to the age at which high salt diet was initiated or to the length of time of hypertension or to both. An evaluation of the distribution of mesangial injury in SHR and DS indicated that deep glomeruli in SHR had consistently more injury than superficial glomeruli, while mesangial injury in DS was similarly distributed (see Figure 4). Indeed, the ratio of mesangial injury score between deep and cortical glomeruli was less than 1.1 in DS, but between 2.5 and 2.8 in SHR with and without FIC respectively. Higher glomerular flows and pressures may have led to greater deposition of immune reactants in all glomeruli in DS with FIC but only in deep glomeruli in SHR with FIC. Strain differences in the immune response to ferritin also may have played a role. Notwithstanding, this difference, if existent, was difficult to discern judging by the similar intensity of immunofluorescence staining in glomeruli of SHR and DS. However, immunofluorescence microscopy is not sensitive enough to differentiate subtle differences, particularly when the intensity of immunofluorescence is high.

To determine if mild glomerular injury in SHR was due at least in part to protective preglomerular vasoconstriction, we performed unilateral nephrectomy in groups of 20-week-old SHR with and without FIC. As immunization with ferritin was stopped at 20 weeks, the role played thereafter by active immune injury in SHR with FIC should have been minimal. This experimental maneuver was predicated on studies that have demonstrated that a 50% reduction in renal mass results in adaptive changes in the remaining kidney that are characterized by afferent arteriolar dilatation and increased renal blood flow and glomerular filtration.

Between 20 and 32 weeks of age, the 2K SHR had only a 10 to 13% increment in mesangial injury score, and glomerulosclerosis did not develop; the predominance of injury in deep glomeruli was maintained. On the other hand, 1K SHR with and without FIC manifested striking changes in the degree of glomerular injury as well as in the distribution of glomerular damage between deep and superficial glomeruli (see Figure 4). This change was due to a disproportionate increment in mesangial expansion in superficial glomeruli, 77% in SHR without FIC and 130% in SHR with FIC. In addition, glomerulosclerosis developed in 1K SHR with or without FIC, a phenomenon that was not observed in 2K SHR. These findings indicate that uninephrectomy in SHR mimics the renal morphological and functional changes that occur naturally in 2K DS, which have high glomerular flows and pressures accompanied by widespread glomerular injury. Indeed, Bank et al. found that uninephrectomy in SHR resulted in a 130% increment in whole kidney glomerular filtration rate and renal blood flow. The single nephron glomerular filtration rate and glomerular blood flow in superficial nephrons only increased by approximately 50%; however, deep single nephron glomerular filtration rate was twofold greater than in superficial ones. Hence, the difference in glomerular damage between deep and superficial glomeruli in SHR may be linked to the disparity in flows and pressures between the two populations of nephrons.

Our results support these contentions and add a new dimension in explaining the pathophysiology of progressive glomerular damage in glomerulonephritis associated with hypertension.

In SHR with mesangial immune injury, unilateral renal ablation resulted in a marked increase in mesangial expansion but no glomerulosclerosis in superficial glomeruli. In deep glomeruli, very high filtration rates, probably the consequences of increased pressure as well as flow, resulted not only in mesangial expansion but also in glomerulosclerosis. Mesangial expansion and glomerulosclerosis are often forerunners of progressive renal destruction. Why the glomerular mesangium, particularly when affected by immune injury, is susceptible to the effects of high pressures and flows is at present unknown. We have previously shown an association between altered glomerular hemodynamic determinants, structural changes, and abnormal mesangial function. In addition, Olson et al. have shown increased trapping of macromolecules in the mesangium of rats with reduced renal mass. It is evident that alterations in flows and pressures disturb the mesangial traffic of macromolecules, which in turn can lead, through unknown mechanisms, to progressive mesangial injury. On the other hand, mesangial injury itself could decrease glomerular compliance or disturb autoregulation of the glomerular blood flow or do both. If this were to occur, the damage induced by high flows and pressures could be magnified. Analogies between experimental models and human disease are always speculative; however, the studies reported herein may offer some insight into the pathophysiology of progressive glomerular destruction in hypertensive patients with nephritis. These studies may also have therapeutic implications.

Based on the changes observed in DS, we submit that given similar degrees of glomerular immune injury, progressive damage will be more severe and occur earlier in hypertensive individuals in whom deficient preglomerular vasoconstriction results in glomerular hypertension early in the course of their disease. This propensity could be genetically determined, owing to a decreased number of glomeruli, and inherited concomitantly with a predisposition to hypertension. Diminished preglomerular vasoconstriction also could be an abnormal inherited response of the glomerular vasculature to injury mediated by an unbalanced production of vasoactive substances. Whatever the mechanism, these patients would belong to those populations in which hypertension has been found to play a clear role in the progression of glomerulopathies. On the other hand, in hypertensive patients with effective preglomerular vasoconstriction, as in the SHR, clinical studies would not reveal a clear relationship between systemic hypertension and progressive glomerular damage. In the latter group hypertension may induce a clinically evident progression in glomerular injury only when (1) systemic hypertension...
is so severe that it overcomes the protective preglomerular vasoconstriction and/or (2) progressive reduction in renal mass induces adaptive changes that expose glomeruli to the effects of systemic hypertension. In any case, our studies indicate that hypertension is a definite risk factor in progressive glomerular damage. They also suggest that (1) patients with deficient preglomerular vasoconstriction may require early, more aggressive, and closely supervised therapy and (2) in the selection of antihypertensive agents for patients with glomerulonephritis consideration should be given to the effects of these agents on glomerular hemodynamics.25,26

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References

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