Development of Vascular Pole-Associated Glomerulosclerosis in the Fawn-Hooded Rat

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Abstract. Fawn-hooded hypertensive (FHH) rats constitute a spontaneous model of chronic renal failure with early systemic and glomerular hypertension, proteinuria, and development of focal and segmental glomerulosclerosis. The goal of the present study was to elucidate a step-by-step sequence of histopathologic events leading from an initial glomerular injury to segmental sclerosis. Segmental sclerosis in the FHH rat is consistently associated with the glomerular vascular pole. The initial injury involves the expansion of primary branches of the afferent arteriole. Apposition of those capillaries to Bowman’s capsule, together with the degeneration and detachment of corresponding podocytes, allows parietal cells to attach to the naked glomerular basement membrane of this capillary, i.e.,, allows the formation of a tuft adhesion to Bowman’s capsule. The adhesion enlarges to a broad synchia by encroaching to neighboring capillaries, apparently based on progressive podocyte degeneration at the flanks of the adhesion. Capillaries inside the adhesion—before undergoing collapse or hyalinization—appear to stay perfused for some time and to maintain some kind of filtration misdirected toward the cortical interstitium. Thereby, a prominent paraglomerular space comes into existence, enlarging in parallel with the adhesion. Toward the cortical interstitium this space is delimited by a layer of sheet-like fibroblast processes, which has obviously been assembled in response to the formation of this space. Toward the urinary space, the paraglomerular space is demarcated by the parietal epithelium and by the interface between the adhesion and the “intact” tuft remnant. Thus, the sclerotic tuft portions all become enclosed within the paraglomerular space. (J Am Soc Nephrol 9: 381–396, 1998)

Male hypertensive Fawn-hooded (FHH) rats are characterized by a mild increase in systemic BP, an elevated glomerular capillary pressure, progressive albuminuria, and the spontaneous development of glomerulosclerosis (1–4). The incidence and severity of the glomerular structural lesions increase with time, but they appear to be present already in young animals (2). Eventually, FHH rats proceed to chronic renal failure at, on average, approximately 1 yr of age. Glomerular hypertension is supposed to be a crucial factor in initiating and maintaining the progressive development of the kidney lesions (5).

Earlier studies (6–8), using different strains of Fawn-hooded (FH) rats, have elucidated several aspects of glomerular degeneration in this model, including prominent damage of podocytes, dilation of glomerular capillaries, and formation of tuft adhesions, but little changes in the mesangium. However, these studies do not provide a complete picture of how sclerosis develops in this model. They contain evidence that the podocyte lesions crucially account for the damaging process (6), but a plausible step-by-step sequence of events eventually terminating in segmental sclerosis has not been elucidated. This question is addressed in the present study, i.e., how, not primarily why, glomeruli degenerate in this genetic model. In agreement with what is observed in a great variety of models (9–16), the development of glomerulosclerosis in the FHH rat is based on the formation of tuft adhesions to Bowman’s capsule (BC), progressing to segmental sclerosis. A characteristic feature of this development in the FHH rat is its constant initiation at primary branches of the afferent arteriole (AA).

Materials and Methods
Experiments were performed in nine male inbred FHH and six male inbred normotensive Wistar Albino Glaxo (WAG) rats, approximately 6 mo old at the time of the study. The FHH rats were bred at the animal facilities of Erasmus University (Rotterdam, The Netherlands). The WAG rats were purchased from Harlan/SD (Zeist, The Netherlands). Animals were housed in standard rat cages. They were fed a normal rat chow containing 24% protein (AM II, Hope Farms, Woerden, The Netherlands) and water ad libitum.

To assess functional renal damage, rats were placed in macrolon metabolic cages for urine collection. After a 2-d adaptation period, two consecutive 24-h urine samples were obtained to determine the urinary albumin excretion; albumin concentration was measured with the bromcresol green method.

The systolic BP (SBP) was measured in conscious rats with the tail-cuff method. The rats were trained to adapt to the procedure. At least three SBP readings were taken on three consecutive days. The mean of the three daily averages was used as the SBP value for each rat.

After completing the urine collection and the SBP measurements, the GFR was determined as the plasma clearance of $^{51}$Cr-ethylenedimi-
amine tetra-acetic acid (17). Plasma samples obtained during the GFR measurement were analyzed with the ELAN system (Eppendorf/Merck, Hamburg, Germany), using colorimetric assays for albumin with brom cresol green, and creatinine with the Jaffé method without deproteinization.

For the structural investigations, kidneys were fixed by total body perfusion as described previously (18). Briefly, after anesthesia with Inactin (100 mg/kg body wt), the abdominal cavity was opened and the kidneys were retrogradely perfused via the abdominal aorta without prior flushing of the vasculature. Perfusion pressure in the perfusing apparatus was 80 mmHg above the last determined SBP (on average 190 mmHg in controls [WAG] and between 220 and 300 mmHg in FHH) and was maintained for 3 min. The fixative contained 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) supplemented with 0.08% CaCl and 0.5 g/L picric acid. After perfusion, the kidneys were removed immediately and immersed in the same fixative for a maximum of 2 d until further processing.

**Light Microscopy**

The right kidney was processed for light microscopy following standard procedures. After embedding in Paraplast, sections of approximately 3 μm thickness were cut and stained with hematoxylin and eosin.

**Transmission Electron Microscopy and High-Resolution Light Microscopy**

Tissue was processed in two ways. First, the classic OsO₄ post-fixation technique was applied followed by dehydration in a graded series of ethanol. Second, a modified post-fixation and staining technique was used, which minimizes treatment with OsO₄ and uses tannic acid as a contrast agent, followed by subsequent dehydration of the specimens in cold acetone (19). All tissues were finally embedded in Epon 812 by standard procedures.

Semi-thin sections (1 μm thick), including several section series, were cut with an appropriate diamond knife on an ultramicrotome, stained with azure II methylene blue, and examined by light microscopy (Polyvar 2, Reichert). Ultrathin sections (gray to silver) were cut with a diamond knife on a Reichert-Jung Ultracut E microtome and placed on Formvar-coated copper grids. The sections were stained in 5% uranyl acetate for 15 min and subsequently in Reynolds lead citrate over 2 min. They were observed under a Philips EM 301 electron microscope.

**Morphometry**

Morphometric analysis was carried out with a semiautomated image analysis system (VIDS IV; Ai Tektron, Düsseldorf, Germany) to determine mean total glomerular (VG) and tuft volumes (VT) based on a procedure described by Weibel (20). Surface areas of the total glomeruli (AG; including BC) and glomerular tufts (AT) were measured in 2- to 3-μm paraffin sections under direct visualization (magnification, ×690) in a blinded manner. The evaluation was carried out on superficial glomeruli (situated within 500 μm from the renal capsule). For each rat, 100 consecutively encountered superficial glomerular profiles were analyzed. Because glomeruli shrink along with the development of sclerosis (21), sclerotic glomeruli were excluded from the analysis.

Mean glomerular volume per animal (VG) and mean tuft volume per animal (VT) were calculated according to the formula (20): \[ V = (\beta/k) (A)^{3/2} \]

where \( \beta = 1.38 \) is the shape coefficient; \( k = 1.1 \) is the size distribution coefficient for spheres; and \( A \) is the mean glomerular or tuft surface area per animal. Because paraffin embedding causes shrinkage of the renal tissue by approximately 48% (22), reported values of glomerular and tuft volumes were corrected for shrinkage.

**Glomerular Damage Score**

To estimate the degree of glomerular damage, all glomerular profiles found in at least three (in controls, two) 1-μm Epon sections from different blocks of each animal (average, 86 profiles per experimental animal; range, 65 to 102; average of 42 profiles in controls) were screened under the light microscope in a blind manner. Injured glomeruli were subdivided into three groups: (a) glomerular profiles exhibiting at least three clearly dilated capillaries (diameter estimated as being at least twice as large as that of the bulk of capillary in this profile); (b) glomeruli with small, circumscribed tuft adhesions; and (c) glomeruli with segmental/global sclerosis. The percentage of the glomeruli (with respect to total number of encountered glomeruli) in group (a) was factored by 1, in group (b) by 2, and in group (c) by 3. The sum of these values in each animal was considered to reflect the degree of damage. The percentage values of group (c) were taken as sclerosis index.

In addition, the glomerular lesions in experimental animals were screened with respect to their topical relationships to the vascular pole (VP). A total of 203 glomerular profiles (average, 23 per animal; range, 17 to 29) exhibiting both the VP and a glomerular injury were evaluated whether the lesions (dilated capillaries [142 cases] and tuft adhesions/segmental sclerosis [61 cases]) were located at the VP or distant from it.

**Statistical Analyses**

Results are reported as mean ± SD. Differences between the two groups were tested with the unpaired t test or the Mann-Whitney U test. Linear regression analysis (either done by Pearson's or by Spearman's correlation test) was used to examine the relation between albuminuria and BP on the one hand and the damage scores (% segmental glomerulosclerosis and weighted damage index) on the other. All statistical analyses were performed with the Statistical Package for Social Sciences analysis program (Chicago, IL).

**Results**

Whole-body data and morphometric kidney data are all summarized in Table 1. FHH-rats show sustained urinary albumin excretion, have higher SBP, and higher filtration rates. These differences are all significant, in agreement with results in previous studies (2–4). There is no difference in plasma creatinine and plasma urea concentration between both animal groups (data not shown); plasma albumin concentrations are significantly lower in FHH rats than in WAG rats.

As has been reported previously (6,8), the kidneys of FHH rats exhibited the histopathologic picture of “classic” focal and segmental glomerulosclerosis. Interstitial fibrosis was also observed, clearly more prominent in areas of sclerotic glomeruli than around seemingly healthy or less seriously damaged glomeruli. The damage index (which included presclerotic lesions), as well as the incidence of sclerosis, correlated significantly with urinary albumin excretion, the damage index, and the degree of hypertension (Table 1). In WAG rats, no sclerosis was found.

Segmental glomerulosclerosis consisted of a broad tuft adhesion to BC associated with the vascular pole (VP) region. Among the glomerular profiles exhibiting both the VP and
Table 1. Clinical and morphometric data\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th>BW (g)</th>
<th>(U_{\text{alb}}) (mg/24 h)</th>
<th>(U_{\text{alb}}) per 100 g BW (mg/24 h)</th>
<th>Systolic Pressure (mmHg)</th>
<th>(V_{\text{Tuft}}) (m(^2) (\times) 10(^3))</th>
<th>(V_{\text{Tuft}}) per 100 g BW (m(^2) (\times) 10(^3))</th>
<th>GFR (ml/min)</th>
<th>GFR per 100 g BW (ml/min)</th>
<th>Damage Scoring</th>
<th>SGS (%)</th>
<th>Weighted Damage Index</th>
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<tr>
<td>25 wk-old FHH rats (n = 9)</td>
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<td>341</td>
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<td>34.8</td>
<td>142</td>
<td>1714</td>
<td>542.67</td>
<td>2.75</td>
<td>0.82</td>
<td>18.65</td>
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<td>102.5\textsuperscript{b,1}</td>
<td>25.1\textsuperscript{b,1}</td>
<td>15\textsuperscript{e}</td>
<td>182\textsuperscript{b}</td>
<td>66.87\textsuperscript{d}</td>
<td>0.21\textsuperscript{b}</td>
<td>0.09\textsuperscript{e}</td>
<td>18.74</td>
<td>44.55</td>
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<td>23 wk-old WAG rats (Control) (n = 6)</td>
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<tr>
<td>mean</td>
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<td>1.4</td>
<td>105</td>
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<td>0</td>
<td>7.28</td>
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<tr>
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<td>6.9</td>
<td>133</td>
<td>70.14</td>
<td>0.32</td>
<td>0.09</td>
<td>0.71</td>
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<tr>
<td>(U_{\text{alb}}) &amp; (U_{\text{alb}}) per 100 g BW</td>
<td>NS</td>
<td>NS</td>
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\textsuperscript{a} The significance of differences between the group means were evaluated by \(t\) test, except those indicated by "1", which were evaluated by the Mann-Whitney \(U\) test. Intragroup correlations were evaluated either by Pearson's correlation test (labeled with "2") or by Spearman's correlation test (labeled with "3"). BW, body weight; \(U_{\text{alb}}\), urinary albumin; \(V_{\text{Tuft}}\), tuft volume; SGS, segmental glomerulosclerosis; FHH, Fawn-hooded hypertensive; WAG, Wistar Albino Glaxo.

segmental sclerosis, the sclerotic tuft area (except for one case out of 61) was always associated with the VP. Certainly, segmental sclerosis may be encountered in glomerular profiles whose VP was not contained in the same section, but when tracing those profiles in serial sections, the sclerotic region consistently extended up to the VP (four such tracings were documented by photography; in many others, the association of the sclerotic area with the VP was verified simply by microscopic inspection).

Lesions Suggested to Precede VP-Associated Segmental Sclerosis

When inspecting 1-\(\mu\)m sections under the light microscope, several alterations in the vicinity of the VP are seen that appear to represent early lesions out of which segmental sclerosis may develop (Figure 1); these include architectural and cellular lesions. Among the architectural lesions, ballooning of primary branches of the AA was most prominent. These vessels are \textit{a priori} the largest glomerular capillaries (23–26). In FHH rats, these primary capillaries were consistently often dramatically dilated (Figure 1), and in some of the FHH rats almost every glomerulus was affected. An evaluation of 142 glomerular profiles that exhibited both the VP and dilated capillaries revealed that in all cases except one, dilated capillaries were associated with the VP; thus, most probably represented primary branches of the AA. As frequently seen in longitudinal sections (or when tracing those vessels in serial sections), the dilation of these primary branches extended to subsequent vessel portions, leading to an unfolding of the capillary pattern up to the urinary pole (Figure 1). Moreover, the "bulging out" of these vessels consistently (again as revealed when tracing such vessels in serial sections) resulted in an apposition to the parietal epithelium of BC. Thereby, the urinary space at the VP underneath the reflection of the podocyte epithelium (covering the glomerular stalk) into the parietal epithelium, \textit{i.e.}, the space representing the "stalk-capsule-corner," frequently became quite narrow (Figure 1). Three dimensionally, the "stalk-capsule-corner" is a recessus surrounding the glomerular stalk, vaulted by the transition of the visceral into the parietal epithelium of BC.

Cellular lesions were encountered in podocytes. Compared with the ubiquity of podocyte injury and in agreement with a previous study (6), lesions in endothelial and mesangial cells were not encountered: no endothelial defects, no mesangioly sis, and little, if any, mesangial cell proliferation. In contrast, podocytes, especially those covering balloononed capillaries, exhibited extensive changes. These podocytes appeared to be stretched out, their cell bodies were attenuated, their foot processes partially effaced; also, pseudocyst formation was often seen. Frequently, those injured podocyte portions were apposed to the parietal epithelium (Figure 1; because these podocyte lesions are fairly identical to those seen in later stages of the disease, we decided not to show them here by transmission electron microscopy).
**Figure 1.** Early vascular pole (VP)-associated lesions. Glomerular profiles exhibiting changes of the primary branches of afferent arterioles (AA) believed to represent the earliest lesions in the formation of a tuft adhesion. (a) Fairly normal glomerular profile with a dilated primary branch (asterisk) of an AA with pseudocyst formation (arrow) of associated podocyte. (b) Fairly normal glomerular profile with a dilated primary branch (asterisk) of AA apposed to Bowman’s capsule (BC; arrow). Most other capillaries have a quite uniform small diameter. (c) Tremendously expanded primary and secondary branches (asterisks) of AA in an otherwise normal glomerular profile. (d) Quite dramatically dilated primary afferent branches and branched capillaries (asterisks) leading to a narrowing of the stalk-capule-corners (arrows). Extensive pseudocyst formation (arrowheads) is apparent at several places. (e) Dramatic expansion of a primary branch of AA extending almost to the urinary pole. Note pseudocyst formation of involved podocytes (arrowheads), as well as the narrow stalk-capule-corner (arrow). (f) Dramatic expansion of primary branches (asterisks) of AA with appositions to BC at two sites (arrows), as well as narrowing of the stalk-capule-corner, with a podocyte (arrowhead) appearing to be compressed within this corner. (g and h) Adhesion of dilated primary branches of AA to BC (arrow in h) in an otherwise fairly normal glomerular profile; the boxed area in g is enlarged in h. Associated podocytes appear to be severely injured (stars), including pseudocyst formation (triangle). Fawn-hooded rat, 6 mo old. Light micrographs, except for b, which is a transmission electron microscopy (TEM). Magnification (approximate): ×320 in a; ×410 in b; ×380 in c, d, f, and g; ×470 in e; and ×910 in h.
Figure 2. Early adhesion of capillary loops to BC; an accompanying drawing helps to illustrate the figure. Three patent capillary profiles (1, 2, and 3) are incompletely deprived of their podocytes (arrows; in the drawing, the glomerular basement membrane [GBM] and mesangial structures are drawn in black, capillary lumina are hatched). The podocytes themselves (crosshatched in the accompanying drawing) show severe maladaptive changes, including foot process effacement, pseudocyst formation, and accumulation of absorption droplets. The parietal epithelium (densely stippled in the drawing) is dissociated from its basement membrane, deviates from a periglomerular course, and extends to the flanks of the adhesion (long hatched arrows). The parietal basement membrane (PBM) is still preserved in its multilayered organization (shown as a multilayered hatched line). Between the PBM on the one side and the deviating parietal epithelium and the attached tuft structures on the other side, a fluid-rich space (loosely dotted) has come into existence. On the outer aspect of the PBM, processes of cortical fibroblasts are arranged as an almost complete cover separating the PBM from the cortical interstitium. Fawn-hooded rat, 6 mo old. TEM (approximate), ×1900.

Structure of Small and Medium-Sized Adhesions

Small circumscribed adhesions were only found near the VP comprising one of the primary branches of the AA (Figures 1, g and h, 2, and 3). However, those small adhesions generally did not include the stalk-capsule-corner. With few exceptions, this corner contained a small partition of the urinary space
Figure 3. Small tuft adhesion to BC, as seen in a section series. An accompanying drawing helps to illustrate the figure. (a and f) Profiles just before and after the adhesion. (b through e) Sections through the adhesion. The adhesion contains a single capillary loop (asterisk in b through e; hatched in the drawings), which enters the adhesion from a and leaves toward f. Note the gap in BC: the parietal epithelium (shown as a simple black line in a1 through e1) deviates from its periglomerular course and attaches to the flanks of the adhesion (best seen in e and e1, arrows). The crescent-shaped paraglomerular space (stippled in the drawings) is separated from the cortical interstitium by a layer of fibroblast processes (arrowheads in e; shown as a hatched line in the drawings). At many places in the glomerular tuft, injured podocytes are seen exhibiting pseudocyst formation. Fawn-hooded rat, 6 mo old. Light microscopy (approximate): $\times400$ in a and d; $\times600$ in b; and $\times700$ in c, e, and f.

being outlined by a parietal cell adhering to the flank of the adhesion and a podocyte covering the glomerular stalk, as well as those capillary portions that were not enclosed within the adherent area (Figures 3 and 4).

A small adhesion consisted of one or two capillary loops devoid (at least partially) of a podocyte cover (Figures 2 and 3). The denuded GBM portion of this capillary directly abutted extracellular matrices outside the parietal epithelium (see be-
The parietal epithelium had disintegrated; parietal cells deviated from their periglomerular extension and made contact with the flanks of the adhesion, i.e., generally to the GBM of the adherent capillaries.

At the site of the capillary attachment to BC the parietal basement membrane (PBM) was consistently expanded. In an early stage of adhesion development, the expansion was restricted to the space between the innermost layer of the PBM and the parietal epithelium (Figure 2). In most cases, expansion of the translucent layers in between the dense layers of the PBM, spreading toward all sides, was prominent. In more advanced adhesions, disintegration of the dense layers of the PBM resulted in the formation of a continuous paraglomerular space (Figures 4 and 5). Eventually, this space appeared to be filled with a proteinaceous fluid containing remnants of former dense layers of the PBM. In addition, at various places of this space, extensive accumulations of membrane-bound granular vesicles were found, which quite obviously represented cell debris (Figures 4 and 7). Toward the cortical interstitium, this space was delineated by a layer of sheetlike processes of fibroblasts (Figures 4, 5, 6, and 7; see below).

Generally, capillaries included in small adhesions were patent even if they frequently showed considerable subendothelial depositions of hyaline. In more advanced adhesions (Figure 4), in addition to few open capillaries, collapsed capillaries or those totally occluded by hyaline material predominated.

**Large Adhesions: Segmental Sclerosis**

A large adhesion comprised at least one glomerular lobule; thus, it represented what is generally called segmental sclerosis. The glomerular profiles shown in Figures 5 and 6 display the essential structural features of segmental sclerosis as is characteristic for the FHH rat. The sclerotic area was associated with the VP of the glomerulus. As its dominant structural component it contained collapsed and/or hyalinized GBM formations representing former capillaries and mesangial areas (Figure 5). Inside these GBM agglomerations, few cells had survived representing former endothelial and/or mesangial cells. They extended with tiny processes into the narrow spaces between opposing collapsed GBM layers. Apart from the hyaline material and the matrix of the GBM itself, there was little other extracellular matrix in these profiles. Macrophages may

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**Figure 4.** VP-associated glomerulosclerosis as seen by TEM; the boxed area in a is enlarged in b. Accompanying drawings (a1 and b1) help to illustrate the situation. In these drawings, the GBM and mesangial areas, including all collapsed and hyalinized GBM formations, are shown in black. Parietal cells are densely stippled; for clarity, podocytes are not drawn. Capillary lumina are hatched, and the paraglomerular space is loosely stippled. Separation of the sclerotic area and the cortical interstitium is indicated by a hatched line in a1; in b1, the fibroblast profiles are vertically hatched. (a) The sclerotic area is crescent-shaped and separated from the cortical interstitium by a layer of fibroblast processes (arrows). It contains collapsed and/or hyalinized glomerular capillaries embedded in the proteinaceous matrix of the paraglomerular space. The partition of Bowman’s space in the stalk-capsule-corner (star) is still existent and associated with a patent primary afferent branch (hatched in a1 and b1), which penetrates into the sclerotic area. The walls of this capillary are partially hyalinized. The parietal epithelium adheres to the flanks of this capillary (clearly seen in b and b1). Note the granular cell debris (arrowhead in b) within this space. Fawn-hooded rat, 6 mo old. TEM (approximate): ×720 in a; ×2100 in b.
Figure 5. Fully developed segmental sclerosis comprising the VP. An accompanying drawing helps to illustrate the figure (symbols as in Figure 4). The sclerotic area is crescent-shaped, contains the collapsed or hyalinized remnants of glomerular capillaries and/or mesangial areas (shown in black in the drawing) representing the “sclerotic tuft portions.” They are embedded into a paraglomerular space (loosely stippled in the drawing) that is separated from the cortical interstitium by a continuous layer of cortical fibroblast processes (arrows; hatched line in the drawing). There are only a few cells within the paraglomerular space, most of them probably representing parietal cells. Toward the urinary pole (two adjacent stars), as well as at the other site of the VP (one star), a continuation of an expanded paraglomerular space is seen; however, the tuft at this site is still distinct from parietal epithelium. Fawn-hooded rat, 6 mo old. TEM (approximate), ×1150.

be encountered: rarely within the collapsed, more frequently within the hyalinized formations. Podocytes have virtually disappeared from the sclerotic area.

The real extension of an adhesion could only be recognized when tracing it in serial sections. Thus, it became clear that the development of a tuft adhesion is quite intimately associated with the enclosure of the sclerotic tuft portions into a space outside the urinary space. Figure 6 displays the extension of this space in four pictures of a section series. At the VP, associated with the adhesion of a primary branch of the AA, a space outside the parietal epithelium was discrete and appeared as an expanded PBM. Toward the center of the adhesion, this space progressively increased in breadth as well as in depth. Finally, it represented a tremendous crescent-shaped paraglomerular space interposed between the parietal epithelium and the cortical interstitium.

The paraglomerular space was clearly separated from the cortical interstitium and from the urinary space (Figures 6 and 8). The interstitium abutting the diseased side of a glomerulus was expanded, including an increase in the number of interstitial cells. From their morphology, we observed that most of them, in addition to some macrophages, were fibroblasts. To-
ward the adhesion, these fibroblasts had established a layer of sheetlike processes discontinuous in small adhesions, becoming a fairly complete barrier already in medium-sized adhesions. As seen in Figure 7, even multilayered elaborations have been encountered, in which gaps in the first layer were closed by a second layer.

Toward the urinary space, this paraglomerular space was separated by the parietal epithelium, which, at the borders of this space, deviated from its periglomerular course and extended to the flanks of the adhesion where it generally attached to the GBM (Figures 3, 4, 6, and 8). Three dimensionally, the parietal epithelium formed vaults overarching Bowman's space. These vaults attached to the adhesion in a line that encircled a gap, through which the sclerotic portions of the tuft came to lie outside the urinary space, whereas the unaffected (or at least nonsclerotic) portions of the tuft remained enclosed within the urinary space (Figures 4, 5, 6, and 8). The attachment line may cross podocytes (generally exhibiting serious lesions), i.e., these podocytes lie partially inside, partially outside the adhesion (Figures 2 and 9c).

**Advanced Glomerulosclerosis**

Glomerular profiles were encountered in stages of degeneration going beyond segmental sclerosis. Glomerular profiles with sclerosis extending around the entire VP up to totally collapsed profiles represented by balls of solid extracellular material were seen. This ultimate collapse of glomeruli has not been investigated in this study because comparatively few glomeruli had progressed to such terminal stages.

Most glomerular profiles in advanced degeneration still exhibited a tuft remnant protruding into a urinary space (Figure 8). In all of those profiles, a prominent paraglomerular space was developed that included the sclerotic tuft portions, i.e., collapsed or hyalinized GBM agglomerations. These solid structures may contain cellular elements (remnants of endothelial and/or mesangial cells) (Figures 5 and 8); in most advanced stages of glomerular degeneration, cells had disappeared. In addition to collapsed and/or hyalinized capillaries, the paraglomerular spaces generally contained from one to a few patent capillaries; they eventually disappeared with the collapse of the paraglomerular space, i.e., with the disappearance of any tuft remnant and the formation of an acellular matrix structure tightly embedded into the cortical interstitium (W. Kriz, A. P. Provoost, et al., manuscript in preparation). Generally, the paraglomerular spaces contained only a few cells that, from their morphologic appearance, mostly represented former parietal cells. Macrophages generally were not encountered inside the paraglomerular space but were seen either inside the GBM (included in hyalinized formations) or in the surrounding interstitium.

**Discussion**

FHH rats develop chronic renal failure at approximately 1 yr of age (1,3). The 6-mo-old rats of the present study were in a stage of balanced renal function, but with considerable urinary albumin excretion and decreased serum albumin levels. The structural damage as reflected by a weighted damage score significantly correlated with urinary albumin excretion and the degree of systemic hypertension (Table 1). Glomerular degeneration followed a pattern known as focal and segmental glomerulosclerosis; the segmental damage was consistently associated with the VP of the affected glomerulus.

**Sequence of Lesions Eventually Leading to Segmental Glomerulosclerosis**

First, we will discuss how glomerular degeneration develops in this model simply from a phenomenologic point of view, i.e., by raising the question how the various lesions can be brought into a step-by-step sequence terminating in glomerulosclerosis. Figure 9 presents schematics showing three stages of this process.

In agreement with other models of sclerosis development (11,12,27–29), the lesions initiating the damage sequence leading to segmental sclerosis appear to be ballooning and unfolding of peripheral capillaries. In the FHH rat, this process consistently and almost exclusively comprises the primary branches of the AA, which are straightened out into voluminous vascular channels, resulting in the apposition of these vessels to BC. Generally, podocytes covering ballooned capillaries exhibit various, frequently quite extensive, severe lesions, including foot process effacement, cell body attenuation, pseudocyst formation, and eventually detachment from the GBM. In agreement with what is known from other models (11–14), we conclude that areas of denuded GBM at bulging capillaries when coming into contact with the parietal epithelium lead to the attachment of the parietal cells to “naked” GBM, i.e., leading to the formation of a tuft adhesion. The affixation of the tuft to BC in early adhesions is consistently established between parietal cells and the GBM (Figure 9b).

Parietal cells, when attaching to the GBM, dissociate from their lateral contacts with adjacent parietal cells. Thereby, a gap in the parietal epithelium comes into existence, which opens a filtration/exudation route toward the cortical interstitium (Figure 9b, arrow). Glomerular capillaries inside an adhesion (being devoid of a podocyte cover) directly attach to the GBM. In contrast, for instance, to the situation in chronic Masugi nephritis (14), capillaries contained in early adhesions in the FHH rat are always patent, suggesting that they are perfused. The pressure gradient across the capillary wall, as well as the fact that the interface does not include a continuous cellular layer, strongly suggests some kind of filtration toward the cortical interstitium, probably accounting for the formation of a paraglomerular space outside the GBM and the deposition of hyaline material inside the GBM. Hyalinosis is generally believed to develop from uncontrolled filtration through podocyte-denuded capillary walls, resulting in the accumulation of large proteins inside the GBM because they cannot pass the GBM (30,31).

The growth of the adhesion appears to be intimately associated with the formation of a paraglomerular space. This space develops between the parietal epithelium and the cortical interstitium by expansion of the space occupied in healthy glomeruli only by the PBM. Initially, the translucent layers of the
Figure 6. Four sections of a series show the extension of the paraglomerular space associated with a fully developed segmental sclerosis; accompanying drawings help to illustrate the figure. The paraglomerular space (loosely stippled in the drawings) contains the sclerotic tuft.
PBM (which is a multilayered basement membrane; reference 32) expand by filling with a proteinaceous fluid, and this expansion spreads toward all sides. Consequently, PBM portions underlying an intact parietal epithelium also will expand and disconnect from the parietal cells. Obviously in response to the formation of a paraglomerular space, proliferation in the adjacent interstitium is seen. Sheetlike fibroblast processes become arranged as a continuous cellular layer separating the cortical interstitium from the paraglomerular space.

The adhesion progressively enlarges toward all sides, accompanied by the formation of a tremendous crescent-shaped paraglomerular space eventually surrounding more than a hemisphere of a glomerulus. In agreement with previous work (11–14), the growth of an adhesion apparently occurs by extension of the adherent area to neighboring capillaries due to progressive podocyte degeneration at the flanks of the adhesion. The parietal epithelium follows, moving along the podocyte-deprived GBM to neighboring capillaries. Thereby, the first capillaries lose their contact with parietal cells, being pushed into the center of a growing adhesion. Inside the adhesion, capillaries either collapse or are progressively filled out by hyaline material. Collapsed and/or hyalinized GBM formations (representing former capillaries and mesangial areas), which are eventually deprived of almost any cellular element and enclosed in a paraglomerular space, most typically represent segmental sclerosis in this model.

The encroachment of an adhesion up to and across the VP is associated with expansion of the paraglomerular space onto the glomerular stalk. Thereby, podocytes covering the glomerular stalk and the stalk-capsule-corner become separated from their GBM, resulting in a narrowing of the stalk-capsule-corner. As may be deduced from the cell debris, frequently seen in this corner, the cells in this area (podocytes/parietal cells) appear to be compressed and finally die. As a consequence, the stalk-capsule-corner becomes part of the adhesion.

The involvement of the podocytes at the stalk-capsule-corner appears to account for the extension of the segmental injury to other glomerular lobules, and, eventually, for the degeneration of the entire glomerulus. At the glomerular stalk, the various glomerular lobules are continuous with each other; podocytes at this site generally serve capillaries belonging to different lobules. Damage to those podocytes, therefore, may start the development of sclerosis in another lobule. The process of sclerosis itself does not appear to be any different from what happened in the first lobule; as ever, it appears to be crucially dependent on progressive podocyte degeneration preceding the adhesion and the collapse/hyalinosis of further tuft portions.

What Underlies the Expansion of Primary Branches of the AA?

The ballooning of primary branches of the AA represents the decisive precondition for sclerosis development in the present model. Dilation of these branches of the AA has been encour-
Figure 8. Advanced glomerulosclerosis extending from the VP (not contained in this section) up to the urinary pole. An accompanying drawing helps to illustrate the figure (symbols as in Figure 4). The glomerular profile consists of an "intact" tuft portion with some patent capillaries protruding into the urinary space (shown in white in the drawing) and an adherent "sclerotic" tuft portion, i.e., collapsed (stars) and/or hyalinized (asterisk) GBM formations (shown in black in the drawing) located in the paraglomerular space (loosely stippled). Also, within the "sclerotic" tuft portions, some capillaries are still patent (hatched). The podocytes (not shown in the drawing) overlying the capillaries of the "intact" tuft remnant are severely injured, exhibiting foot process effacement and pseudocyst formation (triangles). The parietal epithelium (densely stippled) separates the urinary space from the paraglomerular space. Toward the cortical interstitium, the paraglomerular space is separated by a layer of sheetlike fibroblast processes (arrows; shown as a hatched line in the drawing). Note the strict separation into four compartments: tuft, Bowman's space, paraglomerular space, and cortical interstitium. The sclerotic tuft portions are all enclosed within the paraglomerular space. Fawn-hooded rat, 6 mo old. TEM, ×650.

tered previously in other models of sclerosis (11,12,29) and has been interpreted as a pressure-dependent phenomenon based on Laplace's law. Accordingly, wall tension for a given pressure gradient increases with the diameter of the vessel. Thus, the first branches of the AA being a priori the largest capillaries (23,24,26,33) have to develop increasing counterforces to balance the progressing dilation of the vessel lumen.

The crucial question is what causes the initial dilation of the vessel: weakness in capillary wall structure or an increased glomerular capillary pressure acting, initially, on normal structures. Because there is no evidence indicating an a priori defect in the GBM or in podocytes or endothelial cells, we favor the idea that an increased capillary pressure, which has been shown to occur in the FHH rat (2,5), is the culprit. The kind of changes seen in podocytes overlying the dilated vessels are known from previous studies and have been interpreted to indicate mechanical overextension. Among them, foot process effacement may be regarded as an adaptive change leading to an increase in counterforces (34), whereas all subsequent lesions such as cell body attenuation and pseudocyst formation are considered to show serious mechanical overextension (35). We conclude that the lesions seen in these podocytes most probably result from increased stress to the capillary wall. Injured podocytes, in turn, will progressively fail to develop adequate counterforces, resulting in the further dilation of the affected capillaries until the tensile strength limit of the GBM is reached. Such a vicious cycle may explain the extreme ballooning of these capillaries, as well as the degeneration of the corresponding podocytes.
Figure 9. Schematic drawing shows the development of segmental sclerosis in the Fawn-hooded rat. (a) Normal glomerulus with vascular and urinary poles. Smooth muscle, extraglomerular mesangial cells, and mesangial cells proper are hatched; podocytes are shown in blue, parietal cells in red. The GBM is shown in black; the PBM is shown in yellow. Thin black lines indicate its multilayered organization. Tubular epithelia are shown in white. (b) Dilated primary branch of the AA is attached to BC. The attachment is accomplished by the adhesion of parietal cells to the podocyte-deprived GBM. Thereby, a gap in the parietal epithelium has developed that represents a route for fluid leakage (arrow) toward the cortical interstitium. The fluid first spreads within the basement membrane of the parietal epithelium, resulting in the expansion of the intrabasement membrane space. Note that the partition of the urinary space in the stalk capsule-corner is still patent. (c) The adhesion has spread to neighboring capillaries resulting either in the collapse or in hyalinosis (shown in a dark gray pattern) of those capillaries. Podocytes at the flanks of the adhesion degenerate. The parietal epithelium may either appose to those podocytes (arrowhead in c) or attach directly to the GBM at the flanks of an adhesion. Fluid leakage toward the cortical interstitium has created a paraglomerular space (shown in yellow), which contains the sclerotic tuft remnants (i.e., collapsed or hyalinized GBM formations). Toward the cortical interstitium this paraglomerular space becomes separated by a layer of sheetlike fibroblast processes (shown in green). (d) Fully developed glomerulosclerosis comprising the entire VP. An intact tuft remnant protrudes into the urinary space outlined by the parietal epithelium. The sclerotic tuft remnants are located outside the parietal epithelium in a paraglomerular space, which is separated from the cortical interstitium by a complete layer of cortical fibroblasts.
What Underlies the Formation of a Tuft Adhesion and the Advancement of an Adhesion to Sclerosis?

The formation of a tuft adhesion starts by the attachment of parietal cells to the GBM of a peripheral capillary loop. Why parietal cells disconnect from their basement membrane and attach to the GBM, thereby permitting disintegration of the parietal epithelium, is most obscure. It is somewhat surprising that compared with the frequency of adhesions, “bare” GBM areas at ballooned peripheral capillaries are rarely seen. This suggests that parietal cells in cases of podocyte detachment instantly attach to a portion of naked GBM, which approaches the parietal epithelium. It is also possible that the detachment of podocytes from the GBM and the attachment of parietal cells to the GBM are not strictly follow-up events, but that seriously injured podocytes detaching at small circumscribed areas from the GBM (even transiently) may trigger apposing parietal cells to attach to the GBM. The attachment of parietal cells to the GBM appears to be stronger than that of podocytes, leading to the suggestion that the specific attachment of podocytes to the GBM by α3β1 integrins (36–38) may for some unknown reason be comparably loose.

The attachment of “naked” capillaries to the PBM at a gap in the parietal epithelium appears to be followed by some kind of filtration toward the cortical interstitium, leading to the formation of a paraglomerular space (see above). In early stages of adhesion development, the amount of fluid reaching the cortical interstitium along this route may be cleared by the peritubular circulation. Later, however, the establishment of a cellular barrier of cortical fibroblast processes between the paraglomerular space and the cortical interstitial spaces may limit the dissipation of filtered fluid into the cortical interstitium, favoring the further enlargement of the paraglomerular space.

The expansion of the paraglomerular space appears to contribute to further podocyte degeneration. Progressive podocyte detachments at the flanks of an adhesion are the prerequisite for the growth of the adhesion. In addition to mechanisms possibly responsible for this process proposed in previous works (11,14) the present study suggests an additional possibility. As frequently seen (Figure 2; schematically shown in Figure 9c), podocytes at the flanks of an adhesion are partially included into the adhesion and partially attached to the tuft portions outside the adhesion. Thus, these podocytes are exposed to the expandile forces that account for the formation and enlargement of the paraglomerular spaces; this may lead to cell surface deformations near the GBM, which, according to a theoretical model (39), are most effective in lifting podocytes from the GBM. This conclusion is corroborated by observations at the glomerular stalk, where expansion of the paraglomerular space continues into an expansion of the space between the GBM and the corresponding podocytes leading to their detachment.

Patterns of Cell Reactions During Sclerosis Development

In the process of sclerosis development, including the final glomerular degeneration, a finite number of cells are involved (endothelial and mesangial cells, podocytes, parietal cells, and interstitial cells including macrophages), which show quite different patterns of reaction.

As seen in this and a previous study of sclerosis development in the FHH rat (6), in early stages of the disease, i.e., before the formation of a tuft adhesion, structural lesions in mesangial and endothelial cells were not encountered. This differs sharply from other models of segmental glomerulosclerosis, for instance, from Masugi nephritis (14,40,41), in which the disease starts with extensive damage to endothelial and mesangial cells. In later stages of synechia development, endothelial and mesangial cells are becoming enclosed within the collapsed and/or hyalinized GBM formations. Again, no local hypercellularity indicating proliferation of endothelial or mesangial cells is seen. In contrast, these GBM-including cellular elements gradually decrease along with the progression of an adhesion to sclerosis; eventually, the matrix remnants in sclerotic glomeruli are fully deprived of any cellular elements. Obviously, these cells die, and the debris is cleared by macrophages that may be found in areas inside the former GBM.

The cells that keep the process of sclerosis going clearly are the podocytes. The formation and growth of tuft adhesions are based on progressive podocyte damage. Starting with stereotyped lesions in cell shape (as they are seen in many other glomerulopathies; reference 35), podocytes finally undergo necrosis and disintegrate. When dying podocytes are enclosed in the urinary space, their cell debris appears to be carried away by urine flow. When dying podocytes are enclosed in the paraglomerular space, their remnants are encountered as accumulations of membrane-bound granular vesicles. Because macrophages do not penetrate these spaces, these remnants appear to persist for some time. As discussed elsewhere (10,11,13,42,43), podocytes are unable to proliferate. Thus, the only way to balance the function of a lost podocyte is by hypertrophy of an adjacent uninjured podocyte. However, hypertrophy will increase the vulnerability of the remaining podocytes to any challenge, thereby accelerating the degenerative process.

Parietal cells take an active role in the development of sclerosis. Their attachment to the GBM of a peripheral capillary creates the nidus for sclerosis. Why parietal cells tend to separate from their basement membrane in order to attach to the GBM represents a central unanswered question in the process of sclerosis. Parietal cells as well undergo progressive cell death in the evolution of sclerosis; eventually, the parietal epithelium disappears. As with podocytes, cell debris of necrotic parietal cells may be delivered to the urinary space or may become incorporated into the paraglomerular space showing up as granular vesicles for some time.

Fibroblasts surrounding such a degenerating glomerulus were consistently seen to proliferate and to establish a continuous layer of sheetlike cellular processes that appears as aiming at enclosing the locus of injury, limiting fluid leakage into the cortical interstitium. In addition, this layer appears to function as a barrier for the migration of macrophages into the paraglomerular space. As long as a tuft remnant is seen in a degenerating glomerulus, fibroblasts and macrophages are not
encountered within the surrounding paraglomerular space. An approach of those cells onto the sclerotic core of a degenerating glomerulus is seen in association with the collapse of the paraglomerular space in very late stages of the disease.

**Conclusion**

Segmental glomerulosclerosis in the FHH rat is consistently associated with the VP, a pattern seen in a variety of other models presumed to have high intraglomerular pressures (29,44–46). This discrete location derives from an initial lesion at primary branches of the AA. Podocytes serving these largest glomerular vessels (in which wall tension would be greatest) are exposed to increased strain. Podocyte insufficiency (10) in counteracting the expansile forces will lead to greatest) are exposed to increased strain. Podocyte insufficiency (10) in counteracting the expansile forces will lead to significant (29,44-46). This triggers parietal cells to attach to "bare" areas of the GBM, establishing a "beachhead" of parietal epithelium on the tuft. This event represents the first committed lesion inevitably progressing to segmental sclerosis. In this process, fluid leakage into the periglomerular interstitium from perfused capillaries contained in a tuft adhesion plays a prominent role.

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**References**

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