Systemic Factors Are Involved in the Pathogenesis of Proteinuria-Induced Glomerulosclerosis in Adriamycin Nephrotic Rats

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Abstract. This study aims to dissociate the respective roles of systemic nephrosis and of the intrarenal effects of proteinuria in the pathogenesis of focal segmental glomerulosclerosis (FGS) in adriamycin nephrosis. To this purpose, this study examined proteinuria and FGS in bilateral (BAP) and unilateral proteinuria (UAP) in two different rat strains. UAP was obtained by protecting one kidney from exposure to adriamycin by temporary clipping of one renal artery during adriamycin injection. At sacrifice (week 12), FGS was present in BAP and in exposed kidneys in UAP, but not in unexposed kidneys. FGS correlated significantly with proteinuria per kidney in BAP and UAP. Remarkably, for a given proteinuria per kidney, the sclerosis score was higher in BAP than in UAP, reflected by a higher ratio of FGS score per mg proteinuria per kidney (Wistar: 0.09 ± 0.01 in BAP versus 0.05 ± 0.01%/mg protein per d in UAP, P < 0.05; Lewis: 0.12 ± 0.01 in BAP versus 0.07 ± 0.01%/mg protein per d in UAP, P < 0.05), indicating that the local damaging effects of proteinuria are modified by other factors. Cholesterol correlated with total proteinuria in BAP and UAP. FGS score was positively correlated with cholesterol. The latter correlation was similar in BAP and UAP, indicating that cholesterol was a more uniform predictor for FGS than proteinuria per kidney. This was independent of strain-specific factors. On multilinear regression analysis, cholesterol turned out to be the most consistent predictor of FGS in proteinuric kidneys, with a stronger predictive value than proteinuria per kidney. It is concluded that although systemic sequelae of nephrosis do not induce renal damage in nonproteinuric kidneys, they modify the severity of proteinuria-induced FGS in proteinuric kidneys.

Proteinuria is associated with progressive renal function loss in humans as well as in experimental renal disease (1). The severity of proteinuria may be a marker of the severity of renal damage. However, a causal role of proteinuria in the development of focal segmental sclerosis, the alleged final common pathway of renal function loss, has also been hypothesized (2). The mechanism of such a causal role of proteinuria in the development of focal segmental sclerosis is still incompletely elucidated. Exposure of renal tissue to local passage of leaked proteins may evoke structural changes in the nephron leading to focal glomerulosclerosis (FGS) (2–5). On the other hand, proteinuria also induces systemic abnormalities such as dyslipidemia (6–9) and hypercoagulability that by themselves may induce renal damage, regardless of the local effects of protein leakage in the kidney. Thus, several systemic as well as intrarenal mechanisms may be involved in the pathogenesis of proteinuria-associated focal segmental sclerosis.

The relative roles of the systemic and the intrarenal factors have not been well defined. Therefore, in the present study we wish to delineate the respective roles of the condition of systemic nephrosis and of the direct renal effects of proteinuria in the pathogenesis of FGS in the Adriamycin nephrotic rat model.

To dissociate systemic and local factors, we induced bilateral and unilateral proteinuria, respectively. The latter is obtained by protecting one kidney from exposure to adriamycin by temporary clipping of one renal artery during adriamycin injection (3). Thus, both kidneys are exposed to the same systemic nephrotic condition, but the proteinuric load is different for the two kidneys. To assess the effect of the systemic condition of nephrosis, we determined FGS in the nonexposed kidney in unilateral proteinuria. The exposed kidneys in unilateral proteinuria, as well as animals with conventional (bilateral) Adriamycin-induced proteinuria, served as controls, subject to systemic as well as local factors.

Several authors have observed that development of FGS in response to proteinuria is affected by strain-specific characteristics (10–12). To enhance the external validity of our study, therefore, we performed the above study in our usual model of Adriamycin nephrosis not only in the Wistar rat, but also in the Lewis rat strain.
Materials and Methods

Adult male Wistar and Lewis rats were studied (Harlan, Zeist, The Netherlands). Throughout the experiment, the animals were housed in a temperature-controlled room with a 12-h light/dark cycle. All animals were fed a low sodium diet (0.05% NaCl, 20% protein, Hope Farms, Woerden, The Netherlands). They received daily fresh tap water ad libitum. Once a week during the entire protocol of 12 wk, rats were weighed, 24-h urine samples were collected in metabolic cages, with the rats allowed free access to food and water, and BP was measured. Food intake and water intake were measured weekly; these parameters were not different for the studied groups throughout the experiment. At the end of the study, blood was collected, kidneys were perfused with saline, the animals were sacrificed, and the kidneys were harvested for histologic examination.

Protocols

The protocols were conducted in accord with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Committee for Animal Experiments of the State University Groningen.

Protocol 1a: Local versus Systemic Effects of Proteinuria in the Development of FGS. For this protocol the following groups of animals were studied.

Bilateral Adriamycin Proteinuria (BAP, n = 24). In this group of Wistar rats, standard adriamycin nephrosis (i.e., urinary protein leakage from both kidneys) was induced. The animals were anesthetized with isoflurane/O₂ (4% induction, 1.5% during operation, O₂ 1 L/min), the abdominal cavity was opened through a midline incision, and a sham clip was placed on the left renal artery. Adriamycin (1.5 mg/kg) was injected in the penis vein. This dose of adriamycin was chosen to induce a modest and stable proteinuria, as confirmed by prior experiments in our laboratory (13). Twelve minutes after injection, the abdominal incision was sutured. At the time of disease induction, mean body weight was 312 ± 12 g. The overall mortality throughout the experiment was 4%.

Unilateral Adriamycin Proteinuria (UAP, n = 24). In this group of Wistar rats, unilateral proteinuria was induced by exposing only one kidney to adriamycin. Animals were anesthetized as described above. The abdominal cavity was opened through a midline incision and a clamp was placed on the left renal artery. Adriamycin was injected immediately after clipping. After 12 min, the clamp was removed and the abdominal incision was sutured. At disease induction, body weight was 309 ± 14 g. The overall mortality throughout the experimental period was 13%.

Time Controls (CON, n = 12). This group of Wistar rats underwent no surgery or adriamycin injection and served as a healthy control group. At the start of the study, the body weight was 325 ± 9 g. The overall mortality was 8%.

Protocol 1b: Contribution of the Exposed and Nonexposed Kidney to 24-h Protein Excretion in UAP. To assess the contribution of the exposed and nonexposed kidney to 24-h protein excretion, the exposed kidney was removed after stabilization of proteinuria, i.e., 6 wk after induction, in a group of UAP Wistar rats with an initial body weight of 319 ± 17 g (UAPNx, n = 12). After uninephrectomy, proteinuria was measured for an additional 2 wk. The overall mortality was 0%.

Protocol 2: Strain Dependency. In a separate experiment, we studied BAP (n = 11) and UAP (n = 12) in Lewis rats to test for possible strain dependency of our results. To accomplish a comparable proteinuria to Wistar rats, adriamycin was injected in a dose of 2 mg/kg. Otherwise, the protocol was similar to protocol 1a.

Design

Animals were trained before study entry to become accustomed to handling and to the BP measurements. Systolic BP was measured in conscious rats with an automated multichannel system (Apollo 179; IITC Life Science, Woodland Hills, CA). This system uses tail cuffs and photoelectric sensors to detect the tail pulse. The rats were placed in the test chamber in restrainers while temperature was regulated and maintained at 28 to 29°C. During each BP measurement session, three measurements were recorded for each animal. The BP was taken as the mean of these three recordings (14).

Urinary creatinine concentration was determined by HPLC. Urinary protein concentration was determined by a biuret method (Bioquant, Merck, Darmstadt, Germany). Proteinuria per kidney was estimated as 50% of total proteinuria in BAP and as total proteinuria in UAP. Plasma cholesterol concentration was determined by an enzymatic colorimetric test (CHOD-PAP; Boehringer Mannheim, Germany). Kidney weight was determined as wet weight of the kidneys.

Tissue processing was performed as described earlier by van Goor et al. (15). In short, coronal tissue slices were placed in 2% paraformaldehyde at 4°C and fixed for 3 h. After fixation they were washed overnight in phosphate-buffered saline containing 6% sucrose. The next morning the specimens were dehydrated in 100% acetone, then infiltrated in Technovit 8100 solution A (Kulzer, Wehrheim, Germany). Subsequently, one part of the accelerator Technovit Solution B was added to 30 parts of tissue containing solution A. After embedding, paraffin was poured around the block holders to prevent inhibition of the polymerization by atmospheric oxygen. Polymerization was accomplished overnight at 4°C. Two-micrometer sections were cut, and the tissue blocks were stored at −20°C.

For determination of FGS, the sections were stained by the periodic acid-Schiff technique. FGS was scored semiquantitatively on a scale of 1 to 4. FGS was scored positive when mesangial expansion, mesangial cellularity, adhesion formation, and capillary obliteration were present in one segment. If 25% of the glomerulus was affected, a score of 1 was given, 50% was scored as 2, 75% as 3, and 100% as 4. The ultimate score is then obtained by multiplying the degree of change by the percentage of glomeruli with the same degree of injury and adding these scores. Interstitial changes were considered to be present if there was an increase in interstitial volume characterized by interstitial fibrosis and mononuclear cell infiltrate. Interstitial changes were scored semiquantitatively as follows: 0% of the interstitium is expanded; 0 to 10% of the interstitium is expanded; 10 to 20% of the interstitium is expanded; and >20% of the interstitium is expanded. Mesangial cellularity was assessed by counting the number of cell nuclei in each mesangial region. Nuclear count exceeding three per region were considered to represent increased segmental mesangial cellularity. A total of 50 glomeruli per kidney was scored, moving from cortex to medulla.

Statistical Analyses

Data are expressed as mean ± SEM. Statistical analyses were performed by an unpaired t test. Comparison between groups for the correlation between FGS and proteinuria was performed by a non-parametric test (Welch). Statistical significance was assumed at the 5% level. To estimate the independent contribution of intrarenal and systemic factors in the severity of FGS, multilinear regression analysis was performed with FGS as the dependent variable, and proteinuria per kidney (reflecting the contribution of intrarenal factors), BP, and cholesterol (reflecting systemic factors) as independent variables. In addition, to account for effects of possible unidentified differences
between rats with unilateral versus bilateral proteinuria, we included the model characteristics BAP or UAP in the analysis as an independent dummy variable.

Results

Mean values for body weight, kidney weight, systolic BP, proteinuria, urinary creatinine, and plasma cholesterol at the end of the experiment (week 12 after adriamycin) are given in Table 1 for protocols 1a and 2. It shows that body weight was comparable in the adriamycin-treated BAP and UAP groups in Wistar as well in Lewis rats. It also shows that body weight in the adriamycin-treated rats was only slightly lower than in control rats, consistent with a good overall condition of our proteinuric rats.

Kidney weight was higher in BAP compared with UAP in both strains. In BAP, the kidney weight was positively correlated with proteinuria (Wistar, \( r = 0.71, P < 0.01 \)), whereas in UAP the weight of each kidney was not correlated to proteinuria. BP was similar in all groups, and no statistical differences were found throughout the time course of the experiment. Urinary creatinine excretion was comparable in all groups.

As expected, proteinuria was present in both BAP and UAP. Mean proteinuria of the UAP rats was approximately half of that in BAP in both strains. In BAP and UAP, proteinuria was associated with a significantly increased cholesterol, indicating that the proteinuria had induced a state of nephrosis. Accordingly, hypercholesterolemia in BAP was nearly twice as high as in UAP \( (P < 0.001) \) in both strains.

Figure 1 shows the time course of proteinuria in BAP and UAP in Wistar rats. Adriamycin induced a marked proteinuria that leveled off 5 to 6 wk after injection. This figure demonstrates that the difference in proteinuria between BAP and UAP was present throughout the study. In Lewis BAP and UAP, a comparable pattern was observed (data not shown). After removal of the adriamycin-exposed kidney in protocol 1b, proteinuria immediately fell to values almost comparable with control values (Figure 2), suggesting that the exposed kidney indeed accounted almost completely for the observed proteinuria. This was accompanied by a significant lowering of cholesterol to control values (Table 2).

Data on mean FGS scores are given in Figure 3. Glomerulosclerosis was present in BAP and in the exposed kidney in UAP in both strains. In the nonexposed kidney in UAP, glo-

![Figure 1. Time course of urinary protein excretion (mean ± SEM) after injection of adriamycin at week 0 in bilateral (BAP) and unilateral (UAP) proteinuria (Wistar).](image1)

![Figure 2. Time course of urinary protein excretion (mean ± SEM) after injection of adriamycin in UAP uninephrectomized rats (UAPNx) and control rats (CON) (Wistar). In UAPNx, the exposed kidney was removed at week 6.](image2)

Table 1. Parameters at the end of the study for the various groups in Wistar and Lewis rats a

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wistar</th>
<th>Lewis</th>
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<tr>
<td></td>
<td>BAP</td>
<td>UAP</td>
</tr>
<tr>
<td><strong>BW (g)</strong></td>
<td>466 ± 7 b</td>
<td>466 ± 7 b</td>
</tr>
<tr>
<td><strong>KW (g)</strong></td>
<td>2.1 ± 0.1 b,c</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>142 ± 7</td>
<td>143 ± 5</td>
</tr>
<tr>
<td><strong>U prot (mg/24 h)</strong></td>
<td>495 ± 59 b,c</td>
<td>232 ± 32 b</td>
</tr>
<tr>
<td><strong>U creat (mg/24 h)</strong></td>
<td>18 ± 2</td>
<td>17 ± 1</td>
</tr>
<tr>
<td><strong>P chol (mmol/L)</strong></td>
<td>5.9 ± 0.7 b,c</td>
<td>3.1 ± 0.2 b</td>
</tr>
</tbody>
</table>

a Results are given as means ± SEM. BAP, bilateral adriamycin proteinuria; UAP, unilateral adriamycin proteinuria; CON, time controls; BW, body weight; KW, kidney weight (in UAP, kidney weight of adriamycin-exposed kidney is shown); SBP, systolic blood pressure; Uprot, urinary protein; Ucreat, urinary creatinine; Pchol, plasma cholesterol; ND, not determined.

b \( P < 0.01 \) versus CON (Wistar).

c \( P < 0.01 \) BAP versus UAP (Wistar).

d \( P < 0.01 \) BAP versus UAP (Lewis).
merulosclerosis score was not different from control. Glomerulosclerosis score was approximately twice as severe in BAP than in the exposed kidney in UAP in both strains. Interstitial injury score in BAP (Wistar: 0.56 ± 0.11; Lewis: 0.89 ± 0.24) and in the exposed kidneys in UAP (Wistar: 0.42 ± 0.16; Lewis: 0.66 ± 0.22) was significantly different from control (0.05 ± 0.05, P < 0.001). The interstitial injury in BAP tended to be higher than in UAP, but due to the wide scatter this difference did not reach statistical significance in either strain. Interstitial injury was closely and positively correlated to glomerulosclerosis score in both strains (Wistar: BAP, r = 0.72, P < 0.01; UAP, r = 0.87, P < 0.001; Lewis: BAP, r = 0.86, P < 0.001; UAP, r = 0.92, P < 0.001). Mesangial cellularity in BAP (Wistar: 86 ± 6, Lewis: 97 ± 9) and in the exposed kidneys in UAP (Wistar: 72 ± 7; Lewis: 81 ± 8) was significantly increased compared with control (55 ± 2, P < 0.01), and closely correlated to FGS. However, there was no significant difference in mesangial cellularity between BAP and UAP.

The quantitative relationship between proteinuria per kidney and sclerosis score in that kidney was determined in unilateral and bilateral adriamycin-induced proteinuria to obtain an estimate index of the damaging effects of the glomerular protein leakage. This relationship is presented in Figure 4 for Wistar rats (top panels) and for Lewis rats (bottom panels). It shows that a greater urinary protein loss per kidney was consistently associated with more severe glomerulosclerosis in both UAP and BAP. Interestingly, this relationship was twice as steep in BAP as in UAP, indicating that in BAP a given protein leakage per kidney is associated with a glomerulosclerosis score twice as severe as in UAP. This was true for both strains. This was reflected by a significantly higher ratio of FGS/proteinuria per kidney in BAP compared with UAP in both strains (Wistar: 0.09 ± 0.01 versus 0.05 ± 0.01%/mg protein per d, P < 0.05; Lewis: 0.12 ± 0.01 versus 0.07 ± 0.01%/mg protein per d, P < 0.05).

The relation between cholesterol, as an index of the systemic state of nephrosis, and glomerulosclerosis in UAP and BAP is presented in Figure 5 for Wistar rats (top panels) and Lewis rats (bottom panels). A higher plasma cholesterol is consistently associated with more severe glomerulosclerosis in both UAP and BAP. This relationship is comparable in both strains. Furthermore, this relationship was similar for BAP and UAP in both strains, indicating that for a given cholesterol, the FGS score is similar in BAP and UAP. Cholesterol values were higher in BAP than in UAP, with accordingly a higher FGS score in BAP.

Multiple linear regression analysis (Tables 3 and 4) revealed that the severity of systemic nephrosis, as indicated by cholesterol, was the best independent predictor of FGS in both strains. Remarkably, proteinuria per kidney had only a weak predictive value. BP had no predictive value for FGS in either strain. To test for bias due to possible unidentified differences between animals with bilateral versus unilateral proteinuria, we also tested for unilateral versus bilateral proteinuria as a dummy variable, but this appeared to have no independent predictive value.

### Discussion

In this study in unilateral and bilateral adriamycin-induced proteinuria, focal segmental sclerosis was found in both kidneys in BAP. In UAP, focal segmental sclerosis was present in the exposed kidney, but not in the nonexposed kidney, indicating that the systemic manifestations of nephrosis in UAP did not induce sclerosis in the nonexposed kidney. In accord with previous studies, more severe proteinuria was associated with more severe FGS in exposed kidneys (3–5,13,16,17). This is consistent with the assumption that local effects of proteinuria per se play a role in the development of sclerotic damage, i.e., that local protein leakage appears to be a prerequisite for the development of glomerulosclerosis.

We found no significant FGS in nonexposed kidneys in UAP. We have no direct information on proteinuria from the nonexposed versus the exposed kidneys in UAP. However,
removal of the exposed kidney in UAP reduced proteinuria to almost control values, indicating that proteinuria in UAP is almost completely accounted for by the exposed kidney. Total proteinuria was twice as high in BAP than in UAP. Taken together with the disappearance of proteinuria after removal of the exposed kidney in UAP, this strongly suggests that in our model adriamycin exposure induces a similar protein leakage per kidney in BAP and UAP, with absence of proteinuria in the nonexposed kidney in UAP.

Remarkably, compared with UAP, sclerosis score was twice as severe in BAP for a given proteinuria. Thus, for any given value of proteinuria per kidney, the associated sclerotic damage was twice as high in BAP. This strongly suggests that other factors besides the local effects of proteinuria are involved in the development and severity of sclerosis. Interstitial damage was present in both BAP and in the exposed kidneys in UAP in both strains, and closely and positively correlated with FGS. Accordingly, interstitial damage tended to be more severe in BAP than in UAP, but this difference did not reach statistical significance. Mesangial cellularity was increased in BAP and in exposed kidneys in UAP in both strains, but no difference was observed in mesangial cellularity between BAP and UAP.

What factors could explain the more severe FGS in BAP? First, BP was similar in BAP and UAP and therefore cannot explain the differences in FGS. Second, intrarenal hemodynamics should be considered (1,18–21). In adriamycin nephrosis as well as puromycin aminonucleoside nephropathy, proteinuria as such is considered a main factor in the pathogenesis of FGS, whereas the role of glomerular hypertension (18) and/or hypertrophy seems to be relatively minor or absent, at least in the absence of renal ablation (22) or excessive sodium intake (23). A role for renal hemodynamic differences between both kidneys in UAP, however, cannot be excluded, since we did not measure renal hemodynamics. Thus far, no data on intrarenal hemodynamics have been reported for unilateral adriamycin nephropathy, whereas a study in unilateral puromycin aminonucleoside nephropathy reported a normal GFR and single nephron GFR in the unexposed kidney, with a reduced GFR and single nephron GFR in the exposed kidney (24). Third, mesangial cellularity was the same in UAP and

Figure 4. The correlation between proteinuria per kidney and the FGS score in BAP and UAP for Wistar rats (top panels) and Lewis rats (bottom panels). In BAP, data for both kidneys are presented.
BAP, indicating that differences in mesangial cell influx cannot explain the differences in glomerulosclerosis between BAP and UAP. There is evidence to suggest that interstitial changes may be a primary factor in adriamycin-induced glomerular damage (3). The close correlation we found between interstitial damage and FGS could suggest such a cause-and-effect relationship; alternatively, FGS and interstitial damage could both be consequences of proteinuria by distinct mechanisms.

As in our study, the difference in interstitial damage between BAP and UAP was less prominent than the difference in FGS between BAP and UAP—and in fact did not reach statistical significance. Hence, it would not be warranted to attribute the difference in FGS between BAP and UAP to differences in interstitial damage. Finally, systemic metabolic differences between BAP and UAP could be involved. Multiple metabolic abnormalities occur in proteinuria, such as hyperlipidemia and hypercoagulability, that have been implicated in progressive renal damage (6–9). In our study, we measured cholesterol as an index of the severity of the state of systemic nephrosis.

**Table 3.** Best fitting multiple linear regression of FGS in Wistar rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>b</th>
<th>SEM</th>
<th>beta</th>
<th>t</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>−19.31</td>
<td>5.1</td>
<td>−3.76</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>U_prot</td>
<td>0.068</td>
<td>0.03</td>
<td>0.30</td>
<td>2.55</td>
<td>0.013</td>
</tr>
<tr>
<td>P_chol</td>
<td>5.33</td>
<td>1.19</td>
<td>0.52</td>
<td>4.45</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Table 4.** Best fitting multiple linear regression of FGS in Lewis rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>b</th>
<th>SEM</th>
<th>beta</th>
<th>t</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>−18.59</td>
<td>8.2</td>
<td>−2.26</td>
<td>0.032</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P_chol</td>
<td>7.96</td>
<td>1.21</td>
<td>0.78</td>
<td>6.57</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\*n = 44, squared multiple predictor $P_{chol} r = 0.55, P < 0.001$

\*n = 21, squared multiple predictor $P_{chol} r = 0.59, P < 0.001$.

**Figure 5.** The correlation between plasma cholesterol and the FGS score in BAP and UAP in Wistar rats (top panels) and Lewis rats (bottom panels).
Indeed, in BAP and UAP there was a strong and similarly positive correlation between the degree of total proteinuria and cholesterol. Moreover, multiple stepwise regression analysis identified hypercholesterolemia as the major contributor predicting FGS in both strains. Because hyperlipidemia can enhance renal damage (6–9), hyperlipidemia might be a causal factor in the greater sclerotic damage in the BAP kidneys. Alternatively, cholesterol might be a marker for the presence of other potential deleterious factors of the nephrotic state, such as hypercoagulability. Removal of the exposed, proteinuric kidney in UAP led to a decline of both proteinuria and plasma cholesterol, further illustrating the strong relationship between proteinuria and hypercholesterolemia. In UAP, in the nonexposed kidney, sclerosis was not significantly different from control, despite the state of nephrosis, as indicated by the elevated cholesterol. Thus, apparently, the systemic factors that are able to amplify the renal damage in proteinuric kidneys are not able to initiate renal damage in nonproteinuric kidneys.

Finally, we observed a difference in wet kidney weight between BAP and UAP in both strains. In BAP, wet kidney weight was higher than in UAP. In addition, kidney weight was positively correlated with proteinuria in BAP, whereas no such correlation was present in UAP for either kidney. On visual inspection, BAP kidneys were pale and edematous. Kidney weight in BAP presumably reflects the severity of the proteinuria-induced sodium retention. In UAP, the nonproteinuric kidney excretes the excess sodium and thus precludes the development of edema (22). Whether the presence of renal edema in BAP enhances the susceptibility to glomerular sclerosis is unknown at present.

Our findings of a partial dissociation between the severity of proteinuria and FGS in UAP are in accord with those of Bertani et al. (3). These authors found only subtle sclerotic lesions in the exposed kidney in the unilateral adriamycin nephrotic rat, despite severe proteinuria, and suggest that in UAP the severity of proteinuria and sclerotic damage become dissociated. Our data, however, show that in UAP the association between the severity of proteinuria and sclerotic damage is still apparent, but that this relationship appears to be attenuated compared with BAP. This could well indicate that the renal damage is initiated by the local effects of proteinuria, but that systemic factors modify the severity of glomerular focal sclerosis in adriamycin-exposed proteinuric kidneys. However, in our model these alleged systemic factors were not able to induce sclerotic damage in nonexposed, nonproteinuric kidneys.

Our findings are of considerable potential relevance to clinical practice. Proteinuria is the single most powerful determinant of the rate of renal function loss in renal patients. During intervention treatment, unfortunately, proteinuria can often not be reduced completely. The severity of rest-proteinuria predicts the subsequent rate of renal function loss (13,19). Our findings indicate that the degree of renal damage induced by a certain protein leak is in principle modifiable, as systemic factors appear to affect the severity of renal damage. Thus, in addition to proteinuria lowering, lipid lowering, even if proteinuria persists, could have a considerable impact on eventual renal damage. Elucidation of the factors that modify the damaging effects of proteinuria may guide the development of additional renoprotective strategies, such as antilipidemic treatment.

In conclusion, there is a close quantitative relationship between the severity of proteinuria and focal segmental sclerosis, suggesting that the local effects of glomerular protein leakage are important in the development of FGS. However, systemic factors appear to be of prime importance in the pathogenesis of glomerulosclerosis in this model. Although these systemic factors are not able to induce sclerosis in a nonexposed, nonproteinuric kidney, they are able to markedly amplify the severity of sclerosis in a kidney with an adriamycin-induced glomerular protein leakage. Because these findings in the Wistar rat could be reproduced in the Lewis rat, the results are apparently not due to a strain-specific contingency. Additional studies are needed to elucidate the interaction between the systemic condition of nephrosis and the local effects of proteinuria in the pathogenesis of focal segmental sclerosis.

Acknowledgment

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